Physiol Rev
84: 277–359, 2004; 10.1152/physrev.00015.2003.

Brown Adipose Tissue: Function and Physiological Significance

BARBARA CANNON AND JAN NEDERGAARD

The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, Stockholm, Sweden

Cannon, Barbara, and Jan Nedergaard. Brown Adipose Tissue: Function and Physiological Significance. *Physiol Rev* 84: 277–359, 2004; 10.1152/physrev.00015.2003.—The function of brown adipose tissue is to transfer energy from food into heat; physiologically, both the heat produced and the resulting decrease in metabolic efficiency can be of significance. Both the acute activity of the tissue, i.e., the heat production, and the recruitment process in the tissue (that results in a higher thermogenic capacity) are under the control of norepinephrine released from sympathetic nerves. In thermoregulatory thermogenesis, brown adipose tissue is essential for classical nonshivering thermogen-

esis (this phenomenon does not exist in the absence of functional brown adipose tissue), as well as for the cold acclimation-recruited norepinephrine-induced thermogenesis. Heat production from brown adipose tissue is activated whenever the organism is in need of extra heat, e.g., postnatally, during entry into a febrile state, and during arousal from hibernation, and the rate of thermogenesis is centrally controlled via a pathway initiated in the hypothalamus. Feeding as such also results in activation of brown adipose tissue; a series of diets, apparently all characterized by being low in protein, result in a leptin-dependent recruitment of the tissue; this metaboloregulatory thermogenesis is also under hypothalamic control. When the tissue is active, high amounts of lipids and glucose are combusted in the tissue. The development of brown adipose tissue with its characteristic protein, uncoupling protein-1 (UCP1), was probably determinative for the evolutionary success of mammals, as its thermogenesis enhances neonatal survival and allows for active life even in cold surroundings.

I. A MAMMALIAN PREROGATIVE: BROWN ADIPOSE TISSUE

In popular and in formal definitions of the animal group to which we belong, the mammals, our ability to feed our young in a practical way is the one characteristic normally advanced. However, it is not this characteristic alone that has given us an evolutionary advantage. Notably, a unique organ, brown adipose tissue, exists in mammals. Brown adipose tissue is probably the outcome of a single evolutionary development, occurring very early during the evolution of mammals. Although impossible to prove, good arguments can be forwarded that this development, i.e., the acquisition of brown adipose tissue with its new protein, uncoupling protein-1 (UCP1, thermogenin), may have been the one development that gave us as mammals our evolutionary advantage, i.e., to survive and especially to be active during periods of nocturnal or hibernal cold, to survive the cold stress of birth, and probably also by promoting our survival on diets low in essential macronutrients, especially protein. The functional significance of this unique mammalian organ is the subject of this review.

In contrast to other mammalian organs, brown adipose tissue is still scientifically a rather new organ. Although described in certain mammals since 1551 (244), the realization that brown adipose tissue is found in all mammals has occurred within the last century. That heat production is one of the functions of brown adipose tissue has only been formulated for 40 years (751), and the involvement of the tissue in or even its full responsibility for diverse types of metabolic inefficiency (i.e., as a possible antiobesity organ) has only been discussed for some 20 years (680). The identification of UCP1 as the mitochondrial protein responsible for the unique function of brown adipose tissue is of a similar short age (19, 311).

The present review is a further contribution to a series of reviews and books on nonshivering thermogenesis and brown adipose tissue (332, 379, 446, 575, 754, 816). Very detailed reviews of brown adipose tissue function in general, especially in connection with metabolic control, were compiled in the late 1980s (328, 329, 331), and we will not here replicate these efforts.

Brown adipose tissue morphology has been particularly elegantly presented recently (133), but the impacts of the scientific developments of the last decade have not been synthesized into a comprehensive analysis of brown adipose tissue function. The last decade has brought us an understanding of the background of genetically obese phenotypes, the identification of a family of mitochondrial carrier proteins (659) more similar to UCP1 than to any other protein (raising questions concerning the uniqueness of brown fat-derived thermogenesis and metabolic inefficiency) and, as new experimental tools, the development of mice strains deficient in brown adipose tissue (462) or in UCP1 (200), which in their turn have allowed for the demonstration of the essentiality of UCP1 for thermogenesis in the brown adipocytes (491) and for nonshivering thermogenesis in the intact animal (260, 262, 565). We have thus in the present review concentrated on issues developing during the last decade. With now more than 5,000 articles dealing to some extent with brown adipose tissue as such, there is no possibility to be comprehensive, nor is it possible to encompass general biological concepts; we attempt only to reference observations made specifically in brown adipose tissue. We have nevertheless attempted to be conclusive, in the light of available evidence, often to the exclusion of occasional contradictory evidence (which, perhaps, may ultimately become the more correct interpretation); we consequently apologize for misinterpretations, oversights, and omissions.

Certain types of experiments on brown adipose tissue are often performed on particular species of animals, e.g., mice, rats, and Syrian and Djungarian hamsters. We have tried to avoid qualifying each statement as to species, strain, or other condition investigated, provided we have not considered this type of qualification essential. We thus discuss a generic brown adipose tissue. We concentrate especially on the functional significance of brown fat-derived thermogenesis, i.e., to what extent are alterations in metabolism and metabolic efficiency, observed under a broad variety of physiological conditions, explainable through brown adipose tissue thermogenic activity (see sects. V and VI). However, to be able to do this, we initially describe the thermogenic mechanism in the single heat-producing unit, the brown adipocyte, and

how the functional capacity of brown adipose tissue may be altered (i.e., the recruitment processes) (see sects. II–IV). We also discuss the extent to which brown adipose tissue may play a systemically important role in other respects than thermogenesis, by releasing or extracting substances to or from the circulation (see sects. VII and VIII), and we finalize with a short comment (see sect. IX) on brown adipose tissue function in the mammalian species that attracts much of our interest: humans.

To facilitate the more detailed discussion that will follow, a general overview of brown adipose tissue function within the mammalian organism can be seen in Figure 1*A*. Although the thermogenic unit is the brown adipocyte, placed in the center of Figure 1, it is evident from the figure that even within the tissue, the brown adipocyte cannot work in isolation: its activity is controlled by the nerve fibers reaching each cell, and the brown adipocyte is dependent on adequate delivery of oxygen and substrate (lipids) through the capillaries surrounding each cell (212); the delivery of its product, heat, to the organism is equally dependent on the heated blood leaving the tissue. Thus, although the brown adipocytes themselves constitute the main volume of the tissue, the mature brown adipocytes are probably in minority among the cells in the tissue (241), with the largest number of cells being the endothelial cells of the capillaries, and the interstitial cells and preadipocytes that, under conditions of increased thermogenic demand, will divide and differentiate to form new brown adipocytes. In such recruitment phases, not only the number of brown adipocytes but also the capillaries and the nerve terminals have to expand in a coordinated way to fulfil the new demands.

The study of the physiological signficance of the tissue would have been much simpler if brown adipose tissue was only found in one place in the body. However, as summarized in Figure 1*B*, brown adipose tissue is found in defined but dispersed areas in the body, and brown adipocytes may be identified in clusters even within white adipose tissue depots, to a varying degree in different animals or strains of animals. Therefore, the metabolic significance of the tissue in different physiological conditions is still not fully established, but as will be evidenced in the present review, it is an organ with unique functions.

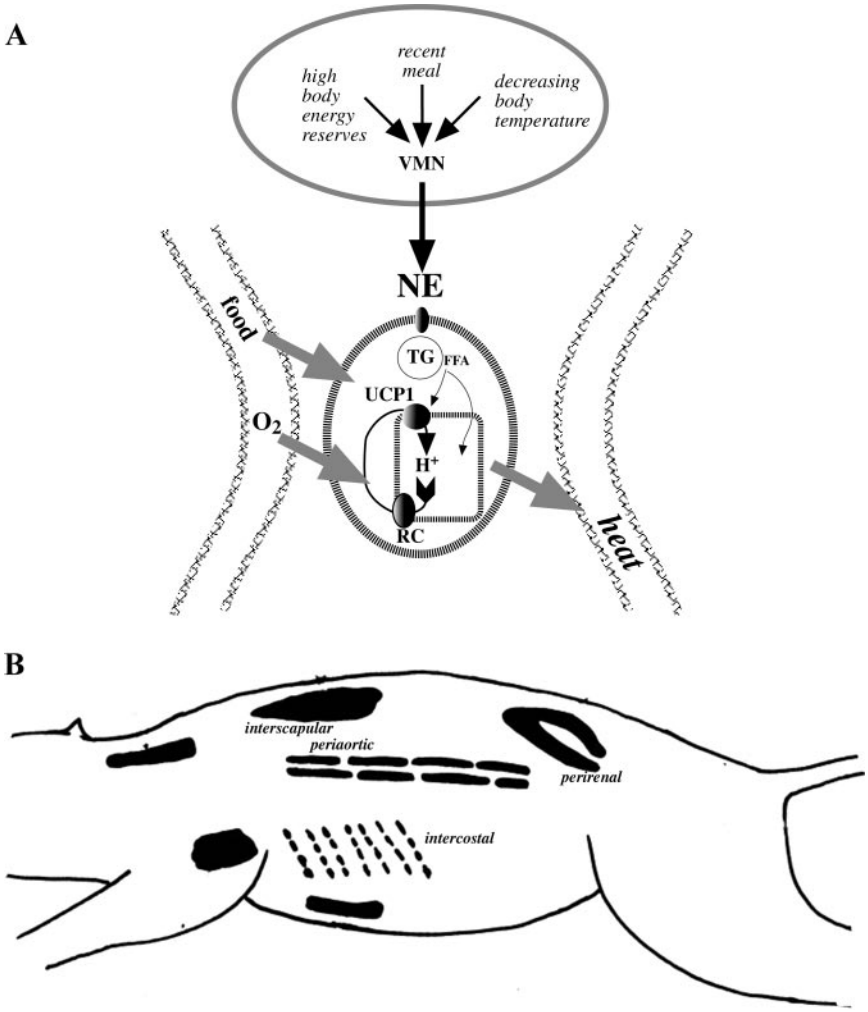

FIG. 1. *A*: an overview of the acute control of brown adipose tissue activity. Information on body temperature, feeding status, and body energy reserves is coordinated in an area in the brain that is probably the ventromedial hypothalamic nucleus (VMN). When there is reason to increase the rate of food combustion (decrease metabolic efficiency) or increase the rate of heat production, a signal is transmitted via the sympathetic nervous system to the individual brown adipocytes. The released transmitter, norepinephrine (NE), initiates triglyceride breakdown in the brown adipocytes, primarily via β_3-adrenergic receptors. The intracellular signal is transmitted via cAMP and protein kinase A, leading to the release from triglycerides (TG) of fatty acids (FFA) that are both the acute substrate for thermogenesis and (in some form) the regulators of the activity of uncoupling protein-1 (UCP1, thermogenin). Combustion of the fatty acids in the respiratory chain (RC) leads to extrusion of H^+, and UCP1 thus allows for mitochondrial combustion of substrates, uncoupled from the production of ATP, by functionally being (the equivalent of) a H^+ transporter. The outcome is that an increased fraction of the food and the oxygen available in the blood is taken up by the tissue and combusted therein, leading to an increased heat production. The participation of brown adipose tissue in total energy metabolism is, at least in smaller mammals, very substantial; at "normal" ambient temperatures, nearly one-half of their energy metabolism may be related to brown adipose tissue activity, and in small mammals living in cold environments, the predominant energy utilizer is brown adipose tissue. The capacity of the tissue for the metabolism of the animals alters thus as an effect of environmental conditions: it atrophies when not needed and it becomes recruited when a chronic, high demand is encountered. *B*: brown adipose tissue distribution in the body.

II. NOREPINEPHRINE CONTROLS THE THERMOGENIC PROCESS

The minimal functional thermogenic unit of brown adipose tissue is the brown adipocyte itself. For an understanding of brown adipose tissue function, and especially for an understanding of how different physiological conditions may lead to an alteration (recruitment or atrophy) in the total thermogenic capacity of the tissue, an understanding of the factors that influence the acute activity of the brown adipocyte, as well as its birth, development, and death, is necessarily of importance. Classical knowledge concerning the brown adipocyte was reviewed in Reference 567.

Among the factors that influence the brown adipocyte, norepinephrine is both the most important and the most well-studied. This effector is most significant physiologically, not only for the acute thermogenic process but also for the control of cell proliferation, advanced cell differentiation, and apoptosis. We therefore first review adrenergic signaling in brown adipocytes, leading towards regulation of the acute thermogenic process. Adrenergic effects on cell proliferation, differentiation, and apoptosis are discussed in section III.

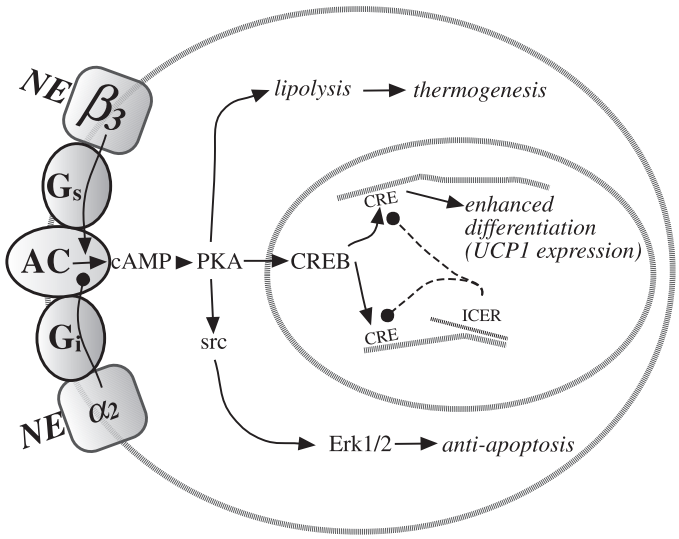

FIG. 2. The β_3- and α_2-adrenergic signaling pathways in mature brown adipocytes. NE, norepinephrine; G_s, stimulatory G protein; G_i, inhibitory G protein (dashed lines with solid circles denote inhibition); AC, adenylyl cyclase; PKA, protein kinase A; CREB, CRE-binding protein; CRE, cAMP response element; ICER, inducible cAMP early repressor (it is the resulting protein that inhibits the stimulatory effect of phosphorylated CREB on its own transcription and on that of certain other proteins).

A. Norepinephrine Signaling Through β_3-Receptors Leads to Thermogenesis

1. β_3-Adrenoceptors in mature brown adipocytes

In mature brown adipocytes, norepinephrine interacts with all three types of adrenergic receptors: β, α_2, and α_1; these receptor types are associated with activation of different signaling pathways in the brown adipocytes, as will be detailed below. The most significant and the most studied pathway is the pathway for β-adrenergic stimulation of thermogenesis (Fig. 2).

Of the three subtypes of β-adrenergic receptors, the β_3-adrenoceptor is the most significant in mature brown adipocytes from rodents. β_1-Adrenoceptors are also expressed in mature brown adipocytes, but they are not coupled to any significant extent to signaling processes in these cells; they are, however, coupled to cAMP production in brown preadipocytes (76) (see sect. III*A*), which means that in membrane preparations from total brown adipose tissue, both receptor subtypes will be functional (126). β_2-Adrenoceptors are not expressed in the brown adipocytes themselves (46), but they are expressed in the tissue (651, 652) and can be observed as binding sites in membrane preparations from brown adipose tissue (438, 676). These β_2-adrenoceptors are probably predominantly localized to the vascular system.

The extent to which the β_3-adrenoceptor mediates the physiological effects of norepinephrine is routinely examined by comparing the effects of norepinephrine stimulation with those of a "specific" β_3-agonist. The β_3-agonists most commonly used are BRL-37344 (25) (which, however, is only a selective β_3-agonist, i.e., at higher concentrations it also stimulates β_2-receptors), CGP-12177 (516) (which is an antagonist on β_1/β_2-receptors), and CL-316243 (336) (which must be considered presently as the most selective β_3-agonist available). It is generally assumed that thermogenesis stimulated by one of these agents (especially CL-316243) in intact animals is indicative of brown adipose tissue thermogenesis, primarily because β_3-receptors are practically only found in white and brown adipose tissue, and because the total thermogenic capacity of white adipose tissue is supposedly so low that it can be neglected in this context [but this assumption has been challenged (275a)].

The existence of a fourth β-adrenoreceptor, the β_4-receptor, has sometimes been discussed (234, 391), also in brown adipose tissue (626). One of the properties of this receptor should be that it is stimulated by CGP-12177 (it is thus difficult to differentiate from the β_3-receptor in normal brown adipocytes). Such a "β_4-effect" has sometimes been ascribed to an atypical activation by CGP-12177 of β_1-receptors in a certain conformation (rather unexpectedly, as CGP-12177 is a high-affinity antagonist on these receptors) (275, 408). There is thus no gene for this "β_4-receptor," and the phenomenon is still not fully clarified.

In addition to being characterized by specific stimulation by "specific" β_3-agonists, β_3-adrenoceptors are also characterized by a very low affinity for classical β-adren-

ergic antagonists, such as propranolol [with a pA_2 of ~9 on β_1/β_2-receptors and ~6 on β_3-receptors, i.e., about 3 orders of magnitude lower affinity (25, 900)]. To eliminate β_3-stimulation in vivo, very high concentrations of propranolol must therefore be used (at least ≥10 mg/kg body wt). Unfortunately, no well-recognized high-affinity selective β_3-antagonist is presently available; SR 59230A has been suggested (585), but the efficacy of this ligand has been criticized. Thus simple questions concerning the significance of the β_3-pathway cannot be answered simply, by experimentally inhibiting this pathway selectively.

2. β_3-Adrenoceptors do not possess properties essential for brown adipose tissue function

The distinct localization of β_3-receptors to brown and white adipose tissue has led to suggestions that the receptors as such may have functional properties necessary or at least advantageous for (brown) adipose tissue function. This does not, however, seem to be the case.

In this respect, it is noteworthy that the guinea pig lacks identifiable, functional β_3-receptors in brown adipose tissue (33), but its brown adipose tissue is nonetheless fully functional (80, 338, 456). Similarly, brown adipocytes prepared from animals in which the β_3-gene has been ablated are fully functional, except that in these cells, it is the β_1-adrenoceptor that mediates the β-response (139, 408, 596). The ability of brown adipocytes from β_3-ablated animals to respond to norepinephrine via β_1-receptors does not indicate that β_1-receptors are normally responsible for stimulation of thermogenesis; rather, there is an induced expression of β_1-adrenoceptors in these animals (781). [The term *compensatory* is often used for such a situation, but this is easily interpreted as implying some nearly conscious act on the part of the cell; however, the increase in β_1-adrenoceptor expression is probably coincidental, as expression of the β_1-gene is under positive adrenergic control (46) and is thus self-inducing under conditions of increased sympathetic tone, which would be expected to occur when insufficient heat is produced due to the absence of β_3-receptors.]

The β_3-receptors distinguish themselves from the β_1/β_2-receptors by lacking most of the amino acid residues that are normally thought to be involved in receptor desensitization (199, 545). It can easily be argued that it would be advantageous for brown adipocytes to possess receptors that were not easily desensitized (because thermogenesis often has to proceed for very prolonged periods). It could therefore be assumed that cells with β_1-receptors would desensitize more rapidly than β_3-expressing wild-type cells, although there is no published evidence for this. In this context, it is also notable that, although the β_3-receptor may not be easily experimentally desensitized, the β_3-expression level (mRNA) is dramatically downregulated (at least transiently) during continuous adrenergic stimulation (47, 276, 398), and this could also result in functional desensitization.

It is sometimes stated that β_3-receptors are less sensitive to norepinephrine than are β_1-receptors. Thus it has been claimed that at low levels of sympathetic stimulation, it would be the β_1-adrenoceptors that would be activated (32, 233, 421). There is, however, no unequivocal evidence for this, neither in transfected systems (in which β_3-receptors have affinities intermediate between β_1- and β_2-receptors, Ref. 797), nor functionally in brown adipocytes [the functional EC_{50} for cAMP formation by norepinephrine in preadipocytes (where the β_1-receptor is dominant) is not lower than it is in mature brown adipocytes (76)]. [There is, however, a lower sensitivity of the β_3-receptor than the β_1-receptor for the pharmacological β-agonist isoprenaline (isoproterenol) (596).] At low norepinephrine concentrations, the thermogenic response is more sensitive to a given dose of propranolol than it is at high norepinephrine concentrations (32), but this is an inherent feature of interaction between agonists and antagonists and does not indicate a shift from β_1-receptors at low to β_3-receptors at high norepinephrine.

It has also been suggested that β_3-receptors could have a dual coupling to the transducing G proteins (to G_i as well as to G_s, see below), but as the splice variant expressed in mouse brown adipose tissue does not have this property (363a), this would seem not to be a general phenomenon in native brown adipocytes.

Thus, at present, there is no evidence that the presence of β_3-receptors (as compared with β_1/β_2-receptors) on brown adipocytes and their coupling to thermogenesis is anything other than coincidental, and the β_3-receptor apparently does not confer to the brown adipocytes any demonstrated physiological advantage. However, the presence of the β_3-receptors predominantly (although not exclusively) on white and brown adipocytes means that these receptors are potentially convenient targets for drugs against obesity, even bearing in mind the lower functional significance of these receptors in human than rodent adipose tissue.

3. Only G_s proteins couple to thermogenesis

β-Adrenergic receptors normally couple to G proteins of the G_s subtype. This coupling has been indirectly demonstrated in brown adipose tissue, since norepinephrine infusion enhances the ability of cholera toxin to ADP-ribosylate the G_s protein (274) and cholera toxin can mimic the effects of β-stimulation (479). G_s proteins exist in $G_s\alpha$L and $G_s\alpha$S forms in brown adipocytes as in other tissues; during differentiation from brown preadipocytes to mature brown adipocytes, the $G_s\alpha$S variant increases, without any change in $G_s\alpha$L (72) and without any functional change being observed.

Based mainly on experiments with ectopically ex-

pressed human β_3-receptors and in cell lines, a particular feature of β_3-adrenoceptors has been suggested to be that they may be dually coupled, i.e., not only to G_s but also to the inhibitory G_i proteins (127, 243, 757). This has been discussed also as being a component in the further mediation of the β_3-signal to the mitogen-activated protein (MAP) kinase system(s) (see below). A parallel β_3-stimulation of G_i would mean that the signal (in the form of cAMP formation) would be self-limiting, and the inhibitor of the G_i pathway, pertussis toxin, should in this case specifically increase β_3-induced cAMP formation in brown adipocytes. However, whereas pertussis toxin does increase cAMP formation, it does so independently of which receptor, adrenergic or not, that cAMP formation is stimulated through (unpublished observations). Thus a high inherent G_i stimulation (endogenous or due to an unknown agonist) may constitutively inhibit cAMP formation. Indeed, when pertussis toxin is given in vivo, a large left-shift of norepinephrine sensitivity is observed (785).

4. Adenylyl cyclase, cAMP, and protein kinase A mediate the thermogenic signal

The further β-adrenergic signaling cascade is mediated via adenylyl cyclase activation: the norepinephrine-induced cAMP formation is fully mediated via β_3-receptors in mature brown adipocytes (899, 900). Correspondingly, all tested β-adrenergic effects, including thermogenesis (144, 709, 778), can be mimicked by the adenylyl cyclase activator forskolin. It is not fully established which of the 10 adenylyl cyclase isoforms that are responsible for mediating the signal in mature brown adipocytes; several are expressed in brown adipose tissue (125, 128), and there are functional indications of a change in active adenylyl cyclase isoform during brown adipocyte differentiation (78).

In other tissues, in addition to its interaction with protein kinase A, cAMP directly activates other proteins [cation channels, exchange proteins directly activated by cAMP (EPACs)]. There is no indication to date that any cAMP effects in brown adipocytes are mediated in ways other than through activation of protein kinase A, the activity of which is increased as an effect of adrenergic stimulation (801); conversely, the inhibitor of protein kinase A, H-89, blocks all effects of β_3-stimulation so far identified and examined in native brown adipocytes (thermogenesis, downstream kinases, gene expression) (107, 226, 227, 450).

5. Protein kinase A-phosphorylated proteins

Through phosphorylation of a series of target enzymes, the activated protein kinase A leads to further mediation of the adrenergic signal.

A) PHOSPHORYLATION OF NUCLEAR-RELATED PROTEINS. Also in brown adipocytes, protein kinase A phosphorylates the transcription factor CREB (802). CREB then supposedly activates the expression of genes, including that for UCP1 (see sect. III*B3*) (Fig. 2). Phosphorylated CREB also induces expression of the transcription factor ICER (801), which is competitive with CREB itself on (certain) CRE sites where it instead acts as a repressor. This successive increase in ICER formation may explain the transient expression of certain genes occurring during sustained norepinephrine stimulation.

The protein kinase A pathway also leads to activation of Src (450), but this cannot be direct, as Src is phosphorylated on a tyrosine residue and is thus not a direct target of protein kinase A; activation of an intermediate tyrosine kinase must therefore be postulated. Activation of Src leads to subsequent activation of one of the three MAP kinase pathways, the Erk1/2 pathway (451, 739), which in its turn couples further to inhibition of apoptosis (451) (see sect. III*C*) (but, in contrast to the CREB pathway, is not linked to control of, e.g., UCP1 gene expression; Ref. 107).

Protein kinase A also induces the activation of a second MAP kinase pathway, the p38 pathway (107). This activation has been suggested to be involved in the adrenergic stimulation of UCP1 gene expression. The third MAP kinase pathway, the stress-activated JNK pathway, is not stimulated by norepinephrine in brown adipocytes in culture; activation is seen in the tissue in vivo during cold exposure, but the cell type and pathway for this activation are unknown (unpublished observations).

The coupling from G protein-coupled receptors (such as the β_3-receptors) to MAP kinases has been proposed in other systems to proceed via a transactivation of a plasma membrane tyrosine kinase receptor, most often the epidermal growth factor (EGF) receptor [or the platelet-derived growth factor (PDGF) receptor] (161, 322, 492). Although the EGF receptor exists and is functional in brown adipocytes (318, 449), it is not activated (phosphorylated) following β-adrenergic stimulation, and inhibition of its activity (by the EGF receptor inhibitor and ATP analog AG1478) does not inhibit norepinephrine-induced MAP kinase activation (although it inhibits EGF-induced MAP kinase activation) (449). There is therefore no indication that transactivation of the EGF receptor is an obligatory step in norepinephrine-induced MAP kinase activation.

B) PHOSPHORYLATION OF CYTOSOLIC PROTEINS. In parallel with its activation of the nuclear proteins summarized above, protein kinase A also phosphorylates (activates) a series of proteins in the cytosol. It probably activates the protein phosphatase inhibitor DARPP (502), in this way potentially prolonging its own action.

Protein kinase A probably also activates/inhibits a series of metabolic pathways in the brown adipocyte, but with the exception of the lipolytic pathway, such pathways have not been studied in brown adipocytes. How-

ever, the lipolytic pathway is the one that leads to thermogenesis in the brown adipocytes, and this pathway is therefore central to the understanding of control of thermogenesis in brown adipocytes (and thus of nonshivering thermogenesis in general).

B. Thermogenesis Is Due to Activation of UCP1 Through Lipolysis

Since the formulation of brown adipose tissue as a thermogenic organ (751), a number of molecular mechanisms to accomplish this thermogenesis have been proposed. These have included futile cycles in a broad sense, such as a lipolysis/esterification cycle or activation of Na^+-K^+-ATPase. These types of suggested mechanisms may be classified together as ATP dependent, since they require that ATP is formed and then used in an "unproductive" way (principally as in muscular shivering thermogenesis), leading to ADP generation and consequently to stimulation of substrate oxidation/oxygen consumption in the mitochondria (i.e., thermogenesis). However, an early observation that inhibition of ATP synthase (with oligomycin) only partly reduced norepinephrine-induced thermogenesis (629), as well as the successive realization that brown fat mitochondria generally have a remarkably low ATP synthase capacity (447) [due to a severe lack of the synthase complex (106, 356), which in its turn results from a specific lack of expression of one of the genes for subunit c, the P1 gene (14, 355)] has led to the conclusion that ATP-consuming mechanisms cannot be responsible for the thermogenic process in brown adipocytes.

The alternative formulation, that no ATP is formed and that oxidation is "uncoupled" (447, 755) in the sense that the word was originally used as describing "an oxidative process not coupled to ATP synthesis," has manifested itself in the identification of "the" uncoupling protein UCP1 (thermogenin). However, the identification of other proteins also classified, at least phylogenetically, as uncoupling proteins, such as UCP2 and UCP3 (see sect. II*B3*C), has meant that even after the identification of UCP1, the question remained as to whether UCP1 is essential for all norepinephrine-induced thermogenesis in the brown adipocytes, or whether other mechanisms could contribute. In this respect, experiments with brown adipocytes isolated from UCP1-ablated mice (Fig. 3*A*) have been conclusive; they clearly demonstrate that in the absence of UCP1, no thermogenesis can be induced in brown adipocytes by norepinephrine (491). There is thus no reason to believe that any processes other than that mediated by UCP1 are by themselves thermogenic in brown adipocytes.

1. Stimulation of lipolysis stimulates thermogenesis

It is a classical observation that the thermogenic process in brown adipocytes can be mimicked by the addition of fatty acids (630, 647) (Fig. 3*B*). That this fatty acid-induced thermogenesis is also completely UCP1 dependent (491) (Fig. 3*B*) makes it likely that, even physiologically, the activation of lipolysis is a sufficient trigger for initiation of thermogenesis in brown adipocytes. Indeed, all manipulations that induce lipolysis in brown adipocytes also induce thermogenesis, and no thermogenesis can be evoked without simultaneously evoking lipolysis.

Lipolysis, observed as glycerol or fatty acid release, is norepinephrine-induced in brown adipocytes (51, 206, 419, 568), just as is thermogenesis (208, 562, 630). Lipolysis is stimulated through β_3-receptors (25, 124) as is

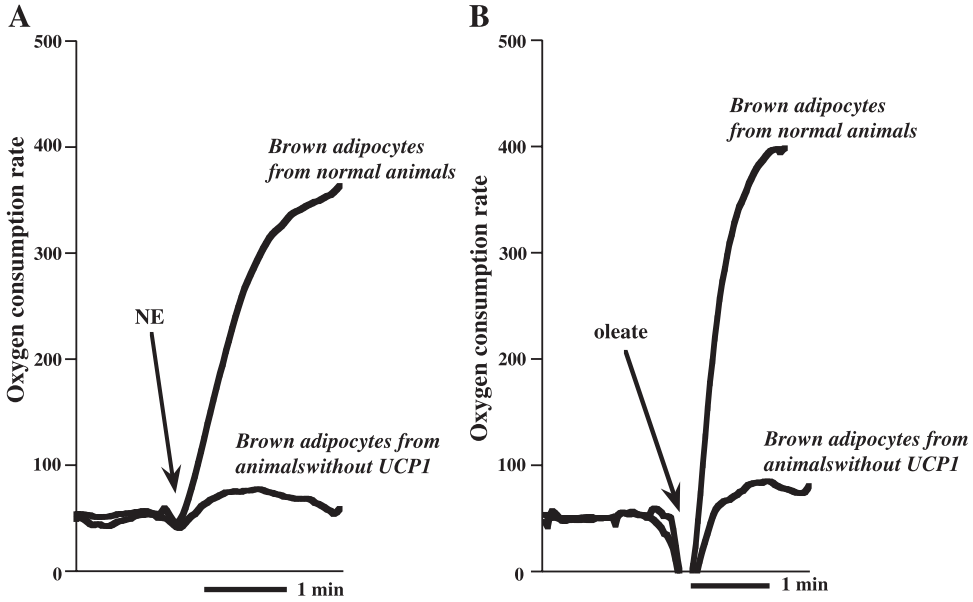

FIG. 3. The unique ability of brown adipocytes to respond to norepinephrine (*A*) or fatty acid (*B*) addition with a nearly 10-fold increase in the rate of oxygen consumption (thermogenesis) is fully dependent on the presence of UCP1. Oxygen consumption rates are in fmol $O_2 \cdot min^{-1} \cdot cell^{-1}$ (the apparent, minor responses occurring in brown adipocytes from UCP1-ablated mice are mainly addition artefacts). [Data adapted from Matthias et al. (491).]

thermogenesis (900). Both processes occur downstream of cAMP formation, as they can be induced also by the adenylyl cyclase activator forskolin (207) or by the addition of cAMP analogs (144, 648, 827).

That lipolysis is due to protein kinase A activation can presently only be deduced indirectly, because thermogenesis is inhibited by the protein kinase A inhibitor H-89 (226). The stimulation of lipolysis is composed of two processes: activation of hormone-sensitive lipase (HSL) and phosphorylation (deactivation) of perilipin (Fig. 4).

Brown adipocytes contain HSL (345), and it has normally been formulated that it is through the norepinephrine-induced phosphorylation of this enzyme that lipolysis is activated (although such phosphorylation has not been directly demonstrated in brown adipose tissue). However, the effect of adrenergic stimulation on lipolytic capacity, measured enzymatically as lipolysis of a triglyceride emulsion in vitro, is very marginal: only an ~50% increase in lipolytic activity is induced by norepinephrine (733), a far lower degree of activation than would be expected.

This low degree of activation is probably explainable as an experimental limitation in this type of experiments. Artificial triglyceride emulsions are not endowed with perilipin, the protein that normally covers the triglyceride droplets within the cell (56). Perilipin protects the triglycerides against HSL activity (485). Activated protein kinase A phosphorylates perilipin (124), the phosphorylated perilipin is dissociated from the triglyceride droplets, and the droplets now become freely exposed to attack by HSL, which is translocated upon phosphorylation to the lipid droplets, at least in white adipose tissue (135, 528) (not as yet demonstrated in brown adipose tissue). This combination of lipase activation and perilipin inactivation may explain the large increase in lipolysis observed within the cell. In accordance with this, (white) adipocytes from perilipin-deficient mice display a high basal lipolysis that cannot be further activated by adrenergic stimulation, and in perilipin-deficient animals, brown adipose tissue appears very lipid depleted. Perilipin-deficient mice also display an increased basal metabolic rate. It is possible that the constitutively increased lipolysis in their brown adipocytes is sufficient to constitutively activate thermogenesis and that it is this extra thermogenesis that explains the lean phenotype of these mice (485) (although this has not been directly demonstrated).

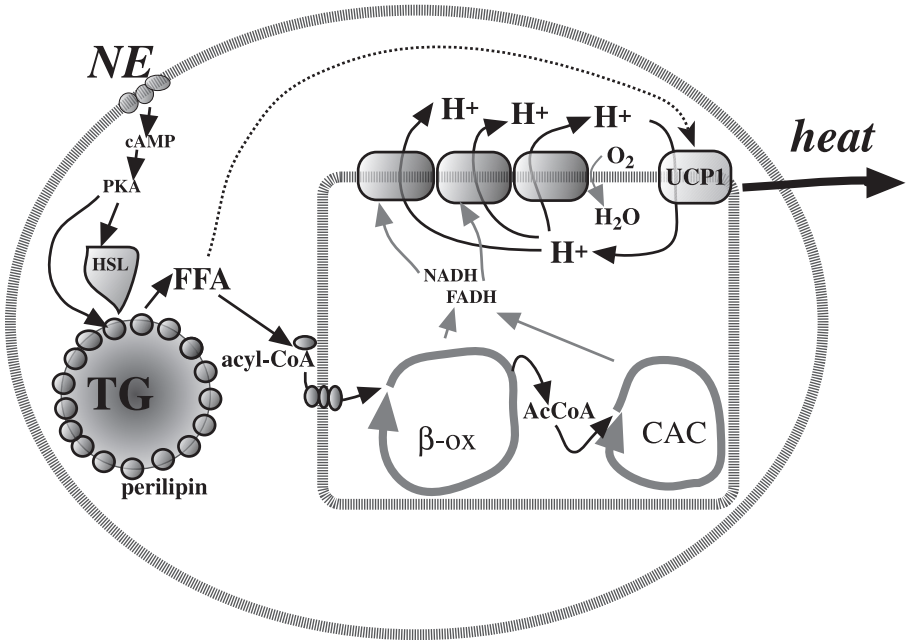

FIG. 4. Norepinephrine-induced stimulation of thermogenesis in brown adipocytes: events downstream of the protein kinase A (PKA) activation illustrated in Fig. 2. HSL, hormone-sensitive lipase; TG, triglyceride droplet; AcCoA, acetyl CoA. Free fatty acids (FFA) activated to acyl CoAs by acyl-CoA synthetase are first transferred to acyl-carnitine by the highly expressed muscle form of carnitine palmitoyltransferase I (M-CPT I), which is the CPT I form found in both brown and white adipose tissue (205) and which is very sensitive to inhibition by malonyl CoA. The acyl-carnitine probably enters the mitochondria through the carnitine transporter (not as yet explicitly described in brown adipose tissue) and is probably reconverted to acyl CoA by CPT II. The ensuing β-oxidation (β-ox) of the fatty acids (acyl CoAs) as well as the activity of the citric acid cycle (CAC) lead to the formation of the reduced electron carriers FADH and NADH, which are then oxidized by the electron transport chain (respiratory chain; here indicated by the series of gray boxes), ultimately through oxygen consumption. This results in a pumping out of protons from the mitochondria and the formation of a proton-motive force that drives the protons back into the mitochondrial matrix through the uncoupling protein UCP1. The energy stored in the proton-motive force is then released as heat.

That HSL is involved can also be seen from studies of HSL-deficient mice (608, 854). In these mice, basal lipolytic activity in brown adipose tissue is not diminished. However, catecholamine-induced lipolysis in white adipocytes is eliminated (854), implying a similar result in brown adipocytes. In these HSL-deficient animals, the white and brown adipocytes become heterogeneously more fat-filled than in wild type, further indicating that lipolysis is diminished (608, 854). The HSL-deficient mice are not more sensitive to an acute cold stress than are wild-type mice (608, 854), but this is not in itself evidence that brown adipose tissue is thermogenically active in these mice, despite the absence of HSL. (As discussed in section v*B*, acute defense against cold is mainly through shivering, and direct examination of brown adipose tissue thermogenic capacity would be required to conclude on a noninvolvement of HSL in the thermogenic response.) The most reasonable conclusion is thus still that HSL is both responsible and obligatory for norepinephrine-mediated lipolysis in brown adipocytes.

2. Fatty acids are the thermogenic substrates

Lipolysis of triglyceride droplets ultimately results in the liberation of glycerol and free fatty acids within the cell. Although some fatty acids may leave the cell (see sect. viii*C1*), most are channelled further within the cell. In the cytosol, they are probably bound to fatty acid-binding proteins. Similarly to other adipocytes, brown adipocytes possess the adipocyte form of the fatty acid binding protein, A-FABP or FABP4 (= aP2) (156). However, in contrast to white adipocytes, brown adipocytes also possess the heart form of this protein, H-FABP (156), and in contrast to what is the case for the A-FABP, gene expression of H-FABP is dramatically induced by norepinephrine. Although mRNA levels are not direct indicators of protein levels, it would seem likely that brown adipocytes possess very high levels of fatty acid binding proteins; accordingly, the level of free fatty acids in the cytosol is probably low, despite high rates of lipolysis.

Although some fatty acids may be degraded initially in peroxisomes (287, 556), most are channelled towards the mitochondria. In the mitochondrial environment, they may have several roles. They are definitely the substrate for thermogenesis, and they are most likely also involved in the regulation and/or function of UCP1.

In their fate as substrates, the fatty acids are transferred into the mitochondria via the general activation/carnitine shuttle system and are then β-oxidized in the mitochondria, with the released acetyl CoA moities being oxidized in the citric acid cycle (Fig. 4). The catabolic pathway is thus not different from that in other cells (although brown adipocytes contain very high amounts of the catabolic enzymes involved, Ref. 218). Also similarly to what happens in the mitochondria of other cell types, the passage of the released electrons through the respiratory chain results in the pumping out from the mitochondrial matrix of protons and the establishment of a mitochondrial membrane potential (Fig. 4). Thus, in the catabolic steps, the mitochondria of brown adipose tissue are as energy-conserving as any other mitochondria. The difference, i.e., the thermogenic ability, results from the existence of high amounts of UCP1 in the mitochondria of brown adipose tissue.

3. The uncoupling protein UCP1

UCP1 (earlier known simply as UCP, or as the uncoupling protein, as thermogenin, as the GDP-binding protein, or as the 32,000-Da polypeptide) is a member of the mitochondrial carrier protein family. Present knowledge on UCP1 has been extensively reviewed (237, 397, 400, 560, 565, 569, 653). We will therefore here only summarize some of the more important points.

As a member of the mitochondrial carrier protein family, UCP1 shares many points of homology with the other members of this large family, including its tripartite structure and amino acid sequences, which are both conserved between the three 100-amino acid sequences and between many members of the mitochondrial carrier family; these features will not be discussed further here (65, 237, 565) (Fig. 5). There are also sequences and residues that are of particular interest for UCP1 function. One topological area is the amino acid residues involved in the binding of purine nucleotides (GDP in Fig. 5); these residues are also found in the sister proteins UCP2 and UCP3 (see sect. ii*B4*c). Other sequences of particular interest are two sequences that are fully conserved within UCP1 from all species as yet characterized but which are not found in any other mitochondrial carrier. These sequences are in the middle of the central loop (probably facing the matrix) and the last part of the COOH terminus (facing the cytosol). The COOH-terminal sequence is also immunogenic, and selective antibodies are preferably designed to react to this sequence. The actual function of these two conserved sequences is not known presently (although there are some indications concerning the histidines in the central loop, Refs. 188, 830a), but their unique and consistent presence in UCP1 indicates that they could be of importance for the functioning of this protein.

a) how is ucp1 activated? The early observation that isolated brown fat mitochondria had high rates of respiration when examined under conditions in which "normal" mitochondria had low respiratory rates (i.e., in the presence of oxidizable substrate but the absence of ADP) indicated that an "uncoupling" mechanism existed in brown fat mitochondria. In a Mitchellian formulation, uncoupling in this sense must correspond to an increased proton permeability. The observation that GDP (or with similar, or even higher affinity, GTP, ADP, and ATP) could inhibit this high proton permeability (574) led experimen-

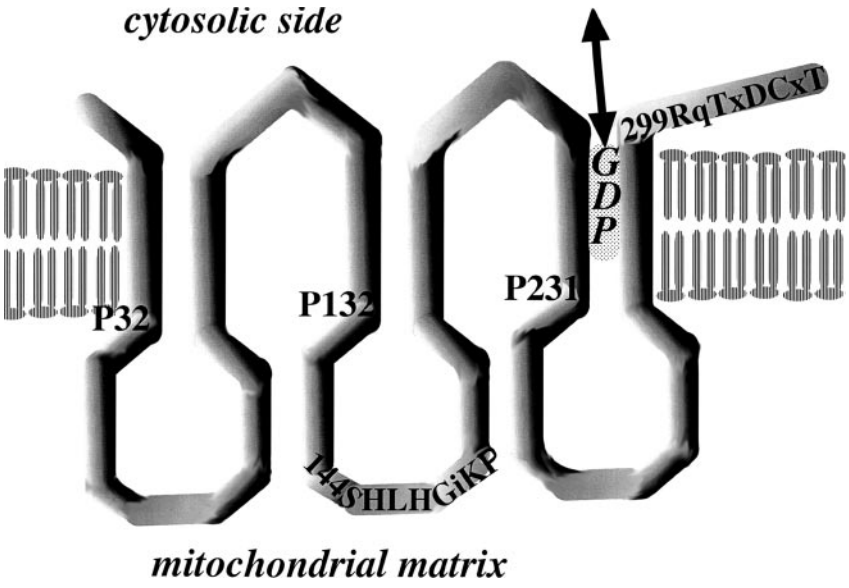

FIG. 5. The structure of UCP1. Only the characteristic proline in each part of the tripartite structure is indicated, as well as the GDP-binding area and the two sequences that are conserved in UCP1 from all examined species but are not found in any other mitochondrial carrier protein, not even the closely related UCP2 and UCP3. (Diagram simplified from Ref. 565.)

tally to the identification of UCP1 (311) and also indicated that a physiological regulatory mechanism for UCP1 action must exist. It is generally accepted that brown fat mitochondria, and thus UCP1, are exposed in the resting state to cytosolic nucleotides and therefore not active (although some authors instead claim that the cytosolic nucleotides do not have this function and that UCP1 is inactive due to the absence of a necessary cofactor: free fatty acids). However, despite nearly four decades of experimentation on brown adipose tissue mitochondria and more than two decades of examination of the responsible protein UCP1, a full and generally accepted understanding of the control of proton (or proton equivalent) transport and the mechanism for this transport (which in some formulations may be said to be the same thing) has not been reached.

The tenet that fatty acids, either as such or as derivatives, are involved in the physiological activation of UCP1 and/or the transport mechanism, is generally accepted. The question is still what exactly the fatty acids do; there are presently at least three formulations: that they act as allosteric regulators, as cofactors, or as proton shuttles (Fig. 6).

In the allosteric interaction model (Fig. 6*A*), the fatty acids interact with a site on UCP1 leading to its activation. In bioenergetic terms, the activation may be formulated

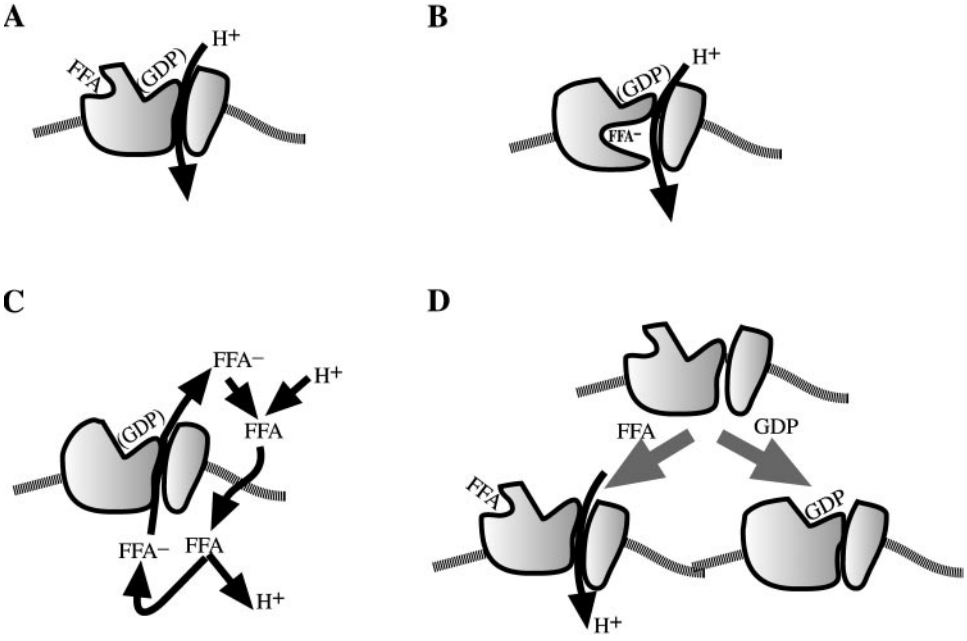

FIG. 6. Hypotheses for the fatty acid-induced activation of UCP1. The nucleotide-binding site is indicated with (GDP), implying that only as long as the site is unoccupied does proton transport take place.

as "lowering the threshold for a Zener diode" (653, 655), but what this means in molecular terms has not been clarified. The presumed fatty acid binding site has not been identified. The allosteric interaction model would become even simpler (competitive, orthosteric) if the free fatty acids could compete away the inhibitory purine nucleotides presumably bound to UCP1 in the resting state and in this way activate UCP1. There are, however, no indications that free fatty acids can do this. The fatty acid derivative acyl CoA ("activated" fatty acids) has the ability to compete with bound purine nucleotides and has been suggested as a competitive activator of UCP1 (105, 768, 769), but convincing evidence for a physiological relevance for such an effect is lacking.

In the cofactor theory (Fig. 6*B*), the fatty acids become localized to binding sites within the proton-conducting "channel" of UCP1, and their acid moieties then function as "stepping stones" for protons as they pass through the membrane (868); again, no interaction with the purine nucleotide-binding inhibitory site is formulated. The intrachannel fatty acid binding sites required for this model have not been identified.

In the shuttling theory (Fig. 6*C*), an observation by Skulachev (17) that other mitochondrial carriers (notably the ATP/ADP carrier), in the presence of free fatty acids, could function as uncoupling proteins has been extended by Garlid and Jezek (237), as being the mechanism also for UCP1 function. In this formulation, it is not protons that are transported by UCP1 over the mitochondrial membrane; rather, protons (re)enter the mitochondria in the form of the undissociated fatty acid, and the fatty acid, in its anionic form, (re)exits the mitochondria carried by UCP1. There is ample evidence that this process can occur in an experimental system (237, 373). The theory has been questioned (268), and objections can be raised of a more theoretical nature. The process as such is not specific for UCP1; rather, a series of mitochondrial carriers (not only the ATP/ADP carrier), physiologically expected to perform other tasks, can be convinced to function as "uncoupling proteins" under similar experimental conditions. If this is the case, it may be asked what the evolutionary advantage is of UCP1. Correspondingly, the conserved unique amino acid sequences (Fig. 5) imply acquisition of specific properties. Also, the role of the inhibitory GDP-binding site on UCP1 is presently unsolved in this model. Considering the high levels of fatty acid binding proteins in the cytosol of brown adipocytes, it may also be questioned as to whether fatty acids can reach sufficiently high free levels necessary for this process. Thus none of the three models presently proposed has been unequivocally demonstrated to fully explain the regulation and protonophoric properties of UCP1.

It was anticipated that examination of brown adipose tissue mitochondria from UCP1-ablated mice would solve some of these issues. Initial studies unexpectedly indicated that the difference in fatty acid sensitivity between brown fat mitochondria with or without UCP1 was minor (341, 490, 566). However, with more optimal substrate (i.e., pyruvate or fatty acids in the oxidizable form of palmitoyl carnitine) and by expressing the effect as a function of the *free* fatty acid level (rather than the added), quite marked effects of the presence of UCP1 are noticable, with UCP1-containing mitochondria being more than 10-fold more fatty acid sensitive than mitochondria without UCP1 (721a). Remarkably, this UCP1-dependent thermogenesis induced by free fatty acids in brown fat mitochondria is competitive with GDP. Because no direct competition between fatty acids and the GDP-binding site exists, the competition must be functional. A formulation by Rial and Nicholls (654) suggests that UCP1 is transformed by free fatty acids and by GDP into two different states (Fig. 6*D*), and this could result in the apparent competition observed.

B) UCP1 IN TISSUES OTHER THAN BROWN ADIPOSE TISSUE. It has long been the general notion that UCP1 expression is a unique feature of brown adipose tissue, indeed to the degree that "brown adipose tissue" may be defined as an (adipose) tissue that has the ability to express UCP1; the word *ability* indicates that actual expression is not necessarily seen, e.g., in brown preadipocytes and even in nonstimulated otherwise differentiated brown adipocytes. There have, however, been occasional reports that UCP1 is found in non-brown adipose tissues.

Concerning reports that UCP1 is expressed in "white" adipose tissue depots (140, 238, 288, 411, 593, 822, 882), this seems to be mainly a question of definition. We prefer to formulate it that any adipocyte that has the ability to express UCP1 is a brown adipocyte, and the occurrence of UCP1 in white adipose tissue therefore does not constitute any change in paradigm, but merely indicates that brown adipocytes can occur sporadically in predominantly white depots.

The situation is more complex in nonadipose tissues. Early reports that UCP1 mRNA was observable in liver of newborn and cold-exposed rats (741) resulted from unspecificity of the cDNA clone used (666). Two reports indicating that chronic stimulation of animals with β_3-agonists leads to UCP1 expression in skeletal muscle (541, 884) were published about the time when it was becoming clear that proteins closely related to UCP1 (i.e., UCP2 and UCP3, see below) were expressed in skeletal muscle; the observations have not as yet been confirmed under experimental circumstances ensuring that cross-reactivity of these UCP1-like proteins could not be the cause of the positive reactions seen. Indeed, chronic treatment of muscle cells (the cell line L6) with β_3-agonists leads to increased expression of UCP2 (542). Thus it seems presently unlikely that UCP1 can be expressed in skeletal muscle following physiological or pharmacological stimulation.

Evidence, so far unconfirmed, has also been presented that UCP1 is expressed in a few, specific cells within the longitudinal muscle layer of (all) peristaltic organs in the body (573); this thus includes the entire gastrointestinal tract, urethra, as well as gonadal tissues (epididymis and vas deferens in males and uterus in females). The evidence has been criticized (692a) and the reported total expression level in these tissues would in any case be very low at the protein level: in isolated mitochondria, only 1/1,000 of the level in brown-fat mitochondria and thus probably even a further factor of 10 lower on an organ basis, due to the low density of mitochondria compared with brown adipose tissue. It is therefore unlikely that this possible extra-adiposal UCP1 expression has any measureable significance for thermogenesis on a whole body basis. Indeed, classical observations demonstrate that animals that have been functionally eviscerated (i.e., the blood flow to all peritoneal organs cut off) respond to cold exposure or norepinephrine injection with a thermogenic response quantitatively identical to that of intact animals (170, 171). This demonstrates that even if UCP1 is present in peristaltic organs it is not of thermogenic significance. In the following we therefore make the assumption that UCP1-dependent thermogenesis of systemic significance entirely emanates from UCP1 in brown adipose tissue.

Whether this possible extra-adipose expression of UCP1 has any functional significance is not known. Adrenergically induced relaxation of precontracted intestinal strips is somewhat impaired in UCP1-ablated mice (722), but whether this is a secondary effect to the absence of UCP1 in brown adipose tissue or a demonstration of a genuine intestinal UCP1 effect is not settled as yet. However, interpretation of results on the expression of transgenes under the control of the UCP1 promoter (such as diphtheria toxin, or Cre for the Cre/LOX systems) may be affected by an expression of UCP1 in cells outside brown adipose tissue, which, in the diphtheria toxin case, would lead to the death of such cells.

C) UCP1 VERSUS OTHER "UNCOUPLING PROTEINS": ONLY UCP1 MEDIATES THERMOGENESIS. Originally from analysis of cDNA libraries of expressed sequence tags (EST clones), it has become evident that UCP1 is not alone (659): a true phylogenetic family consisting of three closely related proteins UCP1, UCP2, and UCP3 exists. In a similarity analysis of all known mitochondrial carriers, the next closest group is the so-called plant uncoupling proteins (sometimes called PUMP, StUCP, and two ArUCPs). Even further from the core UCP family, and not more closely related to UCP1 than to, e.g., the oxoglutarate carrier, other so-called UCP-like genes have been identified, named UCP4 and UCP5 (also known as BMCP-1) (659). However, there is really no phylogenetic reason to consider UCP4 and UCP5 as members of the UCP family (65). Thus, for the discussion of the function of UCP-like proteins in animals, only UCP2 and UCP3 are of interest.

An extensive discussion has been ongoing concerning the function of these proteins. In anticipation of later analysis (see sect. v*B*) and based primarily on results with the UCP1-ablated mouse, it may be stated here that the standpoint in this review is that only UCP1 can mediate classical thermogenesis (i.e., heat production for the purpose of heat production) (see sect. v*B*) and that this process is therefore fully localized to brown adipose tissue. The mRNA levels of UCP2 and UCP3 are also rather high in brown adipose tissue (66, 67, 200, 263, 425, 490, 604), although the proteins may not be well-expressed (616). As cold acclimation-recruited norepinephrine-induced nonshivering thermogenesis is competent in both UCP2- and UCP3-ablated mice (27, 267, 844), these proteins are apparently not essential for the thermogenic process or for brown adipose tissue recruitment. Because no detailed studies of brown adipose tissue or brown fat mitochondria have been reported in UCP2- or UCP3-ablated mice, more subtle effects of the absence of these proteins on brown adipose tissue function may have gone unnoticed.

To which degree UCP2 and UCP3 are true uncoupling proteins when entopically and endogenously expressed is still unknown, as discussed in detail elsewhere (561). They are certainly all uncoupling when ectopically or entopically overexpressed, but so are certain other mitochondrial carriers, such as the oxoglutarate carrier (369) and adenine nucleotide translocator, and these effects have been analyzed as being due to disruptive membrane effects of the overexpressed proteins (770). UCP2 and UCP3 are apparently present at very low protein levels, even in tissues in which they are highly expressed at the mRNA level (616), and even if they, per se, would be equally as uncoupling (proton conducting) as UCP1 itself, their low concentration apparently prohibits experimental observation of thermogenesis as such (151). The long evolutionary history of these proteins, involving at least most chordate animals (including mammals, birds, and fishes) plus plants, indicates that they have important metabolic roles that still remain to be clarified, but these roles can clearly not be directly related to thermoregulatory heat production. Correlations (direct or inverse) between the levels of UCP2, UCP3, etc., and obese or diabetic states can very well exist, even though the physiological function of the proteins proves not to be uncoupling. There is no reason to consider UCP2 or UCP3 as being directly important for the thermogenic function of brown adipose tissue, and they clearly cannot substitute for UCP1 in any thermogenesis of brown adipose tissue origin.

C. The α_2-Adrenergic Pathway Inhibits Thermogenesis

Brown adipocytes (with the possible exception of cells from hamsters, Ref. 501) also possess α_2-adrenocep-

tors that are stimulated by norepinephrine in parallel with its stimulation of the β-receptors (Fig. 2). α_2-Adrenoceptors are coupled to G_i proteins, probably mainly of the $G_i\alpha 2$ subtype, although all three G_i subtypes are present (72). G_i activation leads to an inhibition of the β-adrenoceptor-stimulated activity of adenylyl cyclase. The parallel stimulatory and inhibitory effects of norepinephrine on adenylyl cyclase activity are evident from studies involving addition of α_2-antagonists to norepinephrine-stimulated cells (which leads to a rise in cAMP levels) or from the observation that cAMP levels induced by pure β-agonists are higher than those induced by norepinephrine (76). The physiological significance of the presence of two counteracting systems (β and α_2) presently escapes comprehension, in brown adipose tissue as well as in other systems, although it may be formulated that this balance between the stimulatory β-receptors and the inhibitory α_2-receptors allows the brown adipocyte to modulate its response to the external stimulation by norepinephrine. How and when the cell decides to use this modulatory power has not been formulated.

There is presently no indication that the α_2-pathway governs any other function in brown adipocytes than adenylyl cyclase inhibition, although other G_i-linked processes have been observed in other tissues.

D. The α_1-Adrenergic Pathway and the Cell Membrane Events

Norepinephrine also activates α_1-adrenoceptors on brown adipocytes (Fig. 7). These are primarily of the

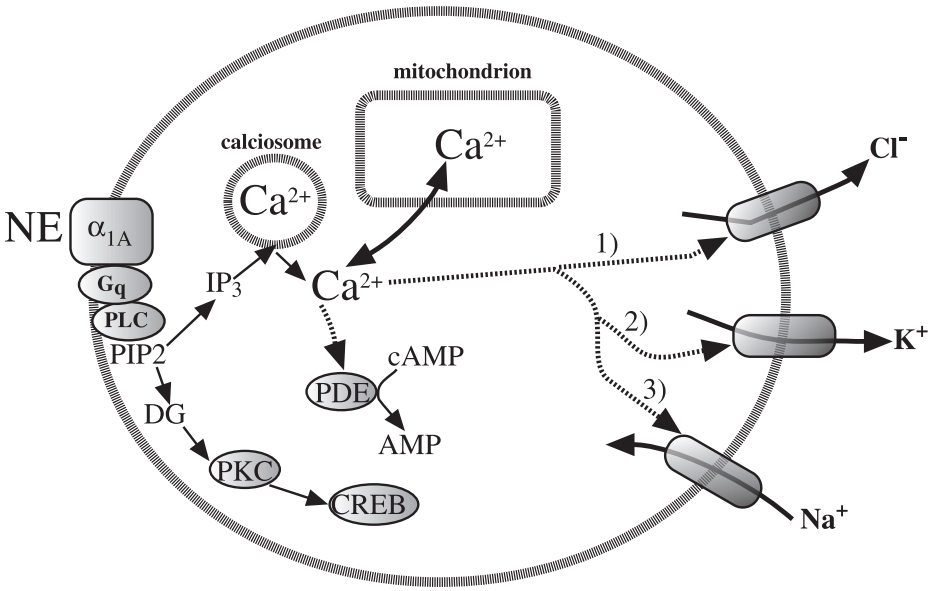

FIG. 7. The α_1-adrenergic pathways and ionic membrane events induced. For discussion of the signal transduction pathway described, see section II*D* in the text. PLC, phospholipase C; DG, diglyceride; PKC, protein kinase C; PDE, cAMP phosphodiesterase. In the figure, the α_1-adrenoceptor-induced plasma membrane electrical events are also summarized. Brown adipocytes possess a negative membrane potential with a resting value about −60 mV (if the buffer contains bicarbonate, otherwise it is only about −25 mV, for unknown reasons). Adrenergic stimulation leads to a series of electrical events in the cell membrane, resulting in depolarization (249, 354, 867). Because no voltage-sensitive Na^+ or Ca^{2+} channels are found in the brown adipocytes (brown adipose tissue is not an "excitable" tissue), the significance of these events is enigmatic. The plasma membrane events are α_1-adrenergically induced, via the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Three successive events occur, affecting sequentially Cl^-, K^+, and Na^+ permeability. *1*) α_1-Stimulation leads first to an activation of a Cl^- efflux/current (160, 609), which leads to a depolarization lasting for ~30 s. Cl^- channels have been identified (694), but it has not been clarified whether it is these Cl^- channels that are involved in the α_1-induced Cl^- efflux. *2*) Second, K^+ channel activity, K^+ currents, and K^+ efflux are observed, probably resulting both from directly Ca^{2+}-activated (apamin-sensitive) K^+ channels (546a, 610) and from depolarization-activated K^+ channels (464), opened due to the initial depolarization. These electrical events are associated with measurable fluxes of both Cl^- (160) and K^+ (546a) out from the cell. Such fluxes are only possible if both cations (K^+) and anions (Cl^-) efflux simultaneously, which should have effects on cellular volume; these fluxes may thus be related to alterations in mitochondrial volume occurring during the transition from the resting to the thermogenic state (174). *3*) The third event is an α_1-induced increase in Na^+ permeability, leading to a small sustained depolarization. This Na^+ permeability results from activation of nonselective cation channels (406) which, under physiological conditions, will be observed as depolarizing Na^+ channels. There are presently no indications as to the physiological significance of these plasma membrane events. Inhibition of Ca^{2+}-induced K^+ fluxes by apamin has only marginal effects on thermogenesis; similarly, acute inhibition of the Na^+-K^+-ATPase leads only to a very marginal decrease in norepinephrine-induced thermogenesis (546a). Earlier indications that the presence of Na^+ in the extracellular medium was necessary for full thermogenesis (553) have been found to be due to inhibitory effects of the ion used to replace Na^+ ($choline^+$); replacement of Na^+ by other large cations ($NMDA^+$) does not result in inhibition of norepinephrine-induced thermogenesis (144). Thus the direct thermogenic significance of the plasma membrane ionic events appears minor.

α_{1A}-subtype (148, 277, 395); the expression level of these receptors is remarkably high in brown adipose tissue, probably the highest in any mammalian tissue.

Although not directly demonstrated in brown adipose tissue, α_1-adrenoceptors probably activate G_q proteins (present in the tissue, Ref. 72) and phospholipase C, leading to phosphatidylinositol 4,5-bisphosphate (PIP_2) breakdown (followed by PIP_2 resynthesis) (518, 713) leading to formation of inositol 1,4,5-trisphosphate (IP_3) (546b) and diglycerides, each with further effects in the cell.

Because IP_3 is formed in brown adipocytes, it is presumably also the agent that releases Ca^{2+} from intracellular stores. It is, however, possible that in brown adipocytes, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is regulated not only positively via the α_1-receptor but that the β-adrenergic pathway also can influence cytosolic Ca^{2+} levels positively (432, 865). This can occur indirectly through β-induced mitochondrial depolarization, which would diminish mitochondrial uptake of cytosolic Ca^{2+}. The combined outcome is still that during norepinephrine stimulation of the cells, mitochondrial Ca^{2+} levels increase (898). In other tissues, intramitochondrial substrate dehydrogenases require elevations of Ca^{2+} levels for full activity (499), and such a function may also be ascribed to Ca^{2+} increases in brown fat mitochondria, explaining a thermogenesis-promoting effect of the α_1-adrenergic pathway (i.e., α_1-stimulation, via $[Ca^{2+}]_i$ increases, augments the degree of thermogenesis induced by a given cAMP level, Ref. 899).

The α_1-induced increase in $[Ca^{2+}]_i$ also activates a phosphodiesterase activity which in turn decreases cAMP levels (78). If the affinities for norepinephrine on β_3-receptors and on α_1-receptors are significantly different, this will lead to semi-bell-shaped curves for cAMP accumulation in the cells as a function of norepinephrine concentration (78), as well as to similar unusual dose-response curves for norepinephrine effects on all events downstream of cAMP.

Calmodulin (CaM) is present in brown adipose tissue, and CaM kinases may be activated, but no downstream effects of this have been reported. Iodothyronine-5′-deiodinase activation (637) and UCP1 gene expression (unpublished observations) show synergistic interaction between β- and α_1-stimulation.

α_1-Adrenergic stimulation of brown adipocytes also leads to a series of cell membrane events of ionic character, summarized in Figure 7. The significance of these ionic events (if any) for acute thermogenesis or for the recruitment process has not been clarified as yet.

Diglycerides released from PIP_2 breakdown lead to activation of one or more isoforms of protein kinase C (39), and this kinase also, directly or indirectly, can lead to CREB phosphorylation (802).

While the pathways activated downstream of α_1-adrenoceptor stimulation in brown adipose tissue are conventional, the significance of these pathways for brown adipocyte function is difficult to evaluate. Direct effects of α_1-stimulation on thermogenesis are small, with reports ranging from not detectable to ~10% of total (87, 517, 714), perhaps being species dependent. However, α_1-stimulation seems equipotent with β-stimulation in leading to CREB phosphorylation (802) and MAP kinase (Erk1/2) activation (451, 739) and should, therefore, be important in control of gene expression and ensuing effects on brown adipocyte differentiation, etc. More functional evidence for this is, however, lacking.

III. THE LIFE OF THE BROWN ADIPOCYTE IS UNDER ADRENERGIC CONTROL

The functional activity of brown adipose tissue in any given physiological condition is determined by two factors: the acute effects of norepinephrine, resulting in stimulation of thermogenesis, through utilization of different degrees of the maximal capacity ("activity state"), and the total thermogenic capacity found at that particular time in the tissue ("recruitment state") (Fig. 8). To understand the physiological significance of brown adipose tissue under any given physiological condition, it is therefore necessary to also understand the events that determine the total thermogenic capacity. The capacity is determined in its turn by the total number of brown adipocytes in the tissue (which is determined by the rates of proliferation and apoptosis) plus the degree of differentiation of the tissue, including the mitochondrial density and amount of UCP1. Parallelly, alterations in vascular supply must take place. All these parameters are responsive to the prevailing physiological conditions.

Although it is possible to observe the recruitment events by studying the tissue in situ during recruitment or

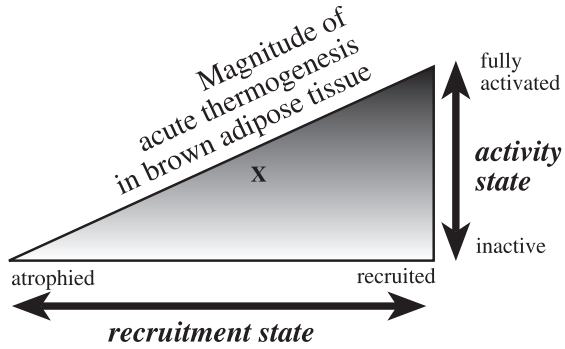

FIG. 8. Activity state and recruitment state. Thermogenesis (here illustrated as gray scale intensity) in brown adipose tissue at a given moment (here illustrated by X) is determined by the degree of activation at that moment (*y*-axis) and can alter within seconds, but the capacity for thermogenesis is determined by the degree of recruitment of the tissue (*x*-axis) and needs days or weeks to be significantly altered. The degree of activity is determined by the acute rate of sympathetic stimulation (norepinephrine release) in the tissue, and the recruitment state is mainly determined by the chronic level of sympathetic stimulation.

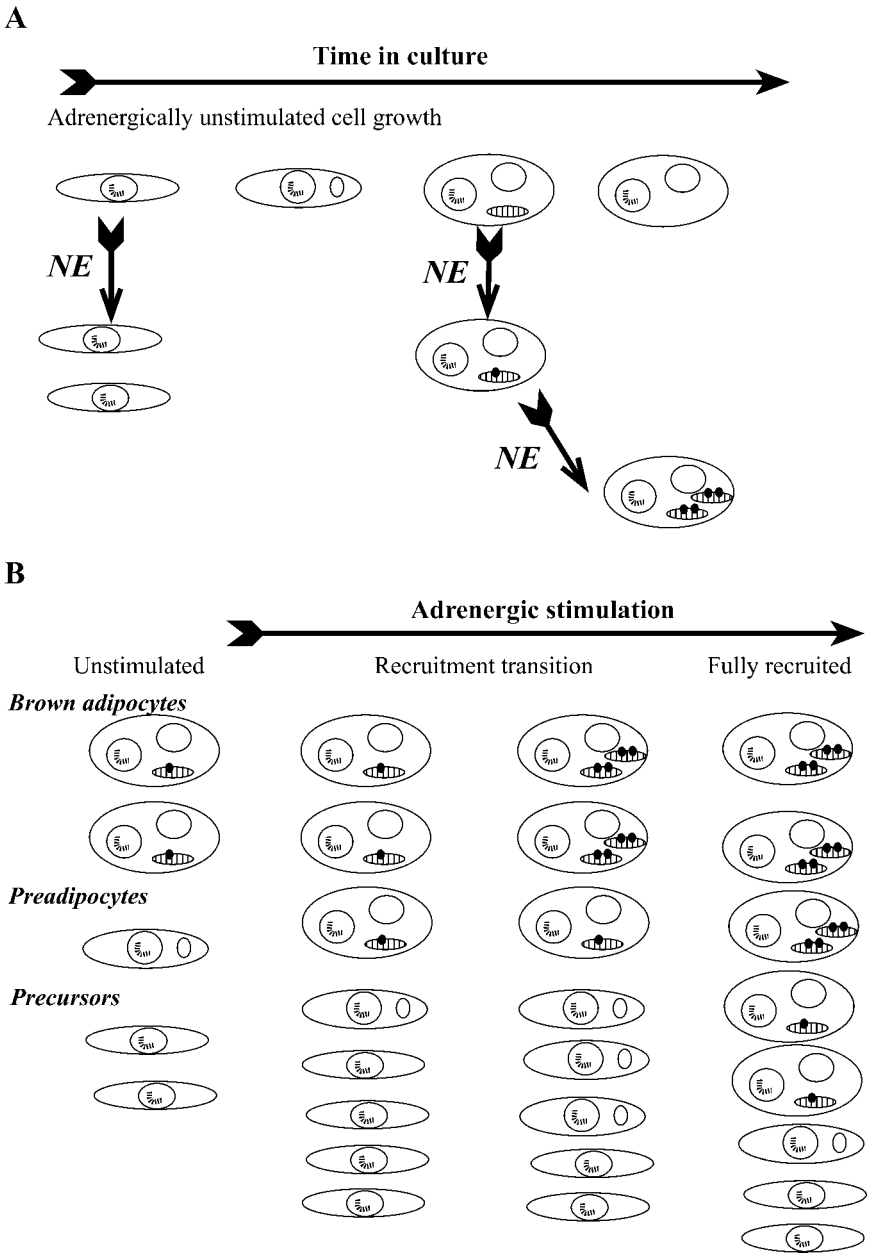

FIG. 9. The composite effects of chronic adrenergic stimulation in brown adipose tissue. In a cell culture system (*A*), the effects of a recruitment modifier (here norepinephrine) can be dissected into effects on, e.g., cell proliferation (*left*) and differentiation (*right*) and distinguished from "spontaneous" cell differentiation. However, in the tissue (*B*), the starting material is a composite of cells in different degrees of development, and it also includes different cell types, e.g., endothelial cells, in addition to the brown precursors, preadipocytes, and adipocytes. The small black dots in the hatched mitochondrial structures symbolize UCP1.

atrophy, the interpretation of such experiments is difficult. This is because in the tissue, at any given time there will be cells present in all states of differentiation. As these may differ in their responses, a very complex picture occurs (Fig. 9). Therefore, these events are more easily studied in cell culture, in which cell life, apparently fairly similar to that in situ, can be followed, but in which all the cells in the culture are at approximately the same state of differentiaton (Fig. 9). We concentrate our discussion here on such studies. We believe that analysis of proliferation and differentiation processes are probably best performed in primary cultures. Systems consisting of surviving differentiated cells are difficult to evaluate, and brown adipocyte-like cell lines tend not to differentiate to the same extent as primary developing cultures and often have alterations in their control of cell proliferation, which make the results difficult to interpret in a physiological context (102).

Brown adipose tissue growth in the developing fetus is probably regulated differently from that in animals after birth. This relates not only to expression of UCP1 but also to agents governing cell growth (e.g., Refs. 798, 800). We will not discuss here the control of ontogenic growth of brown adipose tissue.

From studies performed in primary cultures of isolated brown adipocyte precursor cells from nonfetal animals, a basically rather simple picture can be made, as described below. As seen in this picture, norepinephrine

has a prime role as the agent determining not only the acute rate of thermogenesis but also the degree of recruitment. This does not mean that norepinephrine is the single exogenous component that influences the processes of proliferation and differentiation in brown adipocytes. Classical growth factors [e.g., fibroblast growth factor (FGF; Refs. 407, 877), insulin-like growth factor (IGF; Ref. 622), PDGF (236), EGF (320), etc.] can also influence brown (pre)adipocytes, but the significance of these factors for the physiologically governed recruitment processes is unclear, and conditions under which their concentrations would change have not been described. Studies with these agents have often been performed with fetal brown adipocytes and may reflect control of ontogenic development and basic tissue organization rather than adjustment to different physiological demands on the tissue.

A. In Brown Preadipocytes, Norepinephrine Promotes Proliferation

Cells isolated from the stromal-vascular fraction of brown adipose tissue are fully undifferentiated, as judged by morphological characteristics, but when analyzed for transcription factors characteristic of adipose cells, it is clear that they are already differentiated (see Fig. 10), i.e., they are already committed to become brown adipocytes (62, 549, 550). The molecular basis for this commitment is unknown, but factors present in the brown preadipocyte phase must be part of the determinative process (537). Concerning most of the transcription factors observed, direct functional studies have not been performed in brown adipocytes. It is tempting to extrapolate functional roles from studies in white adipocyte-like cell line studies, where C/EBPδ, C/EBPα, C/EBPβ, and PPARγ2/RXR have been described to interplay in a sequential and self-augmenting process (762), but we do not consider this appropriate: significant differences may be expected, exactly because the two cell types differentiate differently.

Norepinephrine stimulation of brown preadipocytes leads to an increase in cell proliferation, through a β_1-/cAMP-mediated process (77). Norepinephrine stimulation also leads to a decrease in the expression of C/EBPα (649) and PPARγ2 (448) (similar to that observed in situ, Ref. 287), which has been interpreted to be necessary for proliferation to proceed. The further mediation of the norepinephrine effect is unknown, but norepinephrine, via cAMP/protein kinase A, increases the expression of ribonucleotide reductase (227), an enzyme which is often considered the limiting step for DNA synthesis and thus for cell proliferation.

In the brown preadipocytes, UCP1 gene expression is silenced, i.e., norepinephrine cannot induce UCP1 gene expression. The mechanism behind this silencing is not clear, but c-Jun seems to be involved (893). There is also evidence for the participation of other factors such as retinoblastoma protein pRb (632) and p53 (367) in this process.

B. In Mature Brown Adipocytes, Norepinephrine Promotes Differentiation

With time in culture, brown preadipocytes "spontaneously" differentiate morphologically into brown adipocytes, corresponding to "adipose conversion" in white adipocytes. The alteration in morphology is parallelled by and caused by increased expression of genes related to lipid metabolism and, noteworthy for brown adipocytes, of genes related to mitochondrial function. It is also parallelled by an altered expression of a series of transcription factors, as detailed in Figure 10. A "master controller" for this conversion could be perceived but has not been identified.

Although differentiation is a successive process, an understanding is facilitated if the process is seen as a rather abrupt switch from the cells being brown preadipocytes to being mature brown adipocytes. This switch is parallelled by a switch in the type of response of the cells to norepinephrine: from stimulation of proliferation to stimulation of differentiation (and inhibition of apoptosis). All these adrenergic effects make physiological sense because they mean that recruitment is promoted under conditions of a constant demand for thermogenesis, but it is unknown how the switch in norepinephrine effect is achieved at the nuclear level.

Norepinephrine thus promotes the differentiation process in general in brown adipose tissue, through cAMP-dependent processes. The pathways have not been well characterized. In some cases, a classical cAMP/protein kinase A/CREB phosphorylation pathway is probably utilized (see sect. II*A*), leading directly to increased expression of certain enzymes, etc. In other cases, the effect may be indirect, with norepinephrine, perhaps also via CREB phosphorylation, enhancing the expression of transcription factors that in their turn promote differentiation. Thus norepinephrine increases C/EBPβ levels (649). From in vivo experiments, it is also likely that adrenergic stimulation increases PGC-1 gene expression (265, 633), which may largely be causative of the norepinephrine-induced increase in the degree of differentiation. Constant norepinephrine stimulation is thus the mechanism for generating the cellular components of the thermogenic state, including an increased amount of mitochondria (323, 551) well endowed with oxidative capacity for catabolism of fatty acids.

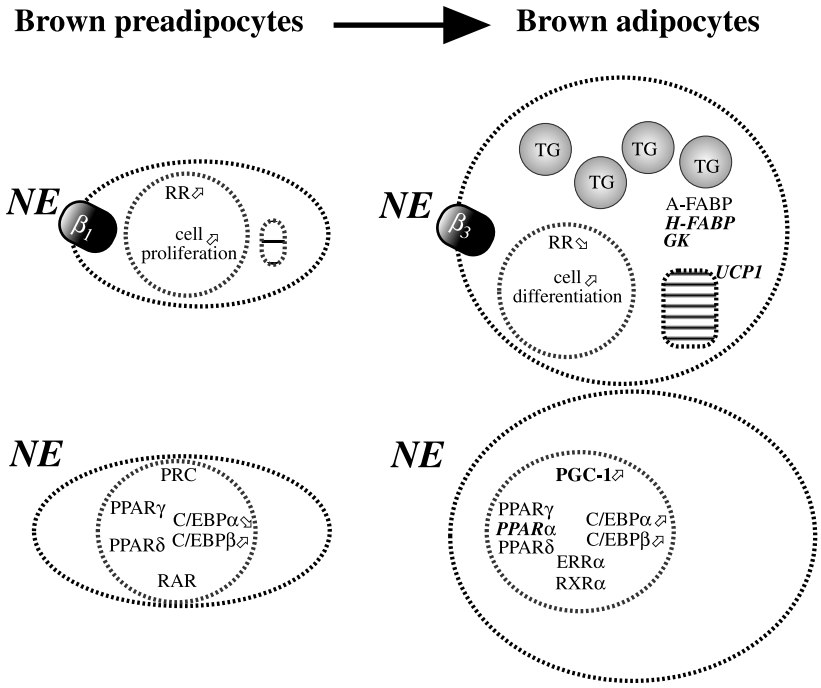

FIG. 10. Differentiation of brown adipocytes: transcription factors. *Top cells*: some characteristic morphological and enzymatic features [RR, ribonucleotide reductase; TG, triglyceride; A-FABP, adipocyte fatty acid binding protein ("aP2"); H-FABP, hepatic fatty acid binding protein]; GK, glycerol kinase. The arrows indicate the effect of norepinephrine on the gene expression level. Bold italics indicate a high brown adipocyte presence combined with an absence in white adipocytes. *Bottom cells*: transcription factors probably involved. Several adipose-related transcription factors are already present in brown preadipocytes (*bottom left*): PPARδ (834) [highly expressed in brown compared with white adipose tissue (204, 273)]; PPARγ (448, 834) (also highly expressed in brown adipose tissue, Ref. 204, 273); the PGC-1 related coactivator PRC (which may be involved in the replication of mitochondria during cell division, Ref. 15); all three RARs (their expression decreases upon differentiation) (8); C/EBPα and C/EBPβ (477, 649, 895) [C/EBPα-ablated mice fail to accumulate lipid in brown adipose tissue and to express UCP1 (852); transgenic mice expressing a dominant-negative A/ZIP/F protein, which prevents C/EBP from binding to DNA, fail to develop brown adipose tissue (519)]. In mature brown adipocytes (*bottom right*), a series of alterations in transcription factor expression has occurred. The expression of estrogen-related receptor α (ERRα, that does not bind estrogens) is increased (842); it binds to an NRRE-1 (nuclear receptor response element) in the promoter of medium-chain acyl-CoA dehydrogenase; this binding is necessary for induction of this dehydrogenase during brown adipocyte differentiation (842). All three retinoic X receptor (RXR) isoforms are found in brown adipose tissue, with the α-form being the major subtype (8) which (together with RXRγ) is induced during cell differentiation (8); other transcription factors (e.g., the PPARs) often bind as heterodimers with RXRs. PPARα is expressed first after differentiation (834); this is particularly noteworthy, as it is expressed at high levels only in brown but not in white adipose tissue (204, 273); PPARα may coordinate expression of fatty acid oxidation enzymes with expression of UCP1 (38). Both PPARγ1 and PPARγ2 are present (834); chronic treatment of animals with thiazolidinediones (glitazones, etc.) that are ligands of PPARγ leads to stimulation of expression of a large number of genes in brown adipose tissue involved in lipogenesis and fatty acid transport and oxidation (791, 858). PPAR-binding protein (PBP) is a coactivator (377), although no functional study has been reported of its action in this tissue. A functionally active coactivator, PGC-1, is found at high levels in brown adipose tissue; the gene was initially reported not to be expressed in white fat (633) but has now been detected there (822); PGC-1 increases the transcriptional activity of a series of nuclear ligand-binding receptors, including PPARα (38), PPARγ (633), thyroid hormone receptor (633), and nuclear respiratory factor-1 (NRF-1) (872). In general, PGC-1 would seem to provide cells with an increased endowment of mitochondrial catabolic capacity; this occurs in brown adipose tissue as well as in muscle (872) and liver (879). The function of PGC-1 in brown adipocytes has not been clarified; in model systems, PGC-1 docks with PPARγ and, through this, recruits both the CREB binding protein (CBP) and the steroid coactivator-1 (SRC-1), both histone acetyltransferases (HAT), to increase transcriptional activity (631) but SRC-1 is not, however, present in brown adipose tissue (377).

C. Norepinephrine Directly Regulates the Expression of the UCP1 Gene

A dramatically increased expression of the UCP1 gene in brown adipose tissue is observed when the recruitment process is initiated (376, 660), and this involves a true increase in the rate of transcription, as evidenced by run-on data (49, 760). As the level of UCP1 protein in brown adipose tissue is mainly controlled pretranslationally (375), i.e., through increased gene transcription (and perhaps through alterations in UCP1 mRNA turnover, Refs. 374, 613, 650) rather than through regulation of protein synthesis as such, much attention has focused on the control of UCP1 gene expression. Studies have been performed with UCP1 promoters in artificial systems (where the experimental background is consequently dif-

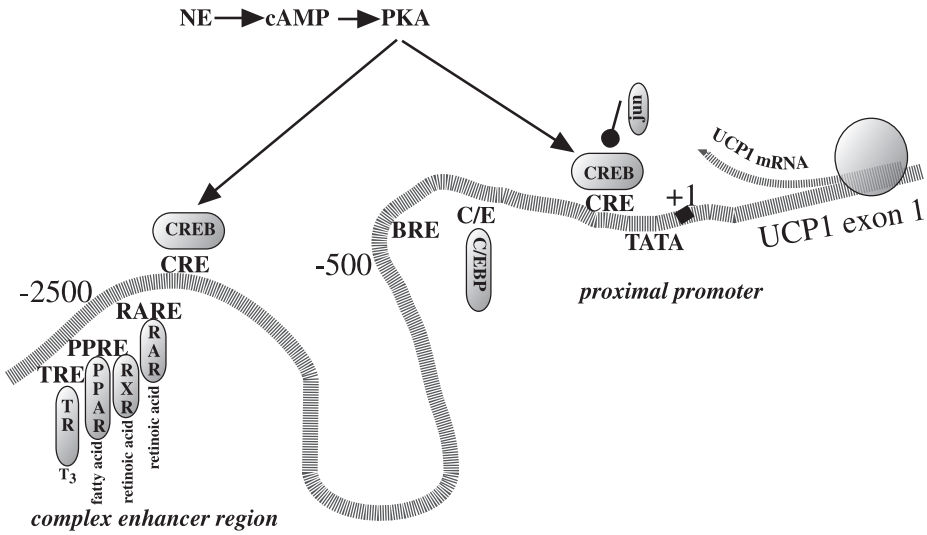

FIG. 11. Factors controlling UCP1 gene expression. The figure summarizes basic principles; more detailed reviews may be found elsewhere (401, 746, 846). The top part indicates the direct pathway for norepinephrine-induced UCP1 gene expression; the bottom part summarizes the presence of other transcription factors and response elements than those involved in the direct norepinephrine effect. Two regulatory regions have been characterized: a proximal promoter and a distal, complex enhancer region. The occurrence of several response element sites for the same factor makes correct alignment of involved response elements (RE) within these regulatory regions unfeasible at this resolution; the figure is thus much simplified. The proximal promoter region contains in addition to TATA and CCAAT elements of the basal promoter a cAMP response element (CRE), two C/EBP sites (C/E) (894), and other sites, one appears similar to that for ETS transcription factors, one may be a brown fat-specific region (BRE) (895). A complex enhancer region exists around −2,500 in rodents (at −3,500 in humans, Ref. 165). This region is a multipartite response element (166) with many response elements within a short sequence, some of which are even overlapping. cAMP response elements (CRE) are found and functional (413, 667). Retinoic acid response elements (RARE) are found, containing three pairs of half-sites for RXR and RAR (8, 9), suggested to be RARγ (640), RARβ, and RXRα (426); retinoic acids (or synthetic ligands) increase transcription of the UCP1 gene in in vitro systems (8, 9, 38, 64, 112, 426, 639, 717) and in vivo (198, 710), but retinoic acid does not increase UCP1 transcription unless the cells are simultaneously induced to differentiate (9). PPAR response elements are found (PPRE) (38, 717); both PPARα and PPARγ can bind here. Treatment of brown adipocytes in culture or of animals with a PPARγ agonist increases UCP1 gene expression (97, 183, 220, 618), but it is not unequivocal whether the induction of UCP1 is a result of increased differentiation of brown adipose tissue or a specific effect on the UCP1 gene; also indirect effects are possible as "new" cAMP response elements are recruited by PPARγ agonist treatment (638). Responsiveness to thyroid hormone (TRE) is also located here, through a type 3 direct repeat (112), an upstream inverted repeat. and a downstream direct repeat (639, 641). These sites amplify the effect of norepinephrine on UCP1 transcription (49, 319). However, ablation of thyroid hormone receptors does not lead to an absence of UCP1 gene expression (259). The unliganded thyroid receptor acts as a repressor of UCP1 expression (639), and the function of thyroid hormone is thus to diminish the repression; in the absence of the thyroid hormone receptor, there is thus no repressing effect.

ferent from that within a brown adipocyte), or in vivo (where it is often difficult to distinguish between direct effects on UCP1 expression and general differentiation-promoting effects), or in brown adipocyte primary cultures (where molecular manipulations are difficult). However, a fairly coherent but complex picture can be given of UCP1 gene expression control, as summarized in Figure 11. As seen, both a proximal promoter and a complex distal enhancer region are involved; the latter is the more studied one. Concerning adrenergic control, it is likely that the cAMP response elements (CRE) are those of greatest importance, together with some elements that have been implied in determining tissue-specific expression of UCP1. The physiological role of the other regulatory elements described in Figure 11 is thus not presently clarified. Some of these may be considered physiologically permissive, in that alterations in the activity/concentration of these regulators is unlikely to occur under physiological conditions.

D. Norepinephrine Is an Apoptosis Inhibitor in Brown Adipocytes

The final step in cell differentiation is regulated cell death: apoptosis. In brown adipose tissue, it is regulated negatively by norepinephrine and positively by tumor necrosis factor-α (TNF-α).

Norepinephrine exerts its anti-apoptotic effect through dual adrenergic pathways, i.e., both β-adrenoceptor stimulation (via cAMP, protein kinase A, Src activation, and activation of the MAP kinase Erk1/2) as well as α_1-adrenoceptor stimulation (through increases in intracellular calcium and also through Src and Erk1/2), lead to

anti-apoptosis (450). Physiologically, the norepinephrine-induced apoptosis inhibition is observable both in the cold, which leads to sympathetic stimulation and inhibition of apoptosis (451), and in obesity, which is associated with reduced sympathetic activity and thus with enhanced apoptosis (75).

In contrast, TNF-α is apoptotic (581, 623, 831). The pathways are not fully clarified, although p38 MAP kinase is suggested to be involved (831). Norepinephrine can also protect against this TNF-α-induced apoptosis (75, 581).

However, apparent effects of obesity on apoptosis in brown adipose tissue may be understood as being secondary to the diminished sympathetic activation of the tissue occurring in all genetic obesities. An anti-apoptotic effect of TNF-α receptor ablation observed in obese animals (579) may indicate that obesity, through increased TNF-α levels, may aggrevate brown adipose tissue atrophy.

IV. HOW SIGNIFICANT IS BROWN ADIPOSE TISSUE?

In a number of physiological (and pathological) conditions, metabolic efficiency and heat production of animals (and humans) are altered, sometimes with pathological consequences (development of obesity due to, or enhanced by, increased metabolic efficiency) but often with advantageous effects (survival of a mammal in the cold). The basic question we address in the following sections is: What is the qualitative and quantitative significance of brown adipose tissue, and especially of brown fat-derived heat production/metabolism, for these metabolic states?

In each such metabolic state, brown adipose tissue participation may be analyzed as being *essential*, if the metabolic phenomenon under study is fully dependent on the heat-generating function of brown adipose tissue. (This means that this metabolic event does not occur if brown adipose tissue is absent or nonfunctional.) If the participation is *optional*, then brown fat activity will be called into effect (often as the first choice, Ref. 81), but in its absence, or if not sufficient, another thermogenic effector (e.g., shivering) can instead be called into action. If *additional* or *coincidental*, it is activated but has no function (it produces an unnecessary extra output of heat). Of course, there are thermoregulatory conditions where brown adipose tissue is not involved, and conditions in which its participation is directly counterproductive, where the activity or recruitment of brown adipose tissue will counteract another thermoregulatory effector.

Although it may appear simple to classify these matters, in many physiological conditions, brown adipose tissue involvement has as yet only been implied based on correlative observations, i.e., as to whether brown adipose tissue activation/recruitment is observed as an effect of treatment. Obviously, such activation could be coincidental and does not necessarily mean that the activation explains the metabolic phenomenon, and especially, the activation could be optional, in that the metabolic alteration could likewise take place equally well using another thermogenic/metabolic effector. Of course, similar reasoning applies where decreased brown adipose tissue activity is invoked to explain states of enhanced metabolic efficiency.

To simplify the following analysis, we first discuss available measures for estimation of activation and recruitment. The relationship between these two measures is illustrated above in Figure 8.

A. Parameters of Activation and Recruitment

1. Parameters of activation

A good acute and continuous measure of brown adipose tissue activition is a recording of the electrical activity in the nerves innervating the tissue, as has been performed (e.g., Refs. 193, 532, 540, 703). This is, however, practically only possible in anesthetized animals, and this limits its use in analysis of e.g., responses to feeding, and the technique cannot distinguish between electric activity in nerves innervating blood vessels versus adipocytes. On a longer time scale, norepinephrine turnover in the synapses can be followed after inhibition of the synthesis of norepinephrine by inhibitory precursor analogs (as e.g., in Refs. 263, 890); however, for these analyses, time periods of several hours are needed, and bouts of brown adipose tissue activity may be much shorter than this.

Because thermogenesis is mainly a mitochondrial event, signs of mitochondrial activity are also good indicators of brown fat activity in general. One such indicator is the increase in mitochondrial size (swelling) that occurs in connection with activation (e.g., Ref. 174), but to obtain this measure is a rather tedious procedure.

A measure that may be related to mitochondrial swelling and that seems to correlate well with mitochondrial activity in brown fat mitochondria is the so-called "unmasking" phenomenon (e.g., Refs. 283, 317, 339, 509). This is a still poorly understood process that reveals itself as an increase in the number of measureable mitochondrial GDP-binding sites, i.e., on UCP1. Such an increase can be observed during a time interval when no synthesis of UCP1 protein can reasonably have occurred, and it can be seen even in the presence of the protein synthesis inhibitor cycloheximide (175). This unmasking process may, at least to some degree, be mimicked by mitochondrial swelling in vitro (559). Similarly to other mitochondrial carrier proteins, UCP1 is thought to exist as a dimer (445), and the unmasking is related to the fact that half of the GDP-binding sites are occupied by purine nucleotide before the addition of the [^{3}H]GDP used for determination

of binding site number (359). However, the cellular steps leading from adrenergic stimulation to unmasking have not been clarified. Despite the lack of knowledge of the molecular basis for the unmasking process, the measure seems to indicate activation from a dormant state, i.e., it functions only provided that the GDP-binding sites are initially masked, a situation that can hardly be expected to exist at temperatures below thermoneutrality (although, somewhat surprisingly, there are several experiments that demonstrate unmasking even at lower temperature).

In an extension of the tenet that all activation is adrenergic, adrenergically induced gene expression may be taken as an activity measure, even though the increased gene expression is not acutely linked to thermogenesis. The interpretation here can also be difficult, in that, even under sustained adrenergic stimulation, the expression of certain genes is not maintained. Good measures of activation would result from monitoring genes with a rapid onset of expression, with a stable level of expression, and with a rapid turnover of mRNA. However, the expression of, e.g., c-*fos* (803) and vascular endothelial growth factor (VEGF) A (30, 225), is only transient, even during chronic physiological stimulation, and UCP1 itself has the problem that the mRNA half-life is relatively long (~18 h in vivo at room temperature and ~3 h in cold, Ref. 374), which can misleadingly indicate tissue activation even after its cessation.

Lipoprotein lipase mRNA (and lipoprotein lipase activity, as the half-life of the protein is very short in the tissue, only ~2 h, Ref. 111) reacts rapidly to activation but is nonetheless not an unequivocal indicator of activation, because lipoprotein lipase gene expression/activity may be increased both through adrenergic activation and through insulin action, which is not directly related to tissue activation. Studies of glucose uptake suffer from the same analytical problem.

However, all the above measures are indirect, in that they would probably be induced even in animals which, for some reason, cannot produce any heat. A quantitative measure of the real product, i.e., heat as such, is not possible. Indeed, the establishment of the relative role of brown fat-derived thermogenesis for a given metabolic condition would have been much simpler had this been possible. The best option is to determine blood flow to the tissue [which can be done with microspheres (221, 222) or laser-Doppler technique (824)] plus the tissue extraction of oxygen (which can be estimated from the degree of oxygen saturation of the blood leaving the tissue); this gives an estimate of the actual amount of oxygen consumed and thus of the heat produced. These procedures are, however, rather cumbersome and nearly impossible to perform with unstressed nonanesthetized animals (although this has been done, Ref. 223). Instead, the temperature in the brown adipose tissue may be followed (preferably as the difference between rectal temperature and brown fat temperature), but this cannot give quantitative information on the significance of brown adipose tissue for a given metabolic alteration.

In the following analysis of different types of thermogenesis, if there is no particular reason to specify the means of observation, we refer to observations of the kind discussed above, simply as indications that a specific event leads to *activation* of brown adipose tissue.

2. Parameters of recruitment

Recruitment state (as illustrated above in Fig. 8) differs from activity state in that it refers to the thermogenic/metabolic capacity of the tissue when maximally stimulated, in contrast to activation, which refers to the processes acutely ongoing in the tissue. The recruitment process is thus the coordinated alterations in the tissue that, taken together, increase the capacity of the tissue for performing nonshivering thermogenesis. Whereas UCP1 is essential for thermogenesis (Fig. 3), it cannot make heat in isolation: an increased thermogenic capacity requires mitochondriogenesis and a general increased differentiation of the cells, and recruitment is normally accompanied by an increased number both of brown adipocytes and of vascular cells, caused by increased cell division and/or inhibition of apoptosis. Brown adipose tissue atrophy is the opposite process.

The most physiologically relevant, and often also the most dramatic, measure of recruitment is the change in the total amount of UCP1 protein per brown adipose tissue depot ("per animal"). This measure encompasses both the increase in the concentration of UCP1 in the mitochondria and the effect of the increased mitochondriogenesis and cell proliferation in the tissue; it is therefore probably the best reflection of the actual thermogenic capacity change occurring in an adaptive situation.

The level of UCP1 mRNA, although easily determined and often used, is a less feasible indicator of recruitment state. It shows an overdramatic, rapid response, i.e., it can be increased manyfold after just a few hours of, e.g., cold exposure. However, there is a long time delay, even up to some weeks, before alterations in UCP1 mRNA levels lead to corresponding alterations in the amount of UCP1 protein. This time delay is a consequence of the rather slow turnover of UCP1 protein in its native environment (discussed in Ref. 375) and includes the synthesis of new mitochondria. Thus a transient alteration in UCP1 mRNA, positive or negative, even if quite large, may be without observable physiological significance.

Less feasible (principally uninterpretable) measures of brown adipose tissue recruitment are the weight of a brown adipose tissue depot, as well as relative contents (%) in the tissue of, e.g., protein or DNA. This is because these parameters are not unequivocal. An increase in wet

weight may be associated with a recruited state, but also with an atrophied state, because, under such circumstances, lipid accumulates in the tissue, increasing its weight; correspondingly, a significant recruitment may occur without an increase (or even with a decrease) in tissue weight, because lipid is exchanged for more active components. Values of relative contents (e.g., per gram) reflect the same problem; dilution by lipid may obscure a true recruitment. Also, the edema observed during the first hours of acute activation (108) may affect wet weight and relative content of protein in a spurious way.

An increase in total (i.e., per depot) mitochondrial enzyme content and enzymatic capacity (often measured as cytochrome-*c* oxidase activity) also accompanies recruitment, as does an increase in total tissue cellularity (i.e., total DNA amount). This increase in DNA reflects increases not only in adipocytes but also in interstitial cells and endothelial cells, allowing new blood vessel formation (89). (In tissue samples taken very early in the recruitment process, the DNA resulting from an increased number of white blood cells being trapped in the tissue as a consequence of the acute increase in blood flow may disturb the results.)

In the intact animal, an increased metabolic (thermogenic) response to injected norepinephrine (or other β-adrenergic agents) may also be considered an indication of brown adipose tissue recruitment. However, this interpretation is only possible if it is first accepted that all recruited norepinephrine-induced thermogenesis emanates from brown adipose tissue. Although we would believe that this is the case, based on present evidence, this has not been examined under all relevant conditions and is still a controversial issue.

3. Is recruitment the effect of chronic activation?

As discussed in section III, norepinephrine stimulation of brown preadipocytes and mature adipocytes will lead to recruitment, and most recruited states can be understood as being caused by chronically increased adrenergic stimulation. Indeed, chronic treatment of animals with norepinephrine leads to all signs of recruitment in brown adipose tissue (68), whereas, as expected, treatment with β_3-adrenoceptor agonist alone will not induce cell proliferation (336) and the degree of recruitment is therefore limited by the number of brown adipocytes present initially. However, this does not mean that the only way to recruitment is that caused by continuous adrenergic stimulation. There are at least two states of recruitment described in which it is unlikely that the recruitment has occurred due to continuous adrenergic stimulation: the late embryonic recruitment seen in precocial newborns (see sect. V*D3*) and the short photoperiod-induced recruitment (see sect. V*F5*).

Correspondingly, signs of activation may be observable but no recruitment may occur, because short, even repeated, periods of activation may not be sufficient to support the recruitment process. For full recruitment, i.e., full adaptation to a new state, several weeks of chronic activation are normally necessary, again due to the slow half-life of, e.g., the mitochondrial complement, including UCP1. Indeed, cold acclimation requires at least 2×2 h of activation per day to occur to the same level as continuous cold exposure (864), and the same minimal requirement for recruitment time per day is probably necessary for other types of intermittent activation.

B. How to Establish Brown Adipose Tissue Involvement

That a physiological condition is correlated with the signs of brown adipose tissue activation or recruitment described above does not demonstrate to which extent, if any, the brown fat activity is responsible for any observed alteration in metabolic efficiency/heat production. The significance of an organ for a particular function is classically examined by observing the effect of its ablation, but as brown adipose tissue is an organ distributed to many sites in the body, it is extremely difficult to excise (and the tissue will tend to compensate for the excision by regrowth in unexcised depots).

To acutely inhibit brown adipose tissue activity, it is possible to inhibit adrenergic stimulation with the β-blocker propranolol. However, the interpretation of such an experiment is difficult. First, the inhibition is not specific: all β-adrenergically stimulated mechanisms are inhibited (which may exaggerate the role of brown adipose tissue or be difficult to interpret because of effects on the circulation). Second, the propranolol may not fully inhibit brown fat-derived thermogenesis. This is because the tradition of testing with propranolol developed at a time when knowledge of the existence of β_3-adrenoceptors as those receptors (mainly) responsible for thermogenesis was still not realized, and a "normal" propranolol dose would then only inhibit β_1/β_2-effects. Very high doses (10 mg/kg of the L-isomer) are necessary to inhibit endogenous adrenergic stimulation of β_3-receptors. Finally, propranolol may have central effects (see sect. V*E*), which could lead to inhibition of the demand for a thermogenic effect, rather than to inhibition of the effector of this demand.

However, for an analysis of the significance of brown adipose tissue for a given type of thermogenesis or altered metabolic efficiency, two types of genetically modified mice have become available in the 1990s: the UCP1-ablated mice and the brown fat-deficient mice. In the UCP1-ablated mice, some of the exons in the UCP1 gene have been replaced with a neomycin-resistance gene (200); in the brown fat-deficient mice, diphtheria toxin is ex-

pressed under the control of the UCP1 promoter, meaning that only in cells with the potential to express UCP1 and which are further stimulated by norepinephrine or other agents will diphtheria toxin be expressed, and these cells will die (462). Thus a lack of effect of diphtheria toxin is to be expected in, e.g., thermoneutral animals (505). In an optimal situation, these two mice strains would be complementary in information: the UCP1-ablated mouse would indicate as to whether brown fat-derived thermogenesis was necessary for a given type of thermogenesis. The outcome should principally be the same in the brown fat-deficient mouse, but if this were not the case, it would indicate that a function of brown adipose tissue, different from that of UCP1-dependent thermogenesis, was necessary for the process.

However, these tools also have their limitations. Both are dependent on the UCP1 gene not being "leaky" (i.e., not being expressed in non-brown adipocytes), but this criterion (see sect. II*B4*B) may not hold, and as discussed Reference 412, interpretation especially of the results with the brown fat-deficient mouse may be heavily influenced by the expression of the diphtheria toxin in sites other than brown adipose tissue, killing cells other than brown adipocytes.

It is a common problem in the interpretation of observations on knock-out mice that compensatory mechanisms may evolve. However, fortunately, this possibility is not really a problem in this particular instance; rather, it is an advantage. It is fully certain that no mechanism develops in brown adipocytes that can reinitiate adrenergic thermogenesis (Fig. 3). Therefore, if alternative mechanisms develop in the intact animal, this is an indication that brown adipose tissue may only be an optional effector, whereas if no compensatory mechanism develops, then brown fat-derived thermogenesis is really essential.

Concerning the UCP1-ablated mouse, the absence of the expected brown fat-derived thermogenesis leads to an intensified (but ineffective) sympathetic hyperstimulation of the tissue so that it becomes hyperrecruited in all other respects than increasing its actual heat output (100a). Although this may complicate characterization of non-UCP1-dependent features of brown adipose tissue, it does not lead to any alternative heat production. The absence of any compensatory response in the form of any other nonshivering thermogenesis results in enhanced muscular training (260). Since the gene ablation itself in this mouse is only partial, a truncated mRNA is formed (200, 263), the expression of which varies in parallel with that of the native gene. It is currently not known if this truncated mRNA is translated and whether the presence of such a truncated protein has any consequences.

Concerning the lack of tolerance to acute cold, the UCP1-ablated and the brown fat-deficient mice behave similarly. However, concerning body weight control, they are considered to be very different, although this may only be a case of degree (see sect. VI*B*); the differences reported between these two models may be exaggerated. The UCP1-ablated mouse is reported not to develop obesity (200), whereas in the brown fat-depleted mouse, the obesity develops rather late in life and seems to be dependent on the diet composition, but this difference may be secondary to the mouse strains in which these modifications are examined (see sects. VI*A* and VIII*C4*).

V. THERMOREGULATORY THERMOGENESIS

Two main physiological purposes of brown fat-derived thermogenesis can be identified. One is what can be understood physiologically as thermoregulatory thermogenesis, the function of which is to produce heat for defense of regulated body temperature. The second is a "metabolic inefficiency thermogenesis," metaboloregulatory thermogenesis, the function of which seems to be to allow for combustion of excess energy intake, perhaps with the purpose of allowing an "extraction" of essential food constituents (notably protein).

We first discuss the significance of brown adipose tissue in physiological conditions that can be encompassed in a broad definition of thermoregulatory thermogenesis.

A. In Acute Cold, Thermogenesis Results From Shivering

When an animal is acutely exposed to a "low" environmental temperature, it needs extra heat to compensate for the increased heat loss, to defend its body temperature. Principally, "low" here means any temperature lower than that referred to as "the lower critical temperature of the thermoneutral zone." For most experimental animals (and for naked humans), this temperature lies close to 30°C (Fig. 12). The extra heat needed to defend the body temperature becomes, however, smaller in relative magnitude the larger the animal becomes. There are two reasons for this: basal metabolism increases, for reasons still unknown, in proportion to the body weight to the power of 0.75, i.e., it is ~11 ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$ irrespective of mammalian species studied, whereas the surface (i.e., the area from which heat is lost to the surroundings) increases, for geometrical reasons, only in proportion to body weight to the power of 0.67. That the power functions are different means that the basal metabolism can provide more and more of the necessary heat the larger an animal becomes. The greater body weight also makes it possible for animals to carry relatively more insulation. The outcome is that under "normal" (room temperature) conditions, common experimental animals constantly maintain a metabolic rate about double their minimal metabolism, merely to defend their body temperature.

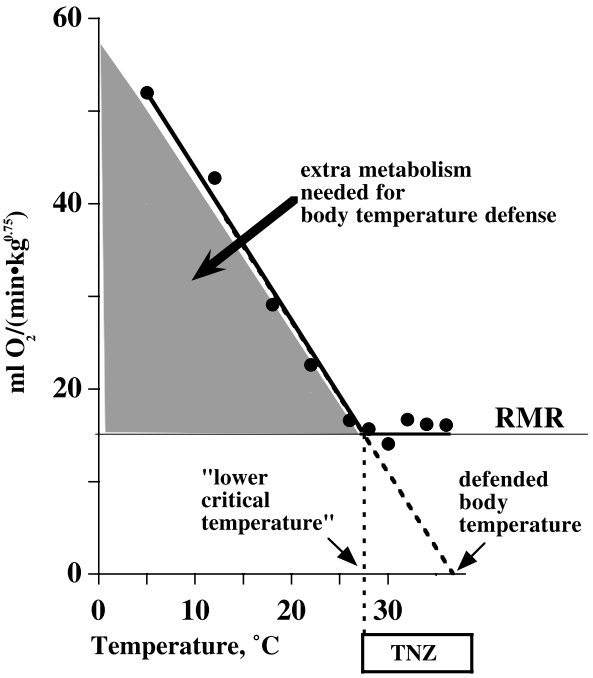

FIG. 12. Thermoregulatory metabolic response to environmental temperature. Principal sketch is based on actual observations (●) on mice (259). RMR, resting metabolic rate; TNZ, thermoneutral zone. The slope of the thermoregulatory line below the TNZ is a measure of the insulation (more insulation, lower slope); for physical reasons, it intercepts the *x*-axis at the defended body temperature. The gray area denotes the extra metabolism needed for body temperature defense; if this amount of heat is not produced, the body temperature cannot be defended and the animal will become hypothermic and eventually die. The heat may be produced by shivering thermogenesis (acutely) or nonshivering thermogenesis (after prolonged exposure to cold) alone or in combination.

Chronically, this exposure to normal room temperature will lead to the recruitment of a certain capacity for nonshivering thermogenesis. Larger animals, including humans, do not normally require this "extra" metabolism for thermal homeostasis and consequently have not recruited such a capacity. It is not impossible that this difference between large and small animals explains some difficulties in extrapolating from experimental animals to humans in these matters.

However, what is normally referred to as "cold stress" is the acute exposure of normal animals (i.e., small animals which have been living at ~20°C) to a temperature of ~5°C. As seen (Fig. 12), this requires a sustained increase in metabolism fourfold above basal metabolic rate. Within the time frame of the experiment (often only a few hours), the animal may either be able to continuously produce this extra heat and thus successfully defend its normal body temperature, or, if not, it will succumb to the cold. The ability of animals to defend their euthermic body temperature under these conditions has recently developed into becoming an "established" method of investigation to analyze for functional activity of nonshivering thermogenesis, a position that this type of experiment does not deserve and that may lead to misleading conclusions. Indeed, quite in contrast, as summarized in Figure 13, shivering is the expected major response to compensate for the increased heat loss following acute transfer from 20 to 5°C, and only during prolonged cold exposure (weeks) would an increased capacity for classical nonshivering thermogenesis develop (see sect. v*B*) (284).

In consequence, the ability to survive acute cold depends on the thermal prehistory of the animals. Thus, in animals previously housed at environmental temperatures higher than ~20°C, the sum of the capacities for shivering thermogenesis and nonshivering thermogenesis may still enable the animal to mount an acute heat production large enough to compensate for heat loss, but the animals do not have the endurance (including lung and heart capacity, as well as skeletal muscle capacity) necessary for a sustained fourfold elevated metabolism, and they eventually become exhausted and body temperature will then fall. In animals housed below ~20°C, the nonshivering thermogenic capacity will be sufficient for this not to happen. A physiologically normal response to cold and/or starvation in small mammals such as mice is to go into torpor, i.e., to allow a controlled decrease in body temperature to occur to conserve energy (see sect. v*F4*), and this will be observed as a decrease in metabolic rate at reduced environmental temperatures. However, if brown adipose tissue is already recruited by "cafeteria feeding" (see sect. vi*B1*), the tissue has an extra capacity that can be used even in the acute cold situation (681). Remarkably, even animals without UCP1 and thus completely lacking the ability to develop nonshivering thermogenesis (see sect. v*B*) can survive many months at 5°C, provided that they are successively exposed to increasing degrees of cold (260), which allows for development of muscle training to support shivering thermogenesis and increased physical endurance in general.

It is therefore not possible to evaluate the capacity of an animal for nonshivering thermogenesis from the ability of the animal to survive acute cold stress. The participation of brown fat-derived thermogenesis in the response to acute cold is clearly optional, i.e., brown adipose tissue will be used if the capacity already exists, but if not, the animal will use other means (shivering) for the same purpose.

B. Classical Nonshivering Thermogenesis Is Entirely Brown Fat Dependent

Although the extra heat needed to defend body temperature initially comes from shivering, chronic cold exposure results with time in an almost complete disappearance of shivering (302) (Fig. 13). As the enhanced meta-

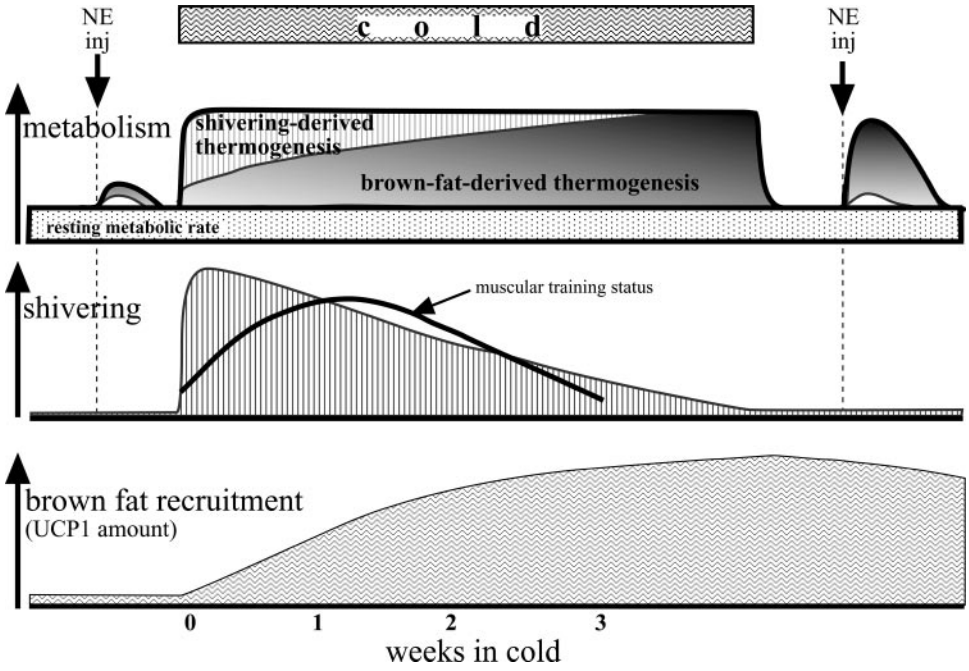

FIG. 13. Metabolic responses to acute and chronic cold. Principal sketch based on an experimental animal (mouse, rat) that has been preacclimated at "normal room temperature" (~20°C), which is below the thermoneutral zone (Fig. 12) and therefore leads to some brown adipose tissue recruitment. In this figure, the animal is first studied within its thermoneutral zone (e.g., at ~30°C) and thus displays only its resting metabolic rate. If the animal is injected with norepinephrine (NE) at this temperature, its metabolism increases, due to both brown fat-derived (gray) and non-brown fat-derived (white) heat production. When the animal is then placed in the cold (~5°C), its metabolism increases ~4-fold (Fig. 12). This is partly accomplished by nonshivering thermogenesis from the amount of brown adipose tissue available; the deficit is obtained from shivering thermogenesis (hatched). During this time, there will be a constant nerve stimulation of the muscles, and the fibers will constantly be contracted and relaxed. This period may therefore be seen as a muscular training period. There are indeed muscular effects of chronic cold exposure, such as an increase in muscle capillary density (756, 782), change in muscle fiber composition (759) and in mitochondrial morphology (45, 282), as well as increases in muscular capacity for β-oxidation (862) and in the oxidative capacity of mitochondria (71, 782, 850, 862). Such effects are very similar to the effects of endurance training on muscle capillary density, fiber composition, and mitochondrial oxidative capacity (52, 131, 291, 861). With time in cold, the capacity of nonshivering thermogenesis (amount of UCP1 in brown adipose tissue) successively increases, and the need for shivering therefore successively decreases. This decreased need is reflected in the transient nature of the characteristics of muscle training seen in the cold (71, 759). Thus observed alterations in muscles during cold exposure can be explained as training effects due to shivering. When the animal is returned to thermoneutral temperature after cold acclimation, the heat production from brown adipose tissue immediately ceases, but the tissue remains recruited and only slowly returns to initial thermogenic capacity. During this time, an injection of norepinephrine will elicit a much larger response than before; this increase is fully due to increased brown fat-derived thermogenesis (gray).

bolic rate persists in the cold (302) (Fig. 13), this must mean that a mechanism for heat production different from that of shivering must exist (150). In the absence of any positive characteristics, this mechanism was originally termed nonshivering thermogenesis (378). Thus "nonshivering" in the expression *nonshivering thermogenesis* is shorthand for "thermogenesis that replaces shivering." Unfortunately, in addition to this strict definition of the term, nonshivering thermogenesis has become widely used to refer to any thermogenic process that does not occur through shivering, irrespective of whether it replaces shivering or not. In this broad definition, nonshivering thermogenesis must then include all metabolic processes in the body, and the expression, as used presently, verges on "basal metabolism." Nonshivering thermogenesis in this broad sense is far too undefined to be analyzed as a general phenomenon. In this section, we will only consider classical nonshivering thermogenesis, which we then define as cold acclimation-recruited, cold exposure-induced nonshivering thermogenesis.

There is ample evidence that prolonged cold acclimation leads to brown adipose tissue recruitment, as analyzed by any parameter (see sect. IV*A*). Notably, the total amount of UCP1 increases some 10-fold or more due to cold acclimation (375, 509), but also an increase in total cellularity, total amount of mitochondria, mitochondrial enzymes, fatty acid oxidizing enzymes, etc., contribute to the vastly enhanced oxidative capacity (31, 88).

That brown adipose tissue is activated in the cold was most clearly demonstrated in the classical blood flow experiments of Foster and Frydman (223). Based on measurements of tissue blood flow distribution and the arte-

rial-venous oxygen difference over the tissue, it was estimated by these authors that brown adipose tissue could use at least some 60% of the extra oxygen used in cold-acclimated animals in the cold, i.e., brown adipose tissue could be responsible for at least 60% of nonshivering thermogenesis. Through this, brown adipose tissue was accepted as the major site for nonshivering thermogenesis.

Thus, for a long time, there has been no doubt that brown adipose tissue is impressively recruited during cold acclimation and that it is activated when cold-acclimated animals are in the cold. The question that has remained unsettled has been as to whether brown adipose tissue is the only site of true nonshivering thermogenesis. Even though Foster and Frydman (223) clearly interpreted their results in the way that the oxygen not directly utilized in brown adipose tissue was used in muscles working to supply brown adipose tissue with oxygen for the thermogenic process, i.e., primarily the respiratory muscles and heart, it was still felt that an alternative mechanism for nonshivering thermogenesis, in addition to that occurring in brown adipose tissue through UCP1 activity, may exist.

From a time before the extent of the thermogenic capacity of brown adipose tissue had been realized, the notion had (and has) remained that there is (also) an involvement of a muscle component in cold-induced nonshivering thermogenesis (59, 270, 379, 461), primarily due to the large mass and high potential metabolic capacity of muscle. However, evidence for the existence of this putative muscle-derived nonshivering thermogenesis has only been circumstantial, and all experimental evidence collected (as described in Fig. 13) is equally compatible with endurance training effects of shivering on muscle.

Instead, experiments with UCP1-ablated mice have been conclusive. The behavior of UCP1-ablated mice is qualitatively distinct from that of wild-type mice. Most impressively, these mice continue to shiver with exactly the same intensity as they did initially, even after several months in the cold (260). This thus means that they are fully unable to recruit any alternative source of nonshivering thermogenesis than that emanating from UCP1 and brown adipose tissue. Thus brown adipose tissue is essential for classical nonshivering thermogenesis, and the only thermogenesis taking place outside brown adipose tissue is that arising as a by-product from the activity of the respiratory and circulatory systems required to supply brown adipose tissue with oxygen (223). There is presently no experimental reason to maintain the tenet that a process of cold acclimation-recruited, cold-induced nonshivering thermogenesis is located to muscle.

The absence of classical nonshivering thermogenesis in muscle does not necessarily mean that no nonshivering thermogenesis, in its broadest sense, exists in muscle. Basal metabolism in its entirety may be considered to represent nonshivering thermogenic processes and, to a large extent, basal metabolism is located to muscle. However, the undiminished shivering intensity in the UCP1-ablated mice in the cold (260) demonstrates that basal metabolism is not adaptive under these circumstances and cannot be increased to obviate the necessity for shivering. The absence of classical nonshivering thermogenesis in mammalian muscle does not necessarily mean that other animal groups (fish, birds, insects) have not been able to evolve alternative mechanisms for muscle thermogenesis, which may be of a nonshivering type. However, at least in fish and insects, these mechanisms are not adaptive (59), and, e.g., thyroid thermogenesis (see sect. v*E5*) in mammals is not facultative. These processes therefore do not represent classical nonshivering thermogenesis as defined here. It is doubtful that nonshivering thermogenesis exists in birds (344), and birds do not possess brown adipose tissue.

Other organs than brown adipose tissue and muscle (e.g., liver) have occasionally also been suggested to be sites of adaptive nonshivering thermogenesis (270, 379). However, that any visceral organ could mediate nonshivering thermogenesis was already dismissed in early experiments showing that nonshivering thermogenesis could take place in animals that were functionally eviscerated (170, 171) (see sect. ii*B4*b), and the experiments with UCP1-ablated mice principally confirm this conclusion (260).

C. Cold Acclimation-Recruited, Norepinephrine-Induced Thermogenesis Is Entirely Brown Fat Dependent

Many substances are thermogenic when injected into an animal (379). There is obviously no physiological significance of such heat production in itself, and the thermogenic responses are only of interest if they reflect physiologically relevant types of thermogenesis. In the question of adaptive nonshivering thermogenesis, the response to injected (or infused) norepinephrine has a unique position. This results from the observation that the acquirement of a capacity for classical nonshivering thermogenesis (measured in the cold) coincides with the acquirement of a much enhanced thermogenic response to an injection of norepinephrine (measured at thermoneutrality) (Fig. 13) (358). Thus the response to norepinephrine at thermoneutrality is quantitatively very similar in acclimated animals to the amount of nonshivering thermogenesis occurring at the acclimation temperatures. Therefore, the response to norepinephrine is now generally equated with the capacity for nonshivering thermogenesis (378).

There is no doubt that exogenous norepinephrine activates thermogenesis in brown adipose tissue. This

was most dramatically demonstrated by Foster and Frydman (222) in blood flow studies that indicated that the major part of the norepinephrine-induced thermogenesis in cold-acclimated animals could emanate from brown adipose tissue. However, the question again remained as to whether all norepinephrine-induced thermogenesis is due to brown adipose tissue activity, or whether also other tissues could respond in a thermogenic way.

Examination of UCP1-ablated mice has enabled analysis of this question. The major outcome is that two different components of norepinephrine-induced thermogenesis can be discerned: a UCP1-independent and a UCP1-dependent component.

1. UCP1-independent thermogenesis

UCP1-independent thermogenesis is what is observable as a norepinephrine-induced thermogenesis even in animals lacking UCP1 (565). A significant characteristic of this thermogenesis is that it is not augmented in animals acclimated to the cold. It can therefore not reflect an adaptive thermogenic process. It is clearly not localized to brown adipose tissue (because brown adipocytes are not thermogenic in the absence of UCP1; Fig. 3). The existence of a non-brown fat-derived adrenergic thermogenesis is supported by observations of a thermogenic response to norepinephrine injection even in animal groups that fully lack brown adipose tissue: amphibians, reptiles, and birds (40, 290, 299, 340). The localization and molecular mediation of this nonadaptive adrenergically induced thermogenesis is not known, but it may be suggested to represent the metabolic summation of the pharmacological stimulation of all adrenoceptors in the body (except the brain), and thus to include contributions from most tissues, including, e.g., muscle. Isolated muscle (504) or perfused rat hindlimb (813) responds to norepinephrine with an increase in metabolism; correspondingly, blood flow to all (nonrespiratory, nonheart) skeletal muscles is increased by ~50% during norepinephrine-induced thermogenesisis, but the blood flow is not augmented after cold acclimation (222). There is no reason to believe that this nonadaptive thermogenesis is the result of "uncoupled" respiration; rather, it more likely represents the metabolic cost for the synthesis of ATP utilized in cellular processes that are stimulated by norepinephrine in different tissues. It cannot be fully excluded that UCP2 or UCP3 may mediate some of this nonadaptive response to norepinephrine, but the metabolic response to injection of β_3-agonist in UCP3-ablated mice is not lower than in wild-type mice (267), implying that such a function for UCP3 is unlikely, and the amount of UCP2 protein in cells and tissues other than those involved in immunological defense is so low that its contribution to total metabolism should be negligable (616). Taken together, we consider the nonadaptive thermogenic effect of norepinephrine to be fully pharmacological, i.e., it does not represent a reflection of a thermoregulatory thermogenic process, and brown adipose tissue is not involved in this nonadaptive adrenergic "thermogenesis."

2. UCP1-dependent (brown fat-derived) norepinephrine-induced thermogenesis

Historically, adaptive adrenergically induced nonshivering thermogenesis was expected to be localized to the muscles, again mainly because of their large mass and high potential metabolic capacity. Muscle has remained a candidate localization of adaptive adrenergic nonshivering thermogenesis (59, 270, 379, 461), especially in larger mammals such as adult humans (who have generally been considered to be principally devoid of functional brown adipose tissue, Refs. 154, 387). However, in spite of numerous attempts, only two reports exist of an increased adrenergic responsiveness in muscle from cold-acclimated animals (286, 742), but such an increased response in vitro may be seen as an effect of muscular physical training (149) and may, therefore, just as the morphological and enzymatic alterations in muscle discussed above (Fig. 13), reflect the training effects of shivering thermogenesis, rather than nonshivering thermogenesis. There is no in vivo effect of persistent shivering "training" on the response to injected norepinephrine (260). Because the idea of a muscle component in adrenergically induced adaptive nonshivering thermogenesis has been maintained, a mechanism for this putative phenomenon has also been sought. Particularly UCP3, which is mainly expressed in muscle (67), has been considered in this respect, but UCP3 gene expression is not chronically elevated in cold-exposed mice (67, 425, 444).

In contrast, UCP1-dependent (brown fat-derived) norepinephrine-induced thermogenesis is vastly augmented as a result of cold acclimation (Fig. 13) (262, 565). Thus brown adipose tissue is essential for cold acclimation-recruited, norepinephrine-induced thermogenesis. As the cold acclimation-recruited, norepinephrine-induced thermogenesis and cold-induced thermogenesis are both fully dependent on brown fat-derived heat and as they develop in parallel, they represent the same phenomenon: norepinephrine injection should be understood as mimicking the sympathetic nervous stimulation of brown adipose tissue, and the norepinephrine has to reach the adrenergic receptors facing the sympathetic nerve synapses. Thus very high, supraphysiological levels of norepinephrine are needed in the blood (~100 nM; Ref. 172) (which may result in the pharmacological effects in other tissues referred to above). The tenet that norepinephrine-induced thermogenesis mimics the physiological nerve stimulation occurring in cold-induced nonshivering thermogenesis is corroborated by the fact that propranolol injections in cold-acclimated animals in the cold inhibit

nonshivering thermogenesis: the animals start to shiver (82, 284, 529, 627, 716).

When an animal is injected with norepinephrine, both the UCP1-dependent and the UCP1-independent thermogenesis are necessarily stimulated in parallel. When adaptive thermogenesis has been recruited (i.e., when the animal is cold acclimated), the dominant part of the thermogenic response is the brown fat-derived, UCP1-dependent adaptive thermogenesis, but in animals housed at higher acclimation temperatures, the nonadaptive, non-UCP1-dependent part makes a significant contribution. The nonadaptive part is probably never activated physiologically, i.e., by endogenously released catecholamines, but its activation during norepinephrine infusion will obscure quantification of the UCP1-dependent adaptive response.

3. Epinephrine-induced thermogenesis

The existence of an epinephrine-induced nonshivering thermogenesis, different in nature from the norepinephrine-induced thermogenesis discussed above, has been repeatedly advocated (379–381).

When "pharmacological" concentrations of epinephrine are injected into animals, an adaptive, epinephrine-induced thermogenesis is observed (324); this thermogenesis is identical in magnitude to the norepinephrine-induced thermogenesis and is fully UCP1-dependent (262). This is then in agreement both with cold acclimation-recruited norepinephrine-induced thermogenesis being fully brown fat derived (see sect. v*C2*), and with the known pharmacology of brown fat cells: epinephrine is able to stimulate thermogenesis in these cells, with an EC_{50} only marginally higher than that of norepinephrine (517). Brown adipose tissue is thus essential for this high-concentration epinephrine-induced thermogenesis (but epinephrine-induced thermogenesis is not distinguishable from norepinephrine-induced thermogenesis).

In addition to this thermogenesis induced by high concentrations of epinephrine, a thermogenic effect of infused epinephrine may exist, considered to mimic a hormonal effect. The interest in this low-concentration epinephrine-induced thermogenesis arises mainly from studies in humans, because such studies have concentrated on the response to epinephrine and have often strived to use doses that are comparable with physiological levels of epinephrine in the blood (subnanomolar levels, Ref. 153); the human studies are therefore necessarily limited to the hormonal aspect of sympathetic thermogenesis. The thermogenic responses in humans are elicited at remarkably low epinephrine levels, in the low (1–5) nanomolar range (476), which result in metabolic increases in the order of ~10%. The very low EC_{50} principally excludes brown adipose tissue from being the effector of low-concentration epinephrine-induced thermogenesis. Conversely, as brown adipocytes could not conceivably respond to the low epinephrine concentrations used, absence of an augmented response following an adaptation process in humans examined with such low epinephrine doses cannot be used to conclude that a brown fat-derived thermogenesis (which would be neuronally stimulated) has not been recruited in a given adaptation process.

Notably, the observed "human" humoral epinephrine-induced thermogenesis is augmented by exceedingly brief periods of cold exposure (436), but this apparent augmentation may be related to adaptive changes in vasoconstriction. The molecular basis for the humoral thermogenesis elicited by epinephrine is unknown, as is the anatomical location, but there is no unequivocal evidence that it has thermoregulatory or metaboloregulatory significance. Brown adipose tissue is thus not involved in this low-concentration epinephrine-induced thermogenesis.

4. Glucagon-induced thermogenesis: does it exist?

Glucagon injection into animals has been reported to be thermogenic and, in studies from the early 1980s, the thermogenic response was reported to be augmented in cold-acclimated animals (184, 185). It could therefore be suggested that the response reflected a type of nonshivering thermogenesis different from the classical nonshivering thermogenesis elicited by sympathetic nerve activity in the cold. There were also reports that indicated that brown adipose tissue was activated (increased blood flow) during glucagon injections (873).

However, although glucagon receptors are expressed in brown adipose tissue (527), it is doubtful that a physiologically relevant brown fat-derived glucagon-induced thermogenesis exists. Unphysiologically high levels of glucagon (~1,000 times higher than plasma levels) are needed to stimulate thermogenesis in isolated brown adipocytes from mice and rats (181, 874), but because no glucagon-induced thermogenesis is observed in hamster cells (181), it is clearly not an essential part of brown adipocyte physiology. Due to the high glucagon levels needed, the possibility exists that it is not the glucagon receptor that reacts to glucagon but, e.g., a receptor for another member of the glucagon superfamily.

The earlier observations of glucagon-induced thermogenesis in intact animals are not easily reproduced today, and the possibility cannot be excluded that the glucagon-induced thermogenesis observed was due to some contamination in the preparations earlier used (181). A pertinent question is thus whether a glucagon-induced (adaptive) thermogenesis really exists.

D. Postnatal Thermogenesis

As long as the fetus is within the mother's womb, it is protected against the thermal whims of nature, and any

induced thermogenesis in the fetus will only add to the thermal burden of gestation. However, after birth, the fetus is directly or successively exposed to the cold and will require thermogenesis to counteract heat loss. Because brown adipose tissue is prominent in newborns, it was earlier thought that nonshivering thermogenesis was a kind of rudimentary embryonal mechanism found in not fully developed animals before they could initiate the "adult" shivering process; we now acknowledge that nonshivering thermogenesis and brown adipose tissue are present because the need for this type of heat production is high and thus nonshivering thermogenesis is an acquired characteristic.

Analysis of the significance of brown adipose tissue for the newborn is facilitated if newborns from different species are classified into one of three groups, with respect to thermoregulatory (and other) properties (as suggested in Ref. 563, where a detailed account of observations on brown adipose tissue in the mammalian newborn is also found): the altricial, the immature, and the precocial newborns. These three groups are distinguished in Figure 14.

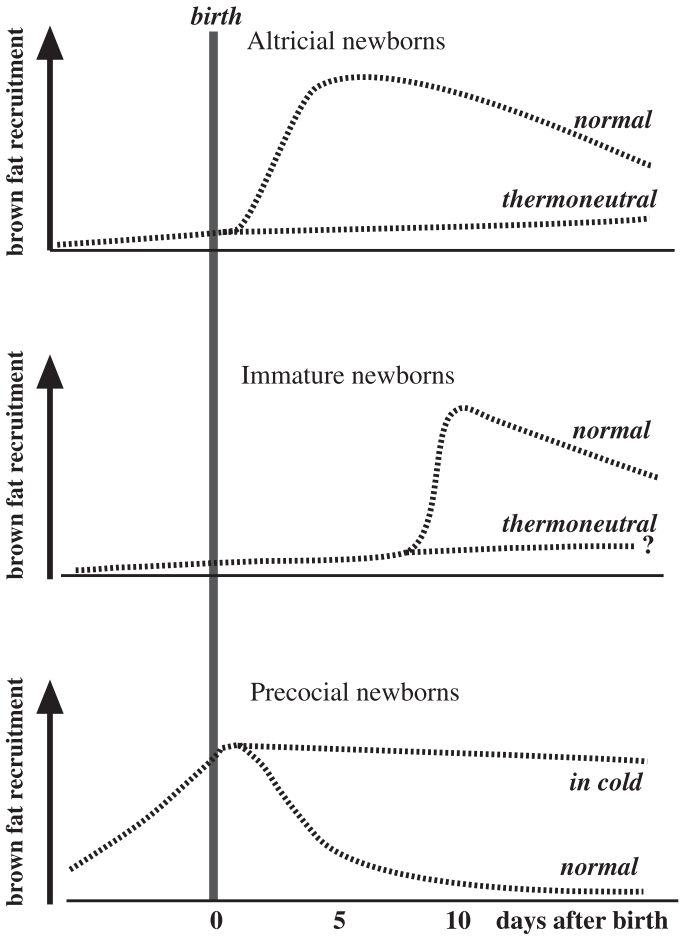

FIG. 14. Brown adipose tissue recruitment in newborn mammals of different degrees of development at birth: altrical (mouse, rat), immature (hamster), and precocial (guinea pig). Postnatal development under conditions of normal animal house temperature (~20°C) and thermoneutral conditions (~33°C) are depicted for altricial and immature newborns; for precocial newborns, development in a normal and a cold (≈5°C) environment are depicted. [Simplified and extended from compilations in Ref. 563.]

1. Altricial newborns recruit brown adipose tissue after birth

Among altricial newborns are the young of the most common experimental species: rat and mouse. There are many pups in the litter, the pups are born blind and naked, and for the first days after birth huddle together in the nest (leading to the name altricial: nest dependent); however, within such a huddle, the pups keep a euthermic body temperature. In these animals, minor amounts of brown adipose tissue are present at birth, and the tissue is successively recruited during the first ~5 days after birth, after which the degree of recruitment slowly regresses. The recruitment process is induced, similarly to the case in adult animals, by the pups experiencing a cold environment. Thus, if born into a thermoneutral environment, the postnatal recruitment is completely inhibited (246, 538, 594, 595). Consequently, there is no reason to postulate any pathway different from that of adrenergic activation of the tissue leading acutely to thermogenesis and chronically to recruitment. There are no direct studies demonstrating the necessity of brown adipose tissue for postnatal survival in these pups, but the survival of UCP1-ablated mouse pups is improved at ambient temperatures higher than normal (unpublished observations). Thus brown adipose tissue may be essential for survival during the first weeks after birth in altricial newborns.

2. Immature newborns recruit brown adipose tissue with a delay

Newborns of a few species belong to the immature group. Characteristic for this group (which includes Syrian hamsters) is that the newborns are very poorly developed, in thermoregulatory, as well as in other, respects. Thermoregulation and brown adipose tissue recruitment occur simultaneously during the second week after birth, but not before (60, 190, 357, 572, 758, 777); until then, the pups behave as poikilotherms, i.e., they are unable to maintain a higher temperature than that of the surroundings. Although there is very good correlation between the acquisition of nonshivering thermogenesis and the recruitment of brown adipose tissue, this is not a strict demonstration that the nonshivering thermogenesis observed is entirely brown fat derived, but this is very likely. It is also clear that no brown adipose tissue recruitment takes place before the thermoregulatory centers have developed; before the second week, hamster pups do not even utilize behavioral means to thermoregulate. Thus the recruitment of brown adipose tissue probably occurs in

these animals in the same way as in altricial newborns (and in adults): that it is secondary to a continuous demand on the tissue from the thermoregulatory centers and does not occur ontogenically ("spontaneously"). However, experiments in which late postnatal development has been followed at high ambient temperatures have not as yet been performed in such "immature" newborns.

The "newborns" of marsupials also belong to this immature group, and not until the young start to leave the pouch would a need for thermogenesis arise. To the extent that brown adipose tissue exists in marsupials, it should become active at this age (460), but its presence is still not firmly demonstrated (352, 576, 674). A problem here may be that the only recognized characteristic of the tissue, UCP1, may be so weakly homologuous to eutherian UCP1 that normal immunological and nucleotide probes may fail to identify it. GDP binding was found in marsupial "brown fat" mitochondria (460). The absence of a response to injection of β_3-adrenergic agonists (576) is not decisive, in view of the absence of β_3-responsiveness of guinea pigs with functional brown adipose tissue (33) and the existence of functional brown adipose tissue in β_3-receptor-ablated animals (781) (see sect. II*A2*). Alternatively, the prolonged "pregnancy" in the pouch may be seen as a consequence of an evolutionary absence of brown adipose tissue, leading to a need for prolongation of thermally protected life.

3. Precocial newborns have recruited brown adipose tissue at birth

Scientifically the most challenging types of newborns belong to the precocial group. These newborns are, as the name implies, very developed at birth; lambs and calves belong to this group, but also guinea pigs. In these newborns, brown adipose tissue is highly recruited at birth and is activated immediately postnatally as evidenced by "unmasking" (643). If the newborns are not transferred to the cold (4, 81), the tissue successively atrophies after birth. The control of recruitment postnatally is thus understandable based on the general model of chronic adrenergic stimulation leading to brown adipose tissue recruitment (or inhibition of atrophy).

However, the prenatal recruitment presents a challenge to the understanding of the mechanism for brown adipose tissue recruitment in general, because the established, adrenergically governed, recruitment model does not seem to hold, mainly because it is very unlikely that the fetus is exposed to a high adrenergic stimulation prenatally. First, there is no reason that the thermoregulatory centers of the fetus (although probably sufficiently developed) should send any sympathetic signal to brown adipose tissue to induce thermogenesis; the temperature of the fetus in the womb is probably even higher than that programmed by these centers and is generally 0.5–1°C higher than in the mother (420). There is good reason to believe that the thermal load of gestation on the dam should not be further enhanced by a purposeless thermogenesis ongoing in the fetus, and there is also some evidence that circulating adenosine may constantly keep the thermogenesis down (36). However, adenosine functions via the G_i pathway leading to inhibition of adenylyl cyclase (see sect. II*C*) and with both proliferation and differentiation in brown adipose tissue being under positive cAMP control (see sect. III, *A* and *B*), an inhibition in this pathway of thermogenesis would necessarily also inhibit recruitment.

Until recently, no nonadrenergic mechanism for brown adipose tissue recruitment had been formulated, but the identification of the stimulation of differentiation via PPARγ2 (791) opens for the possibility that an endogenous activator of this pathway could recruit brown adipose tissue without adrenergic stimulation. What this activator is is not known; it can hardly be (the PPAGγ2 ligands) fatty acids released within the tissue, because this would again demand stimulation of lipolysis and thus also lead to stimulation of the thermogenic pathway. Whether such a recruitment pathway only works in this subgroup of newborns is not known, but that cells isolated from fetal rat brown adipose tissue differ with respect to signal transduction pathways from cells from neonatal rat (see sect. III) could indicate that the differences are more related to the relative timing of birth.

E. Fever, Hyperpyrexia, and Anapyrexia (Stress, Anesthesia, Thyroid Thermogenesis, Exercise)

Mammals, and certain other animal groups, give the impression that they "attempt" to defend a given body temperature. In a simple formulation of this, the "desired" body temperature is referred to as the "set-point," in analogy with what is found in household appliances. However, the idea of a set-point is a pragmatic formulation, to simplify interpretation of thermoregulatory phenomena. There is no set-point, it only appears so. Because the set-point does not exist, it is not "set" anywhere, in a given brain location, but the set-point behavior is the outcome of a series of feedback systems.

Thus, as illustrated in Figure 15*A*, most observations can be understood if a system with independent temperature control is envisaged. The distance between their origins is the central thermal tolerance, which is often only a fraction of a degree. The cold-defense and the heat-defense units are probably separate; their slopes and especially their origin can be independently regulated (i.e., the lower tolerated temperature may be lowered

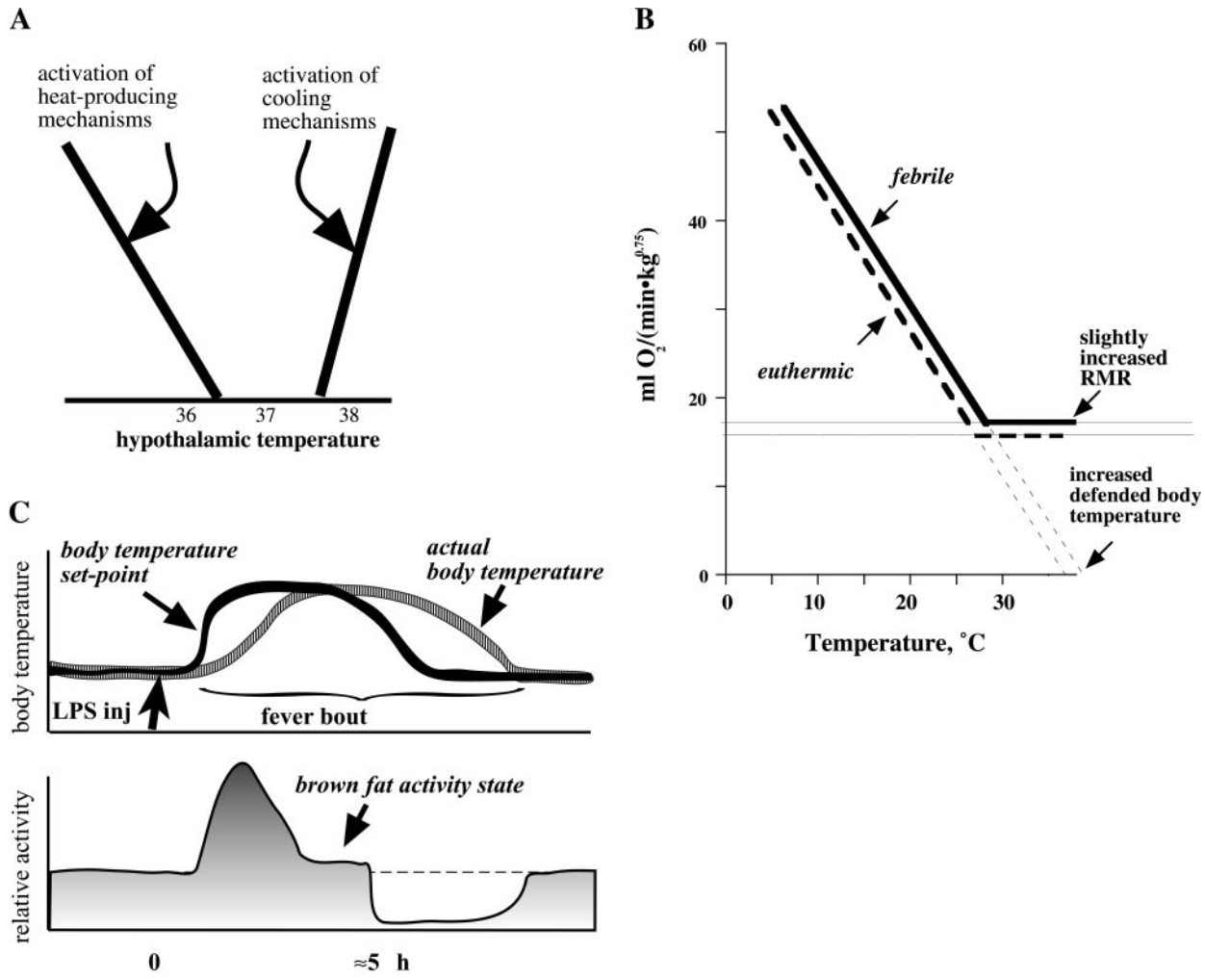

FIG. 15. Metabolic responses to fever. *A*: metabolic responses to deviation from hypothalamic "set-point" temperature. *B*: comparison between metabolism in euthermic and febrile animals at different environmental temperatures (cf. Fig. 12). RMR, resting metabolic rate. *C*: relationship between body temperature set-point, body temperature, and brown adipose tissue thermogenic activity during an experimental fever bout, here induced by lipopolysaccharide injection. Note how body temperature lags behind changes in set-point and that brown adipose tissue is only metabolically highly active during the rise in body temperature.

without the upper necessarily changing in parallel). However, they do often change in parallel (see also sect. V*G*).

The concepts of fever, hyperpyrexia, and hyperthermia, as well as of anapyrexia and hypothermia, are all based on a set-point scenario. According to this formulation, a "euthermic" set-point exists, referred to as "normal" body temperature. The set-point (and subsequently the defended body temperture) can be increased, which we refer to as a "fever" or as hyperpyrexia, or it can be lowered, which we refer to as anapyrexia. If the body temperature is higher than the running set-point, a hyperthermic situation is encountered: the organism will attempt to utilize diverse means to return to the running set-point (it will "defend" its set-point temperature); hypothermia is a similar situation in which the body temperature is lower than the set-point.

1. Classical experimental fevers

Experimentally, fevers are normally induced by injection of lipopolysaccharide (LPS) or other exogenous pyrogens, leading to a transient increase in set-point. As the body temperature is then lower than the new set-point, the organism will strive to increase its body temperature. After some time, only some hours, the set point will return to normal, the new body temperature is now hyperthermic, and the body will attempt to decrease its body temperature (Fig. 15*C*).

The amount of brown adipose tissue recruitment that can be expected for thermoregulatory purposes during a fever attack of this type is very marginal, for two reasons. As illustrated in the semi-principal diagram in Figure 15*B*, one is that the extra amount of heat that is needed to

defend a body temperature even 2°C higher than normal is relatively small, because an animal at normal ambient temperature (e.g., 18°C) is already 12°C below its thermoneutral zone. The movement of this zone upwards by 2°C will mathematically only lead to a 2/12 increase in heat demand; a recruitment of this magnitude is hardly discernable experimentally. The other reason is the transient nature of the event.

Thus, during an experimental (or real) fever, brown adipose tissue may be expected to be markedly activated in the brief transient phase during which the pyrexia is being attained, being only marginally activated during the maintenance of the fever as such, and then being even inactivated during the exit from the fever (Fig. 15*C*). Examination of brown adipose tissue involvement in fever is therefore very time-dependent during this type of experimental fevers. This may explain why certain authors report the "expected" result: an activation of brown adipose tissue by different measures (57, 301, 382, 787), including an increase in GDP binding (unmasking) (383), whereas others report an inactivation (decrease in UCP1 mRNA, Ref. 554).

The activation of brown adipose tissue occurs irrespectively of which of the steps in the pathway from LPS to an increased set-point that is experimentally activated, i.e., in addition to LPS, also interleukin (IL)-1 (applied systemically or intracerebroventricularly) (159), IL-6 intracerebroventricularly (93), and prostaglandin E_1 and E_2 intracerebroventricularly (11, 232, 521, 546) all activate the tissue.

In its attempt to increase body temperature to the new set-point, the animal will use all means available. This means that the participation of brown fat-derived heat is only one of several options. The optional nature of brown adipose tissue in this respect is well demonstrated by the observation that if nonshivering thermogenesis is inhibited by propranolol during a fever bout, the animal will instead start to shiver (58). Thus brown adipose tissue activity is optional for fever onset; it is probably the priority choice (after vasoconstriction to decrease heat loss), but the necessary thermogenesis will be obtained from other sources (i.e., shivering) if the brown fat-derived heat is not sufficient.

In certain disease states, there is constant elevation of body temperature and a small but now chronic demand for heat, and it is thus more likely that signs of recruitment could occur in such states. Malaria infection leads to such prolonged body temperature increases, and the effects of chronic hypothalamic expression of the fever mediator IL-6 are probably of a similar nature (441). In these states, there are measurable indications of brown adipose tissue recruitment (146, 441). However, as pointed out above, the expected increase is rather small, and the observations of recruitment (of ~20%) exceed that needed. The observed recruitment can therefore probably not be explained in this way; a possibility is that it is coincidental, due here to a generalized stress state leading to a general activation of the sympathetic nervous system, which thus results in recruitment. As would be predicted, there is also a decreased metabolic efficiency in such states (notably compared with pair-fed controls) (146).

A) IS BROWN ADIPOSE TISSUE MORE THAN AN EFFECTOR OF FEVER-INDUCED THERMOGENESIS? In addition to being an effector of the thermogenesis needed to induce hyperpyrexia during a fever bout, there are some indications that brown adipose tissue may also influence the magnitude of the bout. Thus, under conditions in which brown adipose tissue is atrophied (e.g., in genetic obesity), the response to a given dose of, e.g., LPS is sometimes (158, 675) but not always (370) lower than normal.

Because brown fat-derived heat is only optional for fever, the amount of heat the tissue can produce should theoretically not influence the magnitude of the fever. One explanation for the phenomenon would therefore be that brown adipose tissue, in addition to responding thermogenically, also influences the set-point setting. There are indications that this could be the case (91, 92, 100). Brown adipocytes contain high levels of the mRNA for the pyrogenic cytokines IL-1 and IL-6, and the levels are markedly increased by LPS, IL-1β, and TNF-α (i.e., the brown adipocytes in this respect function similarly to a leukocyte). It is possible that the production of these cytokines by brown adipose tissue could contribute to their systemic levels, and in this way increase the set-point. However, these pyrogenic substances are also released from white adipose tissue, and the relative contribution of brown adipose tissue is unknown.

More specifically for the tissue, the interleukin gene expression is also stimulated by norepinephrine (91, 92), opening for the possibility that a positive loop could exist in which the increased set-point would activate brown adipose tissue which would increase its release of interleukins, further enhancing the fever. Experiments in UCP1-ablated and brown fat-deficient mice could clarify this point.

B) OTHER TYPES OF "FEVER" (HYPERPYREXIA). A broad definition of fever is any state characterized by a defended increased body temperature; "increased" then means versus some rather undefined "normal" state. Because the word *fever* is so intimately associated with illness, we will use the term *hyperpyrexia* to indicate other conditions with an increased set-point.

A series of acute or chronic conditions are associated with an increased body temperature and an increased oxygen consumption. From a regulatory point of view, one of the questions is the cause-effect relationship: is the increase in body temperature a hyperthermia (due to an acutely increased but unwanted heat production from, e.g., brown adipose tissue) or a hyperpyrexia (which

would then lead to brown adipose tissue activation and increased oxygen consumption)? Hyperpyrexia may be discussed (below) in the analysis of a series of states of altered metabolism, such as stress, hyperthyroidism, and certain effects of food. In a hyperpyrexic state, the prediction would be that brown adipose tissue should become acutely activated during entry into the hyperpyrexic state, but if the heat-producing capacity of the brown adipose tissue is not sufficient, any other means will be utilized (notably shivering) to reach the new set-point. Brown adipose tissue involvement will thus be only optional. As in classical fevers, during the hyperpyrexia, a slight increase in the degree of recruitment may be observed, and the tissue will be inactivated during the return to a euthermic state (or during the entry into an anapyrexic state).

One criterion to distinguish between hyperpyrexia and a direct brown fat-dependent hyperthermic effect is thus that the magnitude of the hyperpyrexia/fever (and thus probably the increase in oxygen consumption, which is often the parameter measured) is not proportional to the heat-producing capacity of brown adipose tissue; if the pyrexia is higher in, e.g., cold-acclimated than in normal animals, this cannot be explained simply in terms of hyperpyrexia.

2. Stress fevers: do they represent hyperpyrexia or hyperthermia?

Stress "fever" (141) is the term used to describe the increase in body temperature under conditions that can be understood only to affect the animal psychologically. In practical terms, one criterion is therefore that it does not occur in anesthetized animals.

In conscious animals, even an injection of saline leads to an increase in body temperature (e.g., Refs. 775, 856) and an increased metabolism (e.g., Ref. 262). This response is the typical "stress fever," and brown adipose tissue is activated. The stress reaction may be controlled from the dorsomedial hypothalamic area (895a). Experimentally, this response complicates analysis of the effect of injection of any substance into conscious animals, and there is no simple way to perform the experiment: the routine experimental design, i.e., to compare the effect of the injected agent with that of injected saline, easily leads to false negatives: because the saline injection itself leads to a large brown fat activation, a true effect of the agent studied may be overshadowed by the saline effect.

It is doubtful that the response to saline injection qualifies (solely) as a fever (hyperpyrexia), because the magnitude of the thermogenic response to a saline injection is influenced by the recruitment state of the brown adipose tissue and by the presence or absence of UCP1 (262). The response seems thus to include an effect of a generalized sympathetic stimulation, where all tissues metabolically responsive to adrenergic stimulation respond without any differentiated central control (a type of W. B. Cannon's "sympathetic" response). Thus brown adipose tissue heat production is additional to the hyperthermia. It cannot be excluded that this stress hyperthermia is some of the explanation for the positive correlation between brown adipose tissue recruitment and experimental fevers discussed in section v*E1*.

Another psychological stress giving elevated body temperature results from immobilization. Also during this stress, the thermogenesis is brown fat-derived and is eliminated by severing the nerves to brown adipose tissue or by sympathectomy (539, 725, 726, 883), and repeated or chronic immobilization even has a recruiting effect on brown adipose tissue (235a, 416, 590), as expected from chronic adrenergic stimulation. Again, brown adipose tissue heat production is additional to the hyperthermia.

A complication in the analysis of the participation of brown adipose tissue in stress hyperthermia is the possibility of a dual effect of propranolol. An inhibitory effect of propranolol is often understood as demonstrating brown adipose tissue involvement due to the antagonist action of (high doses of) propranolol on the β_3-adrenoceptors of the brown adipocytes. However, propranolol may also be antipyretic in a brown fat-independent way: in so-called open-field fevers (hyperpyrexias), propranolol (and other β-blockers) prevents the stress-induced rise in body temperature (493) by acting through central β_1/β_2-adrenoreceptors. Thus a demonstration that propranolol can diminish a stress type of fever/hyperthermia does not necessarily indicate that the hyperthermia is brown fat-dependent; it may be that the set-point is restored.

3. Anesthestic hypothermia

General anesthestics lower the lower threshold temperature for central control of body temperature. Thus, during general anesthesia, normal body temperature will not be defended but will successively decrease (until it reaches the new, much lower threshold temperature). Brown adipose tissue will thus not be activated in spite of the decrease in body temperature; rather, deactivation of brown adipose tissue should be observable (but this has not been demonstrated directly). However, during arousal from anesthesia, when the set-point returns to normal, the body temperature is now below the set-point, and extra heat production is initiated to regain normal euthermia. This situation is similar to that occurring during the initiation of fever; all available thermoeffector processes will be called into action, including nonshivering thermogenesis from brown adipose tissue. In accordance with this, brown adipose tissue is activated during arousal from anesthesia (737). This activation is thus probably an optional contribution; often shivering is (also) encountered during the arousal phase.

In addition to this centrally mediated effect of general anesthesia, there is also an inhibitory effect of certain anesthetics directly on brown adipocytes. All "inhalation anesthetics," i.e., halothane and the analogs isoflurane and enflurane, as well as the classical inhalation anesthetics diethyl ether and chloroform, have direct inhibitory effects on norepinephrine-induced thermogenesis in brown adipocytes (but they do not influence their basal rate of respiration) (599, 600) and accordingly also on norepinephrine-induced thermogenesis in intact animals (179). This inhibition occurs in the range of drug concentrations used for anesthesia in animals and humans. This means that during anesthesia with these agents, an animal is unable to activate nonshivering thermogenesis even when the lower threshold temperature has been reached. Consequently, body temperature may fall below even the lowered set-point. The mechanism for this direct inhibition of brown adipose tissue thermogenesis by inhalation anesthesics involves an inhibition of adenylyl cyclase which is reinforced by an inhibition of mitochondrial oxidation. The effects of inhalation anesthetics on brown adipose tissue are thus opposite to the much studied halothane effects on muscle mitochondria which lead to "malignant hyperthermia" in susceptible human and pig individuals. In malignant hyperthermia, brown adipose tissue thermogenesis is not involved.

Noninhalation anesthetics, such as barbiturates and urethane (599), are devoid of any direct inhibitory effect on brown adipose tissue thermogensis. Serendipitously, most experiments in anesthetized animals have been performed with pentobarbital sodium and similar agents as the preferred anesthetic agent, and there is therefore no reason to reevaluate the outcome of most of these experiments.

4. Thyroid thermogenesis

Chronic treatment of animals with thyroid hormone leads to an increased heat production, normally referred to as thyroid thermogenesis. Hypothyroidism has the opposite effect, and, conceivably, in euthyroid animals, total heat production (metabolism) therefore includes a "thyroid thermogenesis" component. The nature of this thermogenesis is basically unknown, and the significance of any contribution from UCP1-mediated brown adipose tissue thermogenesis is consequently difficult to delineate.

There are both central and peripheral effects of hyperthyroidism that may interrelate with brown adipose tissue, and thyroid hormone may also have direct effects on brown adipose tissue. Centrally, hyperthyroidism leads to hyperpyrexia, i.e., to a defended increase in body temperature of 1–2°C (100, 564, 786). This increased body temperature in hyperthyroidism is often referred to as a hyperthyroid hyperthermia, but this term is misleading, as the new body temperature is defended even at low ambient temperatures (786). In itself, this hyperpyrexia, as all hyperpyrexias (see sect. v*E2*), should lead to some recruitment and activation of brown adipose tissue; brown fat-derived heat would be optional for the process, and several (although not all, see below) reports do indicate recruitment of brown adipose tissue in hyperthyroidism (49, 364, 690, 778, 871).

Hyperthyroidism probably also has peripheral effects associated with an increased basal metabolic rate. This increased metabolism is observed even at thermoneutral temperatures where the increase cannot be due to a need for extra heat to defend the increased body temperature. The increase is perhaps larger than would be expected from passive "Q_{10}" effects (which would correspond to maximally ~10% increase in metabolism per degree C increase in body temperature), but the difference in body temperature between hypothyroid and hyperthyroid animals is some 4–5°C which means that Q_{10} effects could explain a substantial part of the difference in metabolic rate and this would not in itself demand any further molecular explanation. To the extent that additional mechanisms are involved, their molecular basis is unknown. There are many reports of elevated enzyme contents in diverse organs due to hyperthyrodism, and these enzyme changes are sometimes formulated as "explanations" for this peripheral thermogenesis. This cannot, however, be the case; an increased content of, e.g., respiratory enzymes cannot in itself lead to an increased energy utilization. Rather, the increased contents may indirectly be due to an increased demand for substrate during thermogenesis. They may even be due to an increased gene expression of such enzymes as a direct effect of thyroid hormone, but this must be interpreted as a coordinated response to thyroid hormone, leading to both increased substrate utilization and substrate delivery. Only an increased activity of an energy-utilizing process (or processes) can account for increased peripheral thermogenesis. An increased proton conductance in, e.g., liver mitochondria from hyperthyroid animals has been observed (292), but the molecular basis for this has not been established. A suggestion that thyroid thermogenesis could be due to UCP3 activation in diverse tissues (164, 266) has been refuted, both because UCP3 is not expressed in all tissues showing thyroid thermogenesis (notably not in liver, Refs. 427, 796) and especially because UCP3-ablated mice demonstrate thyroid thermogenesis equally well as do wild type (267). However, irrespective of the molecular mechanism, the presence of thyroid thermogenesis in any or all peripheral tissues would lead to a smaller need for UCP1-dependent brown fat-derived heat. Brown adipose tissue should thus be deactivated, leading to atrophy. There are reports indicating this to be the case (2, 300, 312, 662, 707, 776, 820). Thus, based on the central versus peripheral effects of hyperthyroidism, recruitment-promoting versus atrophying effects of hy-

perthyroidism are explainable and observable. Presently, it can only be suggested that other factors must be decisive for whether recruitment or atrophy is observed.

In addition to these effects, it is possible that hyperthyroidism has direct recruiting effects on brown adipocytes; response elements for thyroid hormone receptors are found, e.g., in the UCP1 promoter (see sect. III*B3*). However, a recruiting effect of simulated hyperthyroidism (versus euthyrodism) has not been directly shown in a brown adipocyte culture system. Although most physiological conditions investigated correspond to situations where thyroid hormone is only permissive and the degree of adrenergic stimulation governs the degree of recruitment, conditions have been described in which recruitment state (UCP1 mRNA levels) correlates with thyroid hormone level (73). Because thyroid hormone does not in itself induce thermogenesis in brown adipocytes, such conditions do not automatically correspond to an enhanced thermogenesis from brown adipose tissue.

The experimental hypothyroid state is principally a mirror image of the hyperthyroid state, but even more complex. Hypothyroidism is associated with a lowered body temperature set-point (anapyrexia) (180, 271), which would lead to a decreased demand for brown fat-derived heat, but it is also associated with a lowered peripheral heat production. The latter should lead to brown adipose tissue activation and recruitment, and there are some indications of this (180, 536). However, the increases in total UCP1 amount are smaller than expected. Brown adipocytes freshly isolated from hypothyroid animals are adrenergically desensitized (656, 778). This has been suggested to be a direct effect of lack of thyroid hormone, but it is more likely that it is a secondary effect of the increased sympathetic stimulation occurring in all tissues during hypothyroidism (891); such a chronic stimulation leads to an adrenergic desensitization even in cells from cold-acclimated animals (553, 783, 827). There are no reports that "simulated" hypothyroid brown adipocyte cultures are adrenergically desensitized. The inability of hypothyroid animals to survive in the cold has been ascribed to their poorly recruited brown adipose tissue, but as UCP1-ablated mice can survive in the cold for prolonged periods (260), it is not certain that it is the lack of brown fat-derived heat that is detrimental for hypothyroid animals in the cold. Rather, hypothyroidism is also associated with muscular weakness (384, 494, 495, 866, 896). A lack of muscular shivering endurance may thus be the cause of hypothyroid cold-hypersensitivity.

In animals without hormone-binding nuclear thyroid hormone receptors, there is also a decrease in defended body temperature (an anapyrexia), but even at thermoneutral temperatures, the brown adipose tissue is in a recruited state (259), including a high level of UCP1 (UCP1 is expressed even in the absence of thyroid hormone receptors; see sect. III*B3*). Whether the tissue is metabolically active in thermoneutral conditions is not known. Although this would seem unlikely because of the low basal metabolism observed in these animals, a continuous brown adipose tissue activity would seem unavoidable if the brown adipose tissue recruitment seen is indeed due to a general sympathetic stimulation. Even such thyroid hormone receptor-ablated mice can adapt to moderate cold, and brown adipose tissue becomes recruited just as in wild-type animals, and it would seem to be active in the cold (259). This is understandable if, in the absence of ligand (triiodothyronine, T_3), the thyroid hormone receptors act as repressors of UCP1 gene expression, a repression that is relieved physiologically by the presence of T_3 or experimentally by the elimination of the thyroid hormone receptors (639) (see sect. III*B3*; concerning brown adipose tissue as a producer of systemic T_3, see sect. VIII*C3*).

5. Exercise counteracts brown adipose tissue thermogenesis

Exercise is in itself a heat-generating process. There should therefore be a decreased necessity for brown fat-derived heat in animals during training bouts, and it is thus understandable that brown adipose tissue is hypoactive during the training bouts themselves and shortly thereafter, as observed by e.g., lower UCP1 mRNA level (718). There is therefore no reason to speculate about specifically controlled brown adipose tissue hypoactivity during exercise; it would suffice with normal body temperature control. This effect of exercise is transient, and the exact time elapsed between the end of training and the measurement of brown fat-related parameters may determine whether or not an effect is observed.

Accordingly, the question as to whether training has an effect on the recruitment state of brown adipose tissue is probably mainly a quantitative one. Training bouts normally last only a few hours per day. As experimental animals normally live under thermal conditions that recruit brown adipose tissue during most hours of the day, it would basically be surprising to observe atrophying effects of training, simply for this reason. Atrophying effects of training would also involve a time delay (of greater than a week), as is the case for acclimation to a different environmental temperature. Thus only studies of sustained (but not necessarily intensive) exercise for several weeks could be expected to demonstrate any atrophying effects, but even under such conditions no atrophying effects have been observed (712, 718).

In accordance with this, forced training simultaneously with intermittent cold exposure eliminates the recruiting effect of intermittent cold exposure (26, 298); the animal does not require extra heat, and consequently, brown adipose tissue is neither activated nor recruited.

Exercise through forced swimming demands a spe-

cial note. There are a series of observations that swimming training activates and recruits brown adipose tissue (597, 825). However, even when the animals swim in rather warm water (as high as 35–36°C), they lose body heat, with body temperatures decreasing several degrees (597). Even when body temperature is maintained, a cold stress may well be present, but the animals use thermogenesis (including brown adipose tissue) to counteract the heat loss (cf. the situation in normal cold). There is, however, no simple way to make the "correct" experiment, because increasing the water temperature to that of the body will instead cause a heat load on the animal. Thus a conclusion can only be reached by inference, which would be that swimming in itself, just as other types of exercise, has an atrophying effect, but that, due to the high thermal conductivity of water, there is a heat loss that can result in an apparent recruiting effect of swimming, but this is then not different from the normal recruiting effect of cold (298).

F. Hibernation and Arousal

Brown adipose tissue was originally observed in hibernators (244) and was early referred to as "the hibernation gland" (646). The physiological function of brown adipose tissue is of interest during all four phases of hibernation: prehibernation fattening, entry into hibernation, during each hibernation bout, and during arousal from hibernation (Fig. 16).

1. Prehibernation fattening

In most hibernators (except those which cache food), energy is stored in the body in the form of fat prior to the start of the hibernation season. This prehibernation fattening is an interesting physiological phenomenon in several respects, with a scientific potential still not greatly explored. It is a physiologically induced obesity (414), and as obesity in itself activates brown adipose tissue (see sect. VI*D*), either "adipostat" signals (≈leptin) must be decreased or "leptin resistance" must be physiologically induced. It is a period of hyperphagia, and it is often assumed that hyperphagia should in itself lead to brown adipose tissue recruitment. This would then counteract an accumulation of lipid for hibernation, but it is doubtful (see sect. VI*B8*) that a true hyperphagia-induced activation of brown adipose tissue exists. During the fattening phase, there is nonetheless a recruitment of brown adipose tissue. In nature, the prehibernation phase coincides both with decreasing ambient temperatures and especially with shorter day length (see sect. V*F5*), which may be the cause of this recruitment, but dedicated experiments to evaluate the effects of the different factors have not been performed; thus it is not known whether prehibernation brown adipose tissue recruitment presents a regulatory problem in itself. It has been discussed that the recruitment should occur through non-norepinephrine pathways (173). A decision to enter into hibernation always needs some preacclimation time, and it is possible that hibernation cannot be entered into if the recruitment state of brown adipose tissue is not sufficiently high; how this is evaluated by the animal is not known.

2. Entry into hibernation

Entry into hibernation must include a decision to cease heat production in, e.g., brown adipose tissue (Fig. 16). A drop in body temperature set-point to ~5°C would

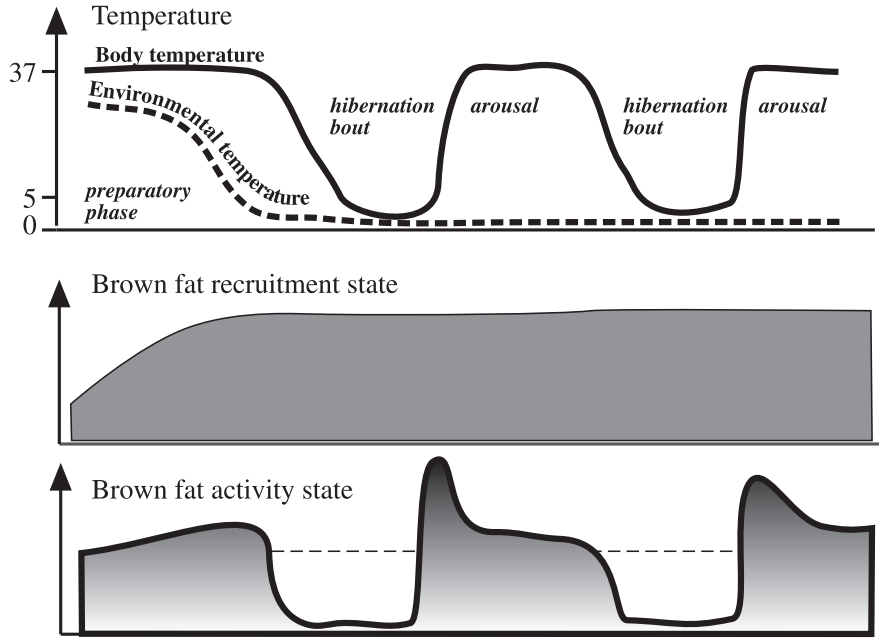

FIG. 16. Hibernation-induced changes in body temperature and in brown adipose tissue recruitment and activity state. The preparatory phase may last for months, whereas each hibernation bout plus arousal cycle will last for about a week, dependent on environmental temperature, etc.

be sufficient to inactivate brown adipose tissue heat production, and thus the cessation of brown fat activity is not a regulatory problem in itself. How this drop in set-point is accomplished is outside the scope of this review and is indeed outside our present understanding.

3. During deep hibernation

During deep hibernation, brown adipose tissue is inactive, as witnessed by, e.g., masked [^{3}H]GDP binding (353, 454, 486, 587) and altered mitochondrial ultrastructure (285, 360). This inactivity is probably mainly coincidental and does not require a specific regulatory mechanism. Because body temperature regulation is not turned off during hibernation but the set-point is simply at a very low setting (~5°C), and because most experimental (and probably natural) hibernacula have a temperature in this range, no extra heat is necessary to maintain the set body temperature. If, however, environmental temperature is further decreased, to 0°C or below, the hibernator will defend its body temperature set-point (316), and this should lead to an activation of thermogenesis in brown adipose tissue, even during deep hibernation (although this has not been directly shown).

4. Arousal depends on brown adipose tissue thermogenesis

In hibernation, it is during arousal that brown adipose tissue plays its main physiological role. Hibernators can rewarm to euthermia even though the ambient temperature remains low. Activation of brown adipose tissue during this phase is evidenced by unmasking (353, 454, 486, 587) and depletion of lipid stores (e.g., Ref. 558) and especially by a large increase in brown adipose tissue temperature; the temperature during arousal may exceed rectal temperature by up to 14°C (752, 753). Again, the control mechanism can be understood as a resetting of body temperature set-point to ~37°C. During the arousal phase, the hibernator will use all available thermogenic mechanisms to reach this temperature. At low body temperatures, shivering can apparently not take place, but when body temperature reaches ~16°C, Syrian hamsters start to shiver intensely; bats can arouse without any contribution from shivering (309). Heat production in brown adipocytes is temperature sensitive, as is principally any chemical process, with a Q_{10} of 2–3 (562). This means that at hibernating temperatures, heat production in the brown adipocyte is ~30-fold lower than at 37°C, and it is surprising that this heat production will eventually be able to rewarm the entire animal against a temperature gradient. Initially, the heat production is probably a local self-reinforcing process, where the tissue mainly heats itself. Because shivering cannot occur in the hibernating hibernator, brown fat-derived heat is essential for arousal from hibernation in mammals.

5. Daily torpor

Especially under conditions of food restriction, several mammalian species exhibit daily torpor: a substantial decrease in body temperature during the resting phase of the day. Because ambient temperature in these types of experiments is often at or above 20°C, the body temperature approaches this temperature but can be lower in lower ambient temperatures. It is unlikely that this particular temperature is regulated; rather, it is simply a reflection of that of the environment. A lowest tolerated body temperature of ~15°C has been arbitrarily defined for torpor (361). Torpor has mainly been observed in small rodents: normal mice, Siberian hamsters, deer mice (Peromyscus) but also in small lemurs. Except for the time frame and more shallow temperature decrease, the phenomenon is not markedly different from hibernation and thus includes periods of entry, torpor, and arousal. During arousal, brown adipose tissue is activated, and the tissue in these animals is recruited (327). Thus food restriction in these animals has a recruiting effect on brown adipose tissue, whereas it leads to atrophy in nontorporing animals (see sect. VI*A2*). An explanation is probably that the repetitive daily activation of brown adipose tissue during arousal has a recruiting effect.

Even mice that genetically lack adipose tissue, including brown adipose tissue (519), enter daily torpor and recover spontaneously from this (239), indicating that brown fat-derived heat is not necessary (is optional) for the torpor-arousal process. (Because body temperature is not extremely low, such animals are capable of coordinated shivering, which can adequately elevate body temperature.)

5. Photoperiod: how does it lead to recruitment?

A short photoperiod has in itself a recruiting effect on brown adipose tissue, independent of the effect of cold (315, 644, 863). In nature, a short photoperiod is an anticipation of winter, so a recruitment effect makes physiological sense, but the phenomenon is not well studied. Traditionally, studies have been performed nearly exclusively in Syrian hamsters (that are hibernators) and Siberian hamsters (that show daily torpor and large photoperiod effects on many phenotypic traits); rats (and mice) seem to show only modest response to photoperiod (424).

Responses to short photoperiod are expected to be mediated by melatonin released from the pineal gland during the dark phase and can therefore be mimicked by melatonin treatment of animals kept in long-day conditions. It would therefore be expected that pinealectomy would make the animals insensitive to short photoperiod, but surprisingly, this may not be the case (43) (although, unfortunately, only effects on brown adipose tissue wet weight were reported). The mechanism of melatonin-induced recruitment is not known. It could be central and

thus associated with increased sympathetic stimulation and decreased metabolic efficiency. Alternatively, it could be a direct effect on brown adipocytes. Melatonin receptors have been described in the tissue, and they may have a direct effect on gene expression (84, 435a, 628). However, there is no stimulatory effect of melatonin on, e.g., cAMP levels, and no indication of melatonin-induced thermogenesis (84, 435a).

G. The Central Regulation of Thermoregulatory Thermogenesis and the Innervation of Brown Adipose Tissue

It is a prevailing concept in this review that sympathetic stimulation is of paramount significance for brown adipose tissue; it is probably the only acute route of activation of physiological importance, and most (but not all) incidences of recruitment of the tissue are caused by prolonged sympathetic stimulation.

The neuronal pathway leading to the sympathetic stimulation may be examined by both anterograde and retrograde investigations. Retrograde studies (that are inherently only anatomical) have been made by examining the transport of neurotropic viruses (pseudorabies virus) through synaptically connected neurons from brown adipose tissue towards the brain (37, 41, 602). Although these studies would appear superior in actually tracing the innervation, a problem, discussed by the above authors, is that the parts of the central nervous system identified are remarkably similar, irrespective of which sympathetic target organ is being examined (see also Refs. 767, 784), thus reflecting more a general sympathetic stimulation of the W. B. Cannon type, rather than the specific selective innervation of a given tissue.

In contrast, anterograde studies are generally functional and consist of manipulations (electrical or chemical stimulation, inhibition or destruction) of nuclei or nerve tracts supposedly involved in the pathway. Coherent pictures are difficult to assemble. Numerous single studies exist, showing effects of electrical stimulation of certain areas of the central nervous system, of their destruction or inhibition, and of injection of substances especially into the third ventricle, but many of these reports are unconfirmed and have not been placed in a mediatory context.

We concentrate here on the thermoregulatory pathway (Fig. 17): its incorporation into the metaboloregulatory system is discussed in section VI*E* (for earlier re-

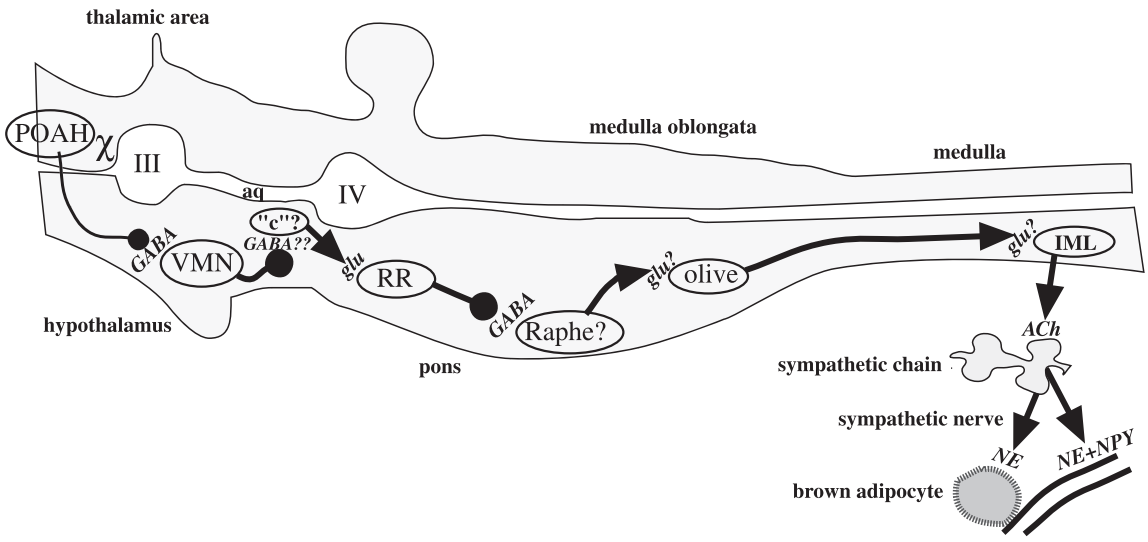

FIG. 17. The central pathway for control of thermoregulatory thermogenesis. In the scheme, a very hypothetical single route of mediation is delineated. However, a single route is basically incongruent with the central nervous system concept, which is characterized not by single pathways but by interactions at all levels; the pathway described should therefore only be seen as a simplistic (and preliminary) representation of the regulatory network involved in the neural control of brown adipose tissue activity. The pathway indicated starts from the POAH [the preoptic chiasma (χ)/anterior hypothalamus area], but the POAH probably receives inputs from the suprachiasmatic nucleus concerning daily rhythm (and thus alterations in body temperature set-point), as well as pyrogenic information from the blood (not shown). The signal from the POAH, which is inhibitory (lines with solid circles) and mediated by the transmittor GABA arrives to the ventromedial nucleus (VMN) below the third ventricle. To draw a coherent picture, it is necessary to introduce an unidentified coupling ("c") that alters the signal to a stimulatory one (lines with arrows) that passes through the periaquaductal gray close to the cerebral aquaduct (aq) and releases the stimulatory transmittor glutamate (glu) at the retrorubral field (RR) from which the signal may pass the raphe nucleus and the olive nucleus on its way to the intermediolateral neurons (IML). The preganglionic nerves release acetylcholine (ACh) in the sympathetic chain, and the sympathetic nerves release norepinephrine (NE) and neuropeptide Y (NPY) at the vasculature of brown adipose tissue and norepinephrine to the brown adipocytes themselves, thus stimulating thermogenesis.

views, see Refs. 304, 333, 338). Our present understanding that all nonshivering thermogenesis emanates from brown adipose tissue (see sect. v, *B* and *C*) means that classical studies in which the end point parameter was "nonshivering thermogenesis" can today adequately be considered examinations of activation of brown adipose tissue, and some of these studies are included here. The pathway outlined must be considered very tentative and open for revision.

1. The temperature control area in the preoptic chiasma/anterior hypothalamic nuclei

An area within the preoptic chiasma/anterior hypothalamic nuclei (POAH), in front of the third ventricle, is accepted as being the center for body temperature control (70); its destruction makes animals unable to thermoregulate (706). Cooling of this area also activates brown adipose tissue (365, 366), whereas warming suppresses activation of brown adipose tissue and nonshivering thermogenesis (130, 229).

The cells responsible for activation are spontaneously firing thermosensitive neurons, the firing frequency of which decreases (warm sensitive) or increases (cold sensitive) as a function of a temperature decrease within the POAH. Very small alterations in temperature elicit large alterations in firing frequency, much more than expected from simple Q_{10} effects. The existence of true cold-sensitive neurons has been doubted; their behavior can alternatively be explained as being due to an inhibitory effect of the warm-sensitive neurons on temperature-sensitive neurons (543).

The relative activity of these temperature-sensitive neurons is influenced by classical fevers (70, 546), and because prostaglandins are involved in mediation of the febrile response, prostaglandin E_2 injection into the POAH (11), as well as intracerebroventricular injection of prostaglandin E_1, activates brown adipose tissue (524). It is likely that nonclassical fevers (hyper- and anapyrexias) (see sect. v*E*) also affect this area, but very little has been demonstrated in this respect.

The POAH also receives inputs from thermosensitive areas elsewhere in the body, and this information is integrated in an unknown way into the final response. It is, e.g., possible to activate brown adipose tissue even in a euthermic animal by cooling the scrotum (442); the exact input pathway is not defined, but could be in the POAH, with the effects evidently being manifest at all descending levels (442).

Electric stimulation of the POAH activates brown adipose tissue (347, 804), but stimulation of POAH with homocysteic acid (that functions as a general chemical activator) decreases activation of brown adipose tissue (130), and inhibition of POAH with local anesthetics stimulates the tissue (366). However, as the outgoing signal from the POAH is integrated from both cold- and warm-sensitive cells, the outcome of such manipulations can be difficult to interpret. More importantly, cutting the connection from the POAH to posterior areas elicits brown fat activation (130). It would thus seem that the thermoregulatory output from the POAH is an inhibitory one; i.e., there is a high outgoing signal at thermoneutral temperatures that is decreased in the cold. The outgoing, inhibitory, nerves are probably GABA releasing, with terminals in the ventromedial hypothalamic nucleus.

2. The ventromedial hypothalamic nucleus

The thermoregulatory signal is probably further mediated by or at least through the ventromedial hypothalamus. The ventromedial hypothalamic area has classically been considered to be associated with feeding control (the "satiety center"). However, detailed analyses have indicated that manipulations (destructions) of this area may have two distinct consequences: both a break in the signaling fibers leading from the arcuate nucleus to the paraventricular nucleus, thus obstructing signaling leading to satiety, with hyperphagic obesity as a consequence, and destruction of the ventromedial hypothalamic nucleus (VMN), which is truely involved in control of metabolism (612). Although many classical studies have broadly addressed the entire ventromedial hypothalamus and thus may have induced dual effects, we think it is possible to ascribe the data mentioned in the following to the effects on the VMN and will here interpret such studies accordingly and will for simplicity refer to the affected area as the VMN. The VMN is not an area markedly identified by retrograde nerve labeling from brown adipose tissue (37, 41, 106a, 602), but as pointed out by some of these authors, it may be so that the few labeled cells may be sufficient to control the thermogenic system. Doubts that the VMN may regulate brown adipose tissue at all have, however, also been expressed (895a).

Electrical stimulation of the ventromedial hypothalamus, in reality probably of the VMN, activates brown adipose tissue (130, 176, 293, 347, 348, 351, 371, 619, 700, 794a, 805, 806, 869). The response observed may be biphasic, i.e., with an initial inactivation of brown adipose tissue (observed as decreased heat production), probably due to vasomotor effects in the tissue (869, 870), followed by an activation. The ability to induce activation of the tissue upon electrical stimulation is specific for the ventromedial hypothalamic nuclei, compared with the lateral hypothalamic nuclei. Stimulation of the VMN leads specifically to nonshivering thermogenesis (brown adipose tissue activation) compared with shivering (808); shivering is controlled via the posterior hypothalamic nucleus (808).

That the VMN is not only able to stimulate brown adipose tissue but is also directly involved in mediation of

thermoregulatory thermogenesis is demonstrated by a series of observations that destruction of the VMN (577) or application of local anesthetics to this area (365, 366, 807) abolishes the ability of external cold or POAH cooling to stimulate brown adipose tissue. [Some of the notable exceptions to this scenario (342, 546, 578, 627, 672) may perhaps be partly explainable by the chemical lesion (in gold thioglucose-treated animals) only affecting certain cells in the nucleus, and/or that alternative pathways may develop with time after lesioning.]

In addition to the "cold" signals from the POAH, other inputs from the POAH [e.g., prostaglandin E_1 (523, 524) or E_2 (11) stimulation in fever-mimicking experiments] also lose their ability to activate brown adipose tissue when the VMN is generally lesioned, has its cell bodies destroyed, or is inhibited by local anesthetics. There are also incoming direct or indirect signals from the circadian rhythm regulator, the suprachiasmatic nucleus, the effects of which on brown adipose tissue are inhibited by application of local anesthetics to the VMN (13), but this input may really be mediated via the POAH. That the input from the POAH is inhibitory is confirmed by the observation that a GABA agonist (muscimol) applied to the VMN abolishes the prostaglandin-induced brown adipose tissue activation (11) (i.e., muscimol mimics the normal chronic inhibition).

The stimulatory areas are found in specifically distributed anatomical areas within the nucleus (393), and individual neurons may be classified [based on their response to peripheral (scrotal) heating/cooling] as cold sensitive or warm sensitive, but this does not reflect a property of the neurons themselves but only their linkage to sensory inputs (443).

In addition to the thermoregulatory inhibitory input from the POAH, there are also stimulatory inputs to the VMN, the origins of which are not clear. Thus glutamate injection into the VMN activates brown adipose tissue (10, 12, 293, 887), and calcitonin-gene related peptide (CGRP) can also act here, possibly through CGRP1 receptors (402, 403).

Thus, in thermoneutral conditions, the VMN cells are constantly inhibited by GABA released from the nerves from the POAH; in the cold, these nerves become less active and therefore the VMN becomes more active.

3. The inhibitory center in the lower midbrain

The signal from the VMN probably passes through the periaquaductal gray (129) and is further mediated via an inhibitory area in the midbrain. For logical reasons, it is necessary to postulate an extra coupling area between the VMN and this inhibitory area ("c" in Fig. 17). This hypothetical "c" area is active at thermoneutrality and stimulates the inhibitory center but becomes inhibited by the nerves from the VMN in the cold. The output from "c" stimulates the midbrain inhibitory area. The presence of this inhibitory area is evident from studies in which this area is either inhibited by local anesthetics, or the descending nerves from this area are cut; such treatments lead to a spontaneous stimulation of brown adipose tissue (48, 728, 729, 829, 830). Accordingly, electrical stimulation of this area leads to inactivation of brown adipose tissue (303, 305). The exact nuclei responsible for the phenomenon have not been identified, but the area involved is the retrorubral field and the rubrospinal tract. The inhibitory effect can be induced by glutamate (730, 731, 829). It is thus likely that the input from the hypothetical "c" area is via stimulatory glutamate neurons. Thus the inhibitory center is chronically activated at thermoneutrality but less so in the cold.

4. Raphe nuclei

Further mediation of the thermoregulatory signal from the inhibitory center is also unclear. Here we suggest that there may be two, probably successive, stimulatory regions in the medulla oblongata area. Thus projections from the inhibitory retrorubral field may reach (some of) the raphe nuclei and release the inhibitor GABA in this area, keeping the area inhibited at thermoneutrality; thus the inhibition of the raphe by GABA is diminished in the cold. Correspondingly, fever-mimicking by injection of prostaglanding E_2 in the POAH leads to activation (increased fos expression) in the raphe (546), and the GABA receptor agonist muscimol injected into the raphe inhibits the activation of brown adipose tissue induced by cooling of the POAH area or by injection of prostaglandin E_2 in the POAH (533, 546). Conversely, injection of a GABA receptor antagonist (bicuculline) into the raphe nucleus activates brown adipose tissue at thermoneutrality (534, 794a).

However, certain investigators (830) have been unable to find evidence for raphe involvement in the thermostimulatory pathway and instead propose a direct connection between the retrorubral field and the olive.

5. Inferior olivary nucleus

The thermoregulatory signal is further mediated by a center in the medulla oblongata, probably in the inferior olivary nucleus. Electric stimulation of this area stimulates brown adipose tissue heat production (731, 830), and the area is activated when the inhibitory areas in the midbrain are inhibited, e.g., by local anesthetics (830). A lesion of this area abolishes the spontaneous brown fat-activating effect of procaine inhibition of the midbrain (731, 830). Glutamate added to this area stimulates brown adipose tissue heat production (731, 830). It is possible that the physiological glutamate signal is an output from the raphe nucleus, although this has not been directly demonstrated. The raphe/olive area(s) are thus tonically

inhibited at thermoneutrality, but this inhibition is released in the cold, and an active "spontaneous" stimulatory signal for brown adipose tissue is generated.

6. The intermediolateral neurons

The activating thermoregulatory signal is passed through an axon down through the spinal cord until it reaches the relevant intermediolateral neurons that connect to the sympathetic chain. The transmittor substance that stimulates these intermediolateral neurons has not been examined, but it is probably glutamate.

7. The sympathetic chain (stellate ganglia)

The thermoregulatory signal is further mediated via cholinergic nerve fibers from the intermediolateral neurons; these fibers terminate in the sympathetic chain where released acetylcholine stimulates the sympathetic neurons. The involvement of these ganglia for the mediation of the signal initiated at higher centers is demonstrated by the inhibitory effect of ganglionic blockers, such as chlorisondamine (10).

The section of the sympathetic chain involved in the control of the interscapular deposit of brown adipose tissue is found in the stellate ganglion. Within the stellate ganglion, two types of nerve cells are identifiable: those that contain both neuropeptide Y (NPY) and the enzymes involved in norepinephrine synthesis (with tyrosine hydrolase as the marker), and those that contain the norepinephrine-synthesizing enzymes but not NPY (103, 415). The thin, unmyelinated fibers from these two nerve groups reach the tissue in bundles (169). Within these nerve bundles there are also thick, myelinated nerves that may contain other neuropeptides (substance P, CGRP, and possibly others) (169), but the functional role of these neuropeptides is still largely unclarified.

The thin unmyelinated fibers that contain both NPY and norepinephrine are those that reach the vasculature of the tissue, especially the arterioles (103, 169, 415, 589, 855). It is not possible to stimulate these nerves selectively, and their function is thus not clarified. NPY has in itself no effect on the thermogenic activity of the tissue, but it augments the activity when added during a nervous stimulation (870); it has been suggested that its function is to redirect blood from the arteriovenous anastomoses to the tissue during thermogenesis (870). How and where the specific regulation of blood flow is coordinated with the regulation of thermogenesis is unknown.

The thin unmyelinated fibers that contain norepinephrine (and not NPY) are those that actually innervate the brown adipocytes themselves (103, 169, 415, 589, 855). They form a dense network within the tissue, being in contact with each brown adipocyte (bouton-en-passant), and their release of norepinephrine acutely stimulates heat production (see sect. II) and chronically leads to brown adipose tissue recruitment (see sect. III).

VI. METABOLOREGULATORY THERMOGENESIS

Thermogenesis, in brown adipose tissue or elsewhere in the body, is necessarily the result of the transformation of the chemical energy in consumed foodstuffs into heat, without storage in other chemical forms. Nevertheless, it was not until around 1979 that it was formulated clearly that heat production in brown adipose tissue is necessarily associated with a decreased metabolic efficiency (i.e., that a lower fraction of the energy consumed is stored in the form of bodily fat when brown adipose tissue is active) and that brown fat activity thus had the potential to be a determinant in metabolic efficiency. Practically simultaneously, two paradigms of metabolic efficiency were then linked to brown adipose tissue activity. One was based on correlated observations that nonshivering thermogenesis was inefficient and brown adipose tissue was atrophied in genetically obese animals (337, 817, 818), and this led to the suggestion that the increased metabolic efficiency observed in genetically obese animals could be due to inactivity of brown adipose tissue (325, 809). The other was the paradigm of "cafeteria feeding" where, in a seminal paper, Rothwell and Stock (680) implicated enhanced brown adipose tissue activity in the reduced metabolic efficiency observed after feeding certain diets. The associations between brown fat activity and metabolic efficiency are still valid today, but, as will be discussed in section VI*D*, the metabolic hyperefficiency in genetic obesity is probably rather a unintentional corollary to the underlying defect in the adipostat than a cause of the obesity.

The basic observations from 1979 have been extended and amply confirmed over the years. However, whereas the correlation between metabolic efficiencies under diverse feeding conditions and brown adipose tissue activity and recruitment is unchallenged, a major and still unresolved question is to what extent these alterations in brown adipose tissue activity are determinative for the metabolic efficiency and thus for obesity. Here we analyze first the thermal effect of a single meal and then discuss the similarities in the diversity of "brown-fat recruiting diets" that have been utilized in this field, and finally discuss to what extent these effects can be encompassed in a general phenomenon of obesity/leptin-induced brown fat-localized thermogenesis.

A. The Acute Thermal Effects of Eating

1. Effects of a single meal

Even in nonfasted animals, a "single meal" leads to a marked but transient increase in metabolism (oxygen

consumption), ~20% in excess of basal metabolic rate. This "specific dynamic effect of food" or "postprandial thermogenesis" is thus an acute response and different from what is normally meant by "diet-induced thermogenesis," which is used as an abbreviation for a diet-recruited increase in metabolic capacity over time (see sect. VI*B*). Single-meal thermogenesis was classically ascribed to the metabolic costs of the handling of the meal, but it has become accepted that there is also another component, i.e., a true thermogenesis.

A single meal leads to activation of brown adipose tissue, as defined by criteria such as unmasking (83, 257, 467), deiodinase activation (257), and a somewhat unexpected "surviving" increase in oxygen consumption in tissue minces (251, 253, 254). There is also a doubling of blood flow to the tissue (256) (an increase is only seen in brown adipose tissue and heart, not, e.g., in liver and muscle) and perhaps an increased norepinephrine turnover in the tissue (252). [In addition to these clearly activation-related parameters, there is also an increase in brown adipose tissue wet weight (253, 254, 256, 257); this is mainly due to refilling of the tissue with lipid and glycogen (254). This refilling process is probably not part of the general activation profile and may be insulin induced, rather than norepinephrine induced, as it is not propranolol sensitive.]

The fact that brown adipose tissue is activated is not in itself evidence that it is responsible for the single meal-induced thermogenesis. However, the magnitude of the response does parallel the capacity of brown adipose tissue, especially the response being higher in cold-acclimated animals (7). [It is also proportional in other states of pretreatment, but as these include feeding situations, the analysis is difficult (478, 678–685, 761).] It has not as yet been examined in UCP1-ablated or brown fat-deficient mice whether the meal-induced heat production is lower, but it seems likely that there is both a true "handling thermogenesis" and a brown fat-derived thermogenesis in the meal response.

The mechanism leading to the response is not fully known, but it seems to be sympathetically mediated (Fig. 18), as are (all) other states of induced brown fat thermogenesis. Although leptin is associated with feeding/fasting, the kinetics of the effects reported here are so rapid that it must be considered less likely that leptin is the mediator; rather, the more acute effects of meals, e.g., increases in serum glucose and insulin, are more likely candidates. When injected into the third ventricle, these

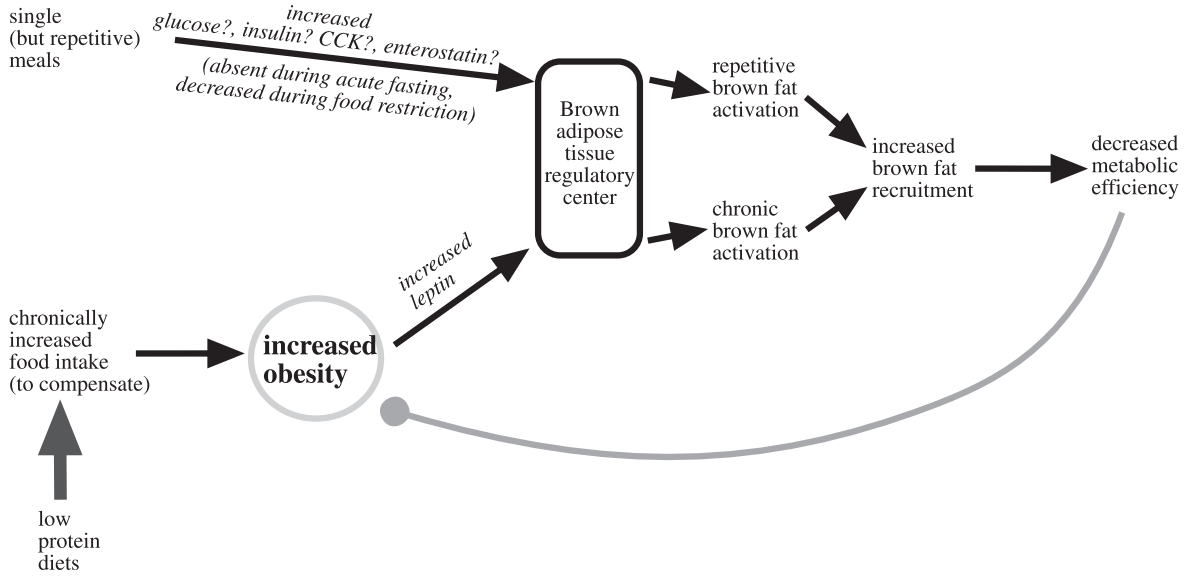

FIG. 18. Two pathways for diet control of brown adipose tissue recruitment status. *Top:* the intake of a meal is signaled to the brain through blood-borne substances that interact with the centers in the brain that regulate brown adipose tissue activity (Fig. 19). Due to the repetitive (although intermittent) effect of single meals, a certain degree of brown adipose tissue recruitment is maintained. *Bottom:* due to overfeeding (which may e.g., be secondary to protein dilution in the diet, as occurs in cafeteria diets and in most high-fat diets), obesity will develop, leading to increased leptin signaling to the centers in the brain that regulate brown adipose tissue activity. This will lead to a positive correlation between degree of obesity and recruitment state of brown adipose tissue. We suggest that this is the basis for so-called "diet-induced thermogenesis" (which thus rather may be considered to be "obesity-induced thermogenesis"). The effect of this pathway is that obesity is reduced due to decreased metabolic efficiency (lines with solid circles). Obviously, failures in the leptin signaling pathway, such as are found in *ob*/*ob*, *db*/*db*, and *fa*/*fa* animals, will lead to augmented obesity as the increased food intake is not counteracted by increased brown adipose tissue activity. Accordingly, different sensitivities of the leptin feedback system may be involved or even be explanatory for the differences between individuals (or between inbred mouse strains) in their responses to high-fat diets.

agents do activate brown adipose tissue, via the sympathetic nerves (701, 704). Other acute messengers influencing feeding, such as cholecystokinin (732, 886) (which is released from the stomach during a meal) and enterostatin (the pentapeptide released upon pancreatic procolipase activation by trypsin) (203) also stimulate the sympathetic nerves to brown adipose tissue (540).

In contrast to the case in other conditions of brown adipose tissue activation (e.g., all thermoregulatory thermogenesis; see sect. v), the functional significance of the single-meal-induced thermogenesis is not easily formulated. Why would an organism develop a mechanism that allowed for combustion of a significant but varying fraction of food energy, apparently rather independently of the composition of the meal? Indeed, the proportionality to the thermogenic capacity of brown adipose tissue gives the impression that the heat production is a passive general sympathetic response, similar to (some of) the "stress fever" (see sect. v*E2*). A physiological function reinvoked several times for single-meal-induced thermogenesis is that it is a signal to the brain to cease a meal (250, 334, 335), referred to sometimes as "thermoregulatory feeding." However, if meal-induced heat does influence meal size, it cannot be heat derived from brown adipose tissue, both because propranolol treatment does not lead to larger meal sizes (255) and because UCP1-ablated mice do not eat excessively. Thus brown fat-derived heat does not signal meal end.

2. Fasting, food restriction, starvation: decreased activity of brown adipose tissue

Fasting (starvation) and food restriction lead to a reduction in metabolic rate, resulting (during food restriction) in increased metabolic efficiency. This is parallelled by an inactivation of brown adipose tissue (684), including a decrease in the amount of UCP1, which is caused by decreased sympathetic stimulation (890).

An increased metabolic efficiency during periods of scarce supply of food seems physiologically relevant, and it could be anticipated that a particular regulatory mechanism would be necessary for this adaptation. However, this may not be the case. As seen above, meals represent an intermittent activation of the tissue. Because animals eat regularly, the baseline is constant repetitive intermittent activation and ensuing recruitment of brown adipose tissue. Thus the atrophy seen in fasting can be considered secondary to the absence of this meal-induced stimulation (Fig. 18) (see sect. vI*A*).

The question is thus whether the reduced activity of brown adipose tissue can explain the increased metabolic efficiency in fasting animals. If a constant fraction of the food is normally channelled into brown adipose tissue, the absence of this combustion in the tissue would be expected to result in a markedly lower basal metabolic rate. Under a normal feeding regime, the UCP1-ablated mouse does not, however, show a reduced metabolic rate, even at thermoneutral temperatures (262). Thus the inactivation of brown adipose tissue during fasting may be considered "optional" for the state of increased efficiency observed during starvation, i.e., if brown fat activity is ongoing, it will be switched off. However, the animal can clearly alter its basal metabolic rate by other means.

3. Basal metabolic rate: a regulated entity?

The nature of basal metabolism is principally unknown (673). The general understanding, more or less explicitly formulated, is that it is the metabolism (and the ensuing heat by-product) that is necessary to "maintain everything running." However, the basal metabolic rate of mammals is at least threefold higher than that of reptiles with the same body weight at the same body temperature, despite the fact that the reptile would also have to keep everything functioning. Thus the high basal metabolism in mammals seems to require an explanation. An alternative is therefore to consider even basal metabolism as a centrally regulated quantity (in parallel with, e.g., body temperature or blood glucose level). This formulation is thus parallel to that of, e.g., fever, implying that such a regulated parameter will be set at the requisite level by utilization of the means available. In such a formulation, the contribution of brown adipose tissue metabolism would be optional, in accordance with the observation that the presence or absence of a heat-producing mechanism in brown adipose tissue does not alter the basal metabolic rate (262). Thus, despite brown adipose tissue being a mammalian prerogative, the tissue is not necessary for the high metabolic rate found in mammals, not even that in small mammals.

B. Recruiting Diets (Obesity, Leptin, Cachexia)

1. Are recruiting diets protein-diluting diets?

The normal diet (chow) offered to experimental animals can be considered healthy, with a rather low fat content, reasonable carbohydrate, an adequate protein content, and much fiber. It is not, however, as can be easily verified, a very tasty or palatable diet. As mentioned in the introduction to this section, a major breakthrough in brown adipose tissue research came with the realization in the late 1970s that access to a so-called cafeteria diet, which tempted experimental animals to overeat and made them obese, was unexpectedly associated with decreased metabolic efficiency and with brown adipose tissue recruitment (680).

Since the introduction of the "cafeteria" diet, a series of "recruiting diets" have been investigated. These include the original cafeteria diet (680) and locally flavored vari-

ants on this theme (721). These types of diet are characterized by the availability to the animals of what can be referred to as "junk food," i.e., food items high in fat and carbohydrate but low in protein. The animals have access to this food in addition to their normal chow and may nearly double their energy intake under such conditions. The brown adipose tissue of these animals becomes recruited, as evidenced by many observations (amply reviewed in, e.g., Refs. 326, 330, 688, 689), including an increased UCP1 level (481, 570).

So-called high-fat diets are generally made by adding extra fat (unfortunately not always clearly specified) to the normal animal chow; the intake is thus not voluntary. This type of diet also leads to recruitment of brown adipose tissue, including increased UCP1 levels (507). Similarly, in high-carbohydrate (sucrose) diet regimes, the animals are either exposed to a diet with extra carbohydrate or are offered drinking water with added sugar, again conditions leading to recruitment, including increased UCP1 levels (90, 434, 530, 779). Also, chronic ethanol forced feeding (i.e., added to drinking water) leads to metabolic inefficiency and brown adipose tissue recruitment (362, 363).

Considering the diversity of these diets, it may be asked what is the common "factor" in each of these feeding regimes that leads to brown adipose tissue recruitment. Expanding from the seminal analysis of Michael Stock (764), we adhere here to the idea that all these "recruiting diets" can be understood within the framework of protein dilution (691). That these different regimes inevitably lead to a protein dilution effect is clarified in a simplified form in Table 1. An "optimal" chow diet is first described, and it is assumed that the amount of protein and the total energy intake reflect the requirements of an animal, during a given time period. When the diet is manipulated, the manipulation routinely consists of the addition of extra low-protein items to the food, either indirectly by giving tasty junk food, or, as exemplified here, directly by fat addition, leading to an increase in lipid energy content from 20 to 43%. Upon recalculation, it appears that with this diet, the animal has to ingest 40% more calories to obtain the same amount of protein. Thus the recruiting diets traditionally reported in the literature can apparently adequately be described as protein-dilution diets, and it therefore does not seem to be of major significance to distinguish between these diets. Indeed, low-protein diets (i.e., reducing the protein energy percentage from ~20% down to 10% or even 5%) are

TABLE 1. *The fat food formulation problem*

	Protein	+	Fat	+	Carbohydrate	=	Total
			A standard "chow" diet				
Composition, g/100 g	20		10		70		100
Energy, kcal	80		90		280		450 kcal/100 g
Energy percent	18		20		62		100
Thus eat 100 g (45 kcal) for 20 g protein substance (defined as "100% food intake"). We assume that this is "need," both for protein and for energy.							
			Many "cafeteria" or "high-fat" diets [just add extra, e.g., fat (directly or disguised as cheap food)]				
Composition, g	20		10 + 20 fat		70		120
(Actual composition, %	17		25		58		100)
Energy, kcal	80		270		280		630, i.e., 525/100 g
Energy percent	13		43		44		100
Thus, if same caloric amount of food is eaten, too little protein is obtained (only 15 g). Instead, eat 120 g (630 kcal) for 20 g protein substance (140% of necessary energy intake). If protein is felt as composition, eat 120 g (140% of necessary energy intake). If protein is felt as relative energy, eat 154 g = 808 kcal (180% of necessary energy intake).							
			High fat with carbohydrate compensation in composition				
Composition, g/100 g	20		20		60		100
Energy, kcal	80		180		240		500
Energy percent	8		36		48		100
Thus, if same caloric amount of food is eaten, too little protein is obtained (only 18 g). Instead, eat 100 g (500 kcal) for 20 g protein substance (111% of necessary energy intake). If protein is felt as relative energy, eat 225 g = 113 kcal (250% of necessary energy intake).							
			High-fat diet compensated to yield same protein energy percent				
Composition, g/100 g	25		24		52		100
Energy, kcal	100		216		206		522
Energy percent	19		41		40		100
Thus, if same caloric amount of food is eaten (450 kcal), adequate protein is obtained.							

in themselves recruiting and also demonstrate the features of the above regimen (186, 691, 860): increased food intake and some degree of obesity but also, by inducing a decreased metabolic efficiency (i.e., the obesity is smaller than would be anticipated from the extra intake of energy), a recruitment of brown adipose tissue, and an increased thermogenic response to injection of norepinephrine. Thus, by the formulation of this protein-dilution hypothesis (764), a physiologically comprehensible role for "diet-induced thermogenesis" is stated. By burning off excess caloric intake, this process allows for survival with a lower obesity load, even under conditions where the quality of the food supply does not meet optimal diet requirements and "extra" food therefore has to be eaten to obtain protein, indeed a situation that many animals are exposed to, and probably earlier most humans.

If the protein-dilution hypothesis is accepted as a common denominator for all recruiting diets and protein dilution thus is the component that leads to hyperphagia, a regulatory question is necessarily raised: What is the sensing mechanism for an alteration in dietary protein content? In general, only little is known concerning this type of food quality sensing. A meal as such has characteristic clues for the brain in the form of glucose and insulin, as well as cholecystokinin and enterostatin, etc. Similar systems relevant for protein and individual amino acids have not been well characterized, although some indications can be found (215, 216, 496). However, in some way, ingestion of a low protein content must be signaled to the brain to increase food intake, to compensate for the low content.

It could also be anticipated that to explain the recruitment effects on brown adipose tissue, there would be a need to transmit the low-protein information to the regulatory centers for brown adipose tissue metabolism, but this may not necessarily be the case. If protein sensing leads to compensatory hyperphagia, this would tend to lead to development of obesity. Thus the successively increasing obesity may be the clue to the brown adipose tissue recruitment, which is then only apparently caused by the diet but in reality by the body weight consequences. This also means that diets that lead to obesity without being protein diluting (by being palatable or by fully compensating for increased fat content by reduction of carbohydrate content) may be able to recruit brown adipose tissue (although this does not seem to have been directly demonstrated as yet).

The question is therefore: Does obesity-induced thermogenesis exist?

2. Is "diet-induced thermogenesis" really obesity-induced thermogenesis?

The answer as to whether "obesity-induced" thermogenesis exists is not immediately evident. Neither of the two models normally used for obesity can be used here: genetically obese mice clearly lack activation of brown adipose tissue (for signaling reasons, see sect. VI*E3*), and in cafeteria-fed animals it is not possible to distinguish between the acute effect of food intake and that of the resulting obesity. There is, however, a suitable model: the postcafeteria animal. After having been fattened through a cafeteria diet, an animal is reexposed to a chow diet. This leads to a state of low metabolic efficiency, lower even than that in the cafeteria-fed state, in spite of the animals being hypo- or euphagic (455, 670). Remarkably, these, still obese, postcafeteria animals maintain recruited brown adipose tissue (455, 670). This can hardly be interpreted other than that the obese state in itself induces brown adipose tissue activation and recruitment.

The obvious candidate for the mediation of this obesity-induced thermogenesis is leptin, the blood level of which is (mainly) determined by the amount of fat in the animal. Leptin treatment of normal (and of leptin-deficient) animals results in decreased body weight for (at least) two reasons: decreased food intake and increased metabolic rate, i.e., the leptin-treated animals lose more weight, even compared with pair-fed controls. As leptin thus reduces food intake, the expected effect on brown adipose tissue is inactivation and atrophy (see sect. VI*A2*). However, on the contratry, leptin treatment, via central mediation, results in the established signs of brown fat activation, such as increased sympathetic nerve activity (136, 747), increased norepinephrine turnover (which is required for the effect) (137, 510), and increases in UCP1 (139, 692, 853) and PGC-1 (388) gene expression and increased glucose uptake (296, 510, 692). The acute thermogenic effect of leptin is fully located to brown adipose tissue: in UCP1-ablated mice, leptin fully loses this effect (138). Thus brown adipose tissue is essential for leptin-induced thermogenesis in rodents.

Recruiting diets also augment the thermogenic response to injected norepinephrine (434, 680), and these animals can thus be said to have increased their "nonshivering" thermogenesis capacity. UCP1-ablated mice are, however, completely devoid of the phenomenon of diet adaptation-recruited norepinephrine-induced thermogenesis (our unpublished observations). Thus brown adipose tissue has a similar role here as it has in classical nonshivering thermogenesis.

There is, however, a principal difference concerning the magnitude of the response. The cold acclimation-recruited response to norepinephrine is exactly of the magnitude needed to combat the heat loss (see sect. V*C*). However, in animals adapted to recruiting diets, the increase in absolute metabolism is small (10–20%), and the increased response to norepinephrine is thus severalfold higher than the increase in metabolism brought about by the diet; indeed, an adaptation to a recruiting diet can even function as a preacclimation to cold (681). Within

the limits of a simple theory for norepinephrine-stimulated recruitment, it is not presently possible to understand how the tissue can become recruited to a higher degree than that which is required for the apparent physiological challenge.

3. Are diet-adapted animals hyperpyrexic?

A further feature of animals adapted to recruiting diets is that these animals exhibit an increased body temperature (682). This was initially considered evidence that they are "overproducing" heat, i.e., the animals were considered hyperthermic. This point has unfortunately not been examined rigorously, but it seems difficult to accept that if the animals were merely overproducing heat, they would not in parallel increase heat loss to the surroundings and thus defend a "normal" body temperature, particularly since they are normally kept in "cool" surroundings (~20°C, cf. Fig. 12) with ample opportunity for heat loss. Thus it seems more likely that the animals demonstrate a "diet-induced fever," i.e., that they are hyperpyrexic.

One argument that may be forwarded for the increased body temperature being a hyperthermia has been the effect of propranolol; propranolol injection into cafeteria-fed animals leads to a decreased body temperature (682), which could be interpreted as indicating that there is a component of β-adrenergically mediated (and thus probably brown fat-localized) facultative thermogenesis; had it been a hyperpyrexia, it would have been expected that other thermogenic mechanisms would have been initiated (vasoconstriction and shivering) to defend the higher body temperature. However, as propranolol has also documented central antipyretic effects (493), propranolol studies cannot distinguish between a hyperpyrexic ("fever") and a hyperthermic effect of recruiting diets.

The increased body temperature, which is in the order of 1°C, is in itself sufficient to account for the increased basal metabolism observed chronically in animals adapted to a recruiting diet, i.e., an increase of ~10%. If this persistently elevated metabolism (which is not a response to acute feeding, since it is observed even in animals fasted overnight, our unpublished observations) is considered as an aspect of hyperpyrexia, it affects the discussion of the participation of brown adipose tissue in this response. It has generally been assumed that the extra metabolism emanates from brown adipose tissue and is dependent on this. Three articles by Foster and Ma (469, 470, 472), based on blood-flow studies, cast serious doubt as to the localization of diet-induced thermogenesis to brown adipose tissue, but the exact experimental conditions were perhaps not optimal for investigating this type of thermogenesis. In contrast, a doubling of blood flow to brown adipose tissue has been observed in otherwise unstimulated cafeteria-fed animals (683), and this would be sufficient to account for the increased metabolism.

However, we have found that even UCP1-ablated mice demonstrate an increased basal metabolism and body temperature when challenged by a recruiting diet (our unpublished observations), and this would indicate that brown adipose tissue thermogenesis is not essential for this persistent hypermetabolism. These apparent controversies become understandable with the hyperpyrexic hypothesis; the increased metabolism is an effect of the defended higher body temperature, and as in other fevers (see sect. v*E*), brown fat-derived heat production will be primarily used to defend this temperature, but if brown fat-derived heat is not available, the organism will utilize other means to maintain the elevated defended temperature. The agent responsible for the increased body temperature set-point is probably leptin; leptin has a hyperpyrexic effect in normal animals (162a, 465).

4. Strain variations in the leptin/brown adipose tissue pathway

The model presented above (Fig. 18) implies that at normal tissue energy status (= normal weight mice), sufficient leptin is available to maintain brown adipose tissue in a somewhat activated state. In well-functioning animals, this system will in principle be self-adjusting, i.e., the metabolic efficiency will decrease in proportion to the amount of fat already accumulated, and theoretically a new steady state will eventually be reached. As a control system, it may be said to be less than optimal, because a rather large deviation from the "adipostat" set-point may be necessary to induce a measurable countereffect. This, though, is what is seen: in cafeteria-diet experiments, the animals become fatter, and this correlates with an increased brown adipose tissue activity, presumably partly counteracting the development of further obesity.

From this model (Fig. 18), it is also evident that if the leptin pathway is not functional or has a decreased function, a series of alterations would be expected. In agreement with this, animals (mouse or rat strains) that have a mutated, unfunctional leptin (*ob*/*ob*) or leptin receptor (*db*/*db*, *fa*/*fa*) generally display a syndrome consisting of decreased body temperature (anapyrexia), low metabolic rate, high metabolic efficiency, an atrophied brown adipose tissue (as it lacks the chronic leptin-dependent activation), and thus an increased acute cold sensitivity, but are still able to adapt to successively increased cold exposure (as the *thermo*regulatory pathway is not affected). When exposed to a recruiting diet, these animals cannot activate brown adipose tissue, which augments their obesity.

In this formulation, other animals (mouse strains) may have a diminished ability to react through this sys-

tem, yielding animals that are obesity prone, such as the C57Bl/6 strain often used in transgenic research. The molecular background is unknown.

Furthermore, other mouse strains may display features implying that the postleptin part of the scheme in Figure 18 is constantly set at a higher level, being rather independent of the degree of activation of the leptin pathway. This is probably the case in the A/J (780) and the Balb/c strains. Indeed, when the *ob/ob* (lept −/−) mutation is placed in the Balb/c background (634), the mice display a higher body temperature, a lower metabolic efficiency, and probably a recruited brown adipose tissue, compared with the case when the mutation is on the traditional C57Bl/6 background. Consequently, some of the variations in metabolic parameters encountered between different mouse strains (34, 369), principally reflecting individual variations in outbred animals, may be manifest as variations in the leptin/brown adipose tissue pathway.

Thus brown adipose tissue is probably optional or perhaps additional for the diet adaptation-recruited increase in "basal" metabolism but is essential for diet adaptation-recruited norepinephrine-induced thermogenesis.

5. *Is obesity due to lack of obesity-induced thermogenesis?*

The corresponding question is thus to what extent genetic or "spontaneous" obesity is due to the absence of obesity-induced thermogenesis.

All genetic models of obesity are characterized by atrophied brown adipose tissue, including a reduced UCP1 content (269), as has been amply reviewed (333, 814). The mechanism behind the atrophy, as seen from the brown adipose tissue point of view, is also well documented: the sympathetic drive to brown adipose tissue is diminished (333). There is thus no need for an independent mechanism leading to brown adipose tissue atrophy in genetic obesities, although such possibilities have been discussed (579).

Irrespective of the cause of the brown fat atrophy, the pertinent question is whether this brown adipose tissue atrophy is (partly) causative of the obesity in genetically obese animals, or whether the atrophy is coincidental (i.e., an unavoidable regulatory consequence of the unfunctional leptin-signaling system, with subsequent decreased sympathetic signaling).

If the lack of brown adipose tissue thermogenic activity were causative of the obesity observed in genetically obese animals, it would be reasonable to expect that UCP1-ablated mice should become spontaneously obese. This is apparently not the case, at least as reported to date (200). In contrast, brown fat-deficient mice do become obese (462). One interesting interpretation of this difference is that brown adipose tissue is doing more than burning off energy; it could also be secreting a satiety factor (506). There could, however, also be more technical reasons for the observed differences. The UCP1-ablation has been studied on mice of the C57Bl/6 background, i.e., mice that are already notoriously obesity-prone in themselves, without the mutation (35, 780, 859), and where a high-fat diet does not induce UCP1 gene expression (857). It may therefore be difficult for the absence of UCP1 to become manifest in animals that are already displaying an "efficient" phenotype. In contrast, the brown fat-deficient mice are on the FVB background, i.e., on a genetic background not known to be inherently prone to obesity. Thus, before it can be concluded that there is a qualitative difference between the UCP1-ablated and the brown fat-deficient mice with respect to obesity proneness, these mutants have to be studied on the same genetic background. Until then it can only be implied from the above that it is still possible that a low or absent function of brown adipose tissue (in the form of thermogenesis) may augment dietary or genetic obesity.

6. *Are age-induced obesity and cold sensitivity due to leptin resistance?*

Not only humans but also (male) rats and mice display with increasing age an increased propensity to become obese. In humans, this is often attributed to alterations in life-style, but these explanations are not readily extended to experimental animals, and the occurrence of a similar phenomenon in animals may therefore bring into question the popular notions of the causes of age-correlated obesity in humans.

Age-induced obesity is parallelled by inactivation of brown adipose tissue and atrophy of the tissue, as evidenced by a series of parameters (including a decreased amount of UCP1, Ref. 709) as reviewed in References 219, 500. With age, the animals also become more sensitive to acute cold, but, as discussed in section v*C*, this may not be directly related to brown fat status but rather to a general loss of physical endurance. Indeed, cold sensitivity is primarily observed in animals that are "senescent" rather than merely old (500), i.e., animals that are in general moribund.

The question may be raised also here as to whether the animals become obese because of a low brown adipose tissue activity or whether this is just a corrollary. Old animals display what is referred to as leptin desensitization (723), i.e., when determined by several parameters, they respond less to leptin treatment than do younger animals. This would also lead to brown adipose tissue inactivation, which also here might augment obesity. It would also seem that chronically elevated leptin can induce a state of leptin resistance, although the thermo-

genic effects of leptin seem less susceptible to leptin resistance than do other effects of leptin (711).

The cause and the biological significance of age-induced leptin resistance are outside both the scope of this review and of present knowledge. One may consider any alteration with age as an inevitable decline. However, if expressed as a tendency to increased metabolic efficiency with age, it is rather a gain of function that is observed. The price is a decreased ability to obtain essential nutrients through extraction from poor food, but perhaps the need is lower in a nongrowing, nonfertile organism.

7. *Activation of brown adipose tissue may not be a general mechanism for all food component deficiencies*

If brown adipose tissue has a physiological role to enable animals to compensate for a diet low in protein by hyperphagia, with only modest effects on body weight, a similar role could be proposed for brown adipose tissue in other nutritional deficiencies.

Dietary deficiencies not demonstrating recruitment of brown adipose tissue include low essential fatty acids (557, 878) and vitamin A (retinoic acid) deficiency (63, 657). However, concerning several other deficiencies, the data are not clear, although some of the effects on brown adipose tissue in the following deficiences may indicate a tendency to a recruitment: riboflavin (614), creatine (231), iron (44, 530, 810), selenium (240, 511), and iodine (240, 511, 615). Concerning several of these, the deficiencies may in themselves have so marked effects on brown adipose tissue function that a recruiting effect could not become manifest.

8. *Lipids containing polyunsaturated fatty acids activate in their own right*

Additionally, or rather in parallel to the protein-dilution effects of most high-fat diets, there is an independent effect of the type of lipid used. It is often difficult in retrospect to establish the exact type of lipid used in a published experiment, but there are some studies in which a direct comparison has been made between different types of lipids, especially saturated/monounsaturated versus polyunsaturated (often lard/tallow vs. safflower or corn oil) diets. Polyunsaturated fat diets lead to lower metabolic efficiency (507) and a lower body fat accumulation under conditions of equal food intake (488, 740). They induce stronger recruitment of brown adipose tissue, as judged by parameters such as higher lipoprotein lipase activity (489), higher mitochondrial oxidative capacity (507, 794), and higher UCP1 content (487b, 694a). Concerning the different types of polyunsaturated fatty acids (*n*-3, *n*-6, etc.), no marked differences have been observed (793, 794). Studies indicating that high-fat diets are more recruiting than high-sucrose diets may relate to the possible presence of polyunsaturated fat (687).

Although experiments of this type are generally performed by comparing high-fat diets of different fatty acid composition, the high fat content as such is not a prerequisite: a high level of polyunsaturates in a normal diet has in itself a recruiting effect on brown adipose tissue, leading to increased UCP1 content (557).

The most probable explanation for the recruiting effects of the polyunsaturates is that they influence, probably as ligands, the transcription factor PPARγ, within the brown adipocytes themselves (see sect. III). They can also increase the degree of sympathetic stimulation of the tissue (488, 892), through unknown central effects. Whether or how the recruiting effect of polyunsaturated fatty acids makes physiological sense is not clear.

9. *Does hyperphagia-induced thermogenesis exist?*

That a phenomenon of hyperphagia-induced thermogenesis should exist is occasionally forwarded, implying that high food intake per se should induce a mechanism leading to activation of brown adipose tissue and its possible recruitment. If such a phenomenon exists, a mechanism must then also be postulated to explain why this does not happen under all physiological conditions associated with a high food intake.

There are cases of association between high food intake and brown fat recruitment. The most obvious is of animals in the cold, which eat about fourfold more than controls and also demonstrate both recruited brown adipose tissue and activated thermogenesis (see sect. V*B*). However, this recruitment is not secondary to the high food intake; brown adipose tissue is recruited even when animals in the cold are pair-fed with controls (417) (clearly a detrimental situation for the animals), and the recruitment is thus not caused by the high food intake. Correspondingly, physical training results in increased food intake, but this does not lead to brown-fat recruitment; rather, a tendency towards atrophy is seen (see sect. V*E5*).

More relevant for the discussion is the recruitment encountered in animals exposed to cafeteria/high-fat diets, but, as noted in section VI*B*, the brown fat recruitment seen here seems to be mainly attributable to dilution of dietary protein and the development of obesity. There are thus no direct reports that overeating as such induces brown fat recruitment.

10. *Cancer cachexia and brown adipose tissue*

Certain tumors in both experimental animals and humans result in profound weight loss, cachexia, as a consequence of decreased energy intake and tissue breakdown but also of increased energy expenditure. The increased energy expenditure has been proposed to be the

result of brown adipose tissue activation and recruitment (79, 671). The recruitment has been suggested to be caused by the action of a tumor-derived product, lipid mobilizing factor (LMF = Zn-α2 glycoprotein), which has been reported to act through β_3-adrenergic receptors and induce lipolysis in isolated adipocytes (693). This may be interpreted to increase substrate supply to the tumor. Whereas the wasting syndrome as such is thus not dependent on brown adipose tissue, the LMF would also stimulate brown adipose tissue through β_3-adrenergic receptors, leading to upregulated UCP1 expression (55) and probably thermogenesis. The brown adipose tissue involvement here would thus be additional.

C. Influence of Sex Hormones on Brown Adipose Tissue

1. Androgen-induced thermogenesis

Treatment with testosterone or the androgen precursor dihydroepiandrosterone (DHEA) leads to reduced body weight in experimental animals (790) and generally reduces body fat (while increasing muscle mass). However, as the reduced body weight is combined with a lower food intake, it does not necessarily depend on an activation of brown fat-derived thermogenesis. There is, however, also a reduced metabolic efficiency in androgen-treated animals (433). Because decreased food intake leads to brown adipose tissue atrophy (see sect. VI*A2*), and because brown adipose tissue recruitment state appears unchanged following androgen treatment, it can be suggested that androgens maintain (i.e., de facto promote) brown adipose tissue recruitment state (and probably activity), despite the reduced food intake (1, 433, 515, 790). However, direct experiments testing this, including pair-feeding animals to androgen-treated animals and examining the relative degree of recruitment of brown adipose tissue, have not been performed.

2. Estrogen-induced thermogenesis

In addition to the effects normally associated with estrogens, they also affect metabolic efficiency. This is routinely examined as the effect of estrogen (routinely estradiol) after ovariectomy. Ovariectomized animals become spontaneously obese, primarily through overeating (42), but brown adipose tissue is atrophied (617). Even in the absence of the hyperphagia, i.e., in pair-fed animals, ovariectomized animals become obese. Estrogen treatment counteracts the obesity, demonstrating alterations in metabolic efficiency, and indeed recruitment status in estrogen-treated ovariectomized animals is higher than in ovariectomized animals pair-fed to these, indicating a true effect of estrogen (617). Mechanistically, the estrogen effects may be centrally or directly mediated. A direct effect could be mediated through estrogen receptors present in brown adipose tissue (847). Because ablation of the classical estrogen receptor α does not lead to any effect on brown adipose tissue weight (whereas white adipose tissue depots are augmented) (313), the receptor subtype expressed in brown adipose tissue may be the nearly ubiquitous estrogen receptor β. It is not known whether this receptor mediates any of the reported estrogen effects directly, but there are effects of estrogen treatment of brown adipocytes in culture (620, 636, 669).

3. Gestational brown-fat atrophy is caused by fetal heat production

During gestation, the brown adipose tissue of the dam becomes successively inactive and atrophied (3, 16, 247, 483, 715, 848). Fetal growth is energy consuming. Towards the end of gestation, fetal metabolism, plus the energetic cost of fetal growth, place a thermal burden on the dam; her total energy utilization (heat production) is higher than in virgin animals (635). As the pregnant dams normally live at temperatures below thermoneutrality, they would as virgin animals have had a recruited and activated brown adipose tissue to produce the extra heat needed in the relatively cool environment. Even before an increase in total heat production becomes evident, fetal growth and metabolism would constitute an extra and gradually increasing source of heat, leading to a decreasing need for brown fat-derived heat. Hence, there would be a decreased sympathetic stimulation, leading to atrophy of brown adipose tissue. Although other mechanisms responsible for this atrophy have been discussed, there is presently no need to invoke other regulatory mechanisms than purely thermoregulatory ones to explain the gestational atrophy of brown adipose tissue.

4. Lactational atrophy is caused by lactational heat production

During lactation, the brown adipose tissue of the dam is inactive and atrophied (122, 410, 483, 484, 526, 586, 618, 815, 819, 845). It is not known how this inactivation takes place, and complex hypotheses have been forwarded. However, most probably it is secondary to the energetics of milk production as such. Milk production is not very efficient (~60%) (668) and therefore results in a large extra heat production. The "basal" metabolic rate (oxygen consumption) of lactating animals is increased, and their thermoneutral zone therefore shifted to much lower temperatures. Because brown adipose tissue is normally recruited at normal ambient temperatures, any extra heat production diminishes the need for brown fat-derived heat, and the tissue atrophies. Although the lactation-induced atrophy has attracted much interest, there is presently no reason to invoke other mechanisms than

normal thermoregulatory control to explain the phenomenon.

Lactation is also associated with a large increase in food intake (122), but as noted above, hyperphagia per se does not recruit brown adipose tissue (see sect. VI*B8*), and the absence of brown fat recruitment (i.e., the atrophy seen) does not require any explanation.

D. Central Regulation of Metaboloregulatory Thermogenesis

Although the central pathway regulating brown adipose tissue activity in connection with body temperature control may be considered complex and only partly clarified (Fig. 17), this pathway must be considered simple and well-explored when compared with the central pathways involved in regulation of brown adipose tissue in metaboloregulatory thermogenesis. We try here very tentatively to coordinate some of the available information with respect to regulation of brown adipose tissue activity and recruitment state (Fig. 19). The regulation of brown adipose tissue activity in these connections is evidently closely associated with the regulation of feeding behavior in general, but the data discussed here are only those directly shown to lead to effects on brown adipose tissue. For the control of feeding, etc., other reviews are available (5, 142, 197).

1. All metaboloregulatory control may come together in the VMN

At some point in the pathway that finally ends in the sympathetic nerves having direct contact with the brown adipocytes, the thermoregulatory and the metaboloregulatory inputs have to come together. The ventromedial hypothalamic nucleus, which also seems to be involved in the regulation of thermoregulatory thermogenesis, may be this junction point. Although many studies referred to below have broadly addressed the entire ventromedial hypothalamus, we think it is possible to ascribe the data mentioned in the following to the effects on the VMN and will here interpret such studies accordingly, and for simplicity, we refer to the affected area as the VMN.

2. Effects of acute meal signals may be directly on the VMN

The acute thermogenic signals, in the form of increased levels of glucose, insulin, cholecystokinin (CCK), and enterostatin (see sect. VI*A1*), may interact directly with receptors in the VMN area. This possibility is supported by the ability of each of these substances to increase activity of brown adipose tissue when they are administered either directly into the VMN, or into the third ventricle, from which the substances could interact with the VMN (see sect. VI*A1*). Gold-thioglucose specifically destroys cells of the VMN, and this leads to obesity and brown adipose tissue atrophy (196, 343), an outcome that is in accordance with glucose sensing taking place directly in the VMN.

3. Leptin activates brown adipose tissue via the activating melanocortin system

The origin of leptin is predominantly peripheral, and accordingly, peripheral injection of leptin activates brown adipose tissue (136, 186a, 308). However, the leptin receptors responsible for mediation of the thermogenic response to leptin are centrally located. Leptin receptors are found in several brain areas (503), including the arcuate nucleus (where there is direct contact with the blood) but also, e.g., in the VMN (to which it may arrive via specilialized ependymal cells); where the thermogenically linked leptin receptors are found is not established, but it could be those in the VMN area. Indeed, injection of leptin into this area or into the brain ventricles activates brown adipose tissue (139, 177, 487, 510, 708, 723). Correspondingly, it is due to the absence of functional leptin receptors that brown adipose tissue is atrophied in Lepr(−/−) animals (*db/db* mice and *fa/fa* rats). Apparently, leptin normally has a tonic effect on the VMN, leading to a certain degree of "basal" brown adipose tissue recruitment.

The thermogenic effect of leptin is linked via the melanocortin system, with melanocyte stimulating hormone (MSH) as the released agent. Stimulation of this system in itself, by melanocortin-4 receptor agonists, activates brown adipose tissue (307, 866a). Correspondingly, inhibition of melanocortin-4 receptors, either pharmacologically (by SHU9119) or physiologically (by agouti-related peptide) or by their ablation (94, 749), leads in itself to deactivation of brown adipose tissue and also inhibits the ability of leptin to activate brown adipose tissue (708, 763).

4. Glucocorticoid inhibits leptin-sensitive cells

The leptin-sensitive, MSH-releasing cells are under a tonic depressing effect by glucocorticoids, released from the adrenals (475). These glucocorticoids increase the level of suppressor of cytokine signaling-3 (SOCS-3) and thus dampen the effect of leptin. In adrenalectomized animals, this dampening is eliminated, and the postleptin pathway is thus augmented (even in the absence of leptin or leptin receptors). Although brown adipocytes possess glucocorticoid receptors (213) and there are direct effects of glucocorticoids on isolated brown adipocytes (760, 823), it is likely that the observations of the brown fat-(re)activating effect of adrenalectomy under conditions of, e.g., genetic obesity (346, 478, 841) are explainable through this central effect of glucocorticoids.

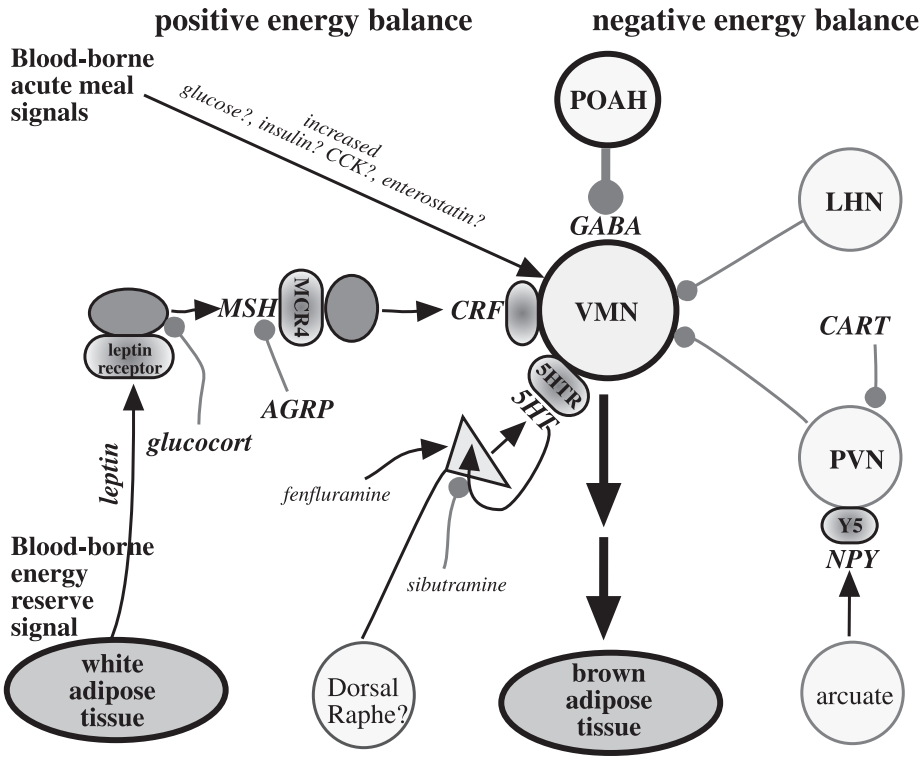

FIG. 19. The central pathway for control of metaboloregulatory thermogenesis in brown adipose tissue: a tentative scheme. The pathways shown here are intimately associated with those involved in control of feeding, but only those demonstrated to affect brown adipose tissue have been included here. The central axis relates to the thermoregulatory pathway from the preoptic anterior hypothalamus (POAH) via the ventromedial hypothalamic nucleus (VMN) to brown adipose tissue, sketched in Fig. 17. Blood-borne acute meal signals [glucose, insulin, choleocystokinin (CCK), enterostatin] may interact directly with receptors in the VMN. The leptin signals from plentiful white adipose tissue interact with leptin receptors in the brain, but the exact anatomical localization of the leptin receptors involved in control of brown adipose tissue has not been established (indicated here as an undefined region); it could be in the arcuate nucleus or in the VMN itself. The cells with leptin receptors are tonically depressed (lines with solid circles) by glucocorticoids from the adrenals but upon stimulation still release melanocyte-stimulating hormone (MSH) that stimulates melanocortin-4 receptors (MCR4); this stimulation can be competitively inhibited by agouti-related protein (AGRP). The signal is further transmitted by the release of corticotropin-releasing factor (CRF) and finally stimulates the relevant cells in the VMN and thus thermogenesis in brown adipose tissue. From an area in the dorsal raphe nucleus, fibers connect to the VMN and there release serotonin (5-HT), which also leads to stimulation of brown adipose tissue; the serotonin release can be pharmacologically enhanced by fenfluramine, and serotonin reuptake can be inhibited by sibutramine or amphetamine, thus having similar effects. When the organism is in negative energy balance ("hungry"), cells from the arcuate nucleus, in direct contact with the blood, sense this and release neuropeptide Y (NPY) that interacts with NPY5 receptors (Y5) in the paraventricular nucleus (PVN); this leads to inhibition of the VMN and thus of brown adipose tissue activity. The inhibition can be overcome by cocaine- and amphetamine-regulated transcript (CART) (which is thus stimulatory for brown adipose tissue). Also, signals from the lateral hypothalamic nucleus (LHN) may inhibit the VMN and thus brown adipose tissue activity.

5. *Further mediation of the leptin signal from the melanocortin receptors involves corticotropin-releasing factor*

Corticotropin-releasing factor (CRF) (which should be considered here in a role as a general transmittor substance and not in its classical role in the adrenal axis) when applied centrally can activate brown adipose tissue (23, 435), even in leptin (receptor)-deficient animals (21, 314, 350). This implies that the effect of CRF is either parallel to or downstream that of leptin. The demonstration that leptin-induced brown adipose tissue activation can be inhibited by a CRF antagonist (147, 487a) indicates that CRF is in the leptin pathway. Although the issue has not been directly investigated concerning brown adipose tissue activation, it is likely, based on investigations of leptin-induced feeding control (191, 482), that CRF comes last in the chain leptin/MSH/CRF. CRF is thus the agent so far identified that is closest to the brown fat-regulating cells in the VMN. If CRF interacts directly with these cells, the effect of CRF on the VMN could be suggested to be

inhibitory, just as the POAH seems to control the VMN via the inhibitory transmitter GABA.

6. *Serotonin from dorsal raphe and the action of weight-reducing agents*

Electrical stimulation of the dorsal raphe area (i.e., a raphe area different from the raphe area possibly involved in the efferent pathway from the hypothalamus to brown adipose tissue; see sect. v*G*) leads to activation of brown adipose tissue and to an increased body temperature (162, 178). The signal mediating this is probably serotonin (5-HT) released in the ventromedial area (162). This released serotonin probably interacts with the VMN, leading to activation of brown adipose tissue thermogenesis. Serotonin injection into the VMN in itself leads to activation of brown adipose tissue (702); correspondingly, the serotonin antagonist metergoline attenuates the thermic effect of a single meal (686), and the serotonin synthesis inhibitor *p*-chlorophenylalanine leads to decreased brown adipose tissue activity and atrophy of the tissue (230).

This serotonin pathway is considered central in the regulation of satiety, and an increase in the serotonin concentration has therefore weight-reducing effects. The induced satiety, and the resulting anorexia, are normally considered the main effects of current weight-reducing agents. However, the response to the reduced food intake is not of the type encountered during fasting, where brown adipose tissue inactivation and atrophy occur (see sect. vI*A2*); rather, it is of the type encountered during a single meal (see sect. vI*A1*), where thermogenesis and a feeling of satiety are induced in parallel (see, e.g., Ref. 498 for an experimental demonstration of this difference). Thus this type of weight-reducing agent normally induces both anorexia and decreased metabolic efficiency (activated brown adipose tissue), although the anorexic effect tends to be the one observed and measured.

The weight-reducing drugs in this system may thus be serotonin reuptake inhibitors or inducers of serotonin release or serotonin agonists. Among serotonin reuptake inhibitors (notably used pharmacologically mainly as antidepressive drugs), sibutramine (Reductil) has been especially studied. Sibutramine leads to activation of brown adipose tissue indirectly through activation of the sympathetic nervous system (143, 310, 765); it is, however, likely that inhibition of reuptake of central norepinephrine is also necessary for its thermogenic action (143, 310, 765). Serotonin release is stimulated by the drug fenfluramine, and this similarly leads to activated, and with time recruited, brown adipose tissue and to decreased metabolic efficiency (22, 439, 466, 468, 473, 677).

It is probable that brown adipose tissue activity is thus essential for the thermogenic effect of these types of substances in rodents. However, as they are also anorexic, brown adipose tissue activation is not essential to obtain the weight-reducing effect. Even in humans, sibutramine increases thermogenesis; the direct mechanism for this is unknown, but it is associated with increased adrenergic activity (295). This may be seen as an indication that even in humans an adrenergic thermogenic response may be induced due to activation of the central pathways that in animals undoubtedly activate brown adipose tissue.

Also the increased appetite, metabolic efficiency, and body weight in gonadectomized animals of either sex (see sect. vI*C*) may be related to this pathway, as sex hormones decrease the enhanced serotonin levels observed in the VMN in gonadoectomized animals (463).

Antipsychotic drugs (neuroleptics) (which are not functionally related to the serotonin pathways) often lead to increased body weight, through pathways not presently clarified. The possibility that an increased metabolic efficiency, (partly) due to inactive brown adipose tissue, may fortify the appetite-promoting effect of these drugs has not been studied.

7. *Lateral hypothalamic nucleus inhibits*

Electrical stimulation of the lateral hypothalamus is without effect on brown adipose tissue (or inactivates somewhat) (347, 392, 700, 772, 792). However, there are inhibitory inputs from the lateral hypothalamic to the ventromedial nucleus. These inputs exhibit a chronic attenuating effect that is evident from the fact that lesion of the lateral hypothalamus increases stimulation of brown adipose tissue (20, 167, 349, 520, 522, 611, 880). Physiologically, it is possible that low glucose may be sensed in the lateral hypothalamic area and activate it, thus leading to inhibition of brown fat activation (194).

8. *The paraventricular nuclei mediate the brown fat-inhibitory NPY-borne signal*

Electrical stimulation of the paraventricular nuclei (PVN) does not affect brown adipose tissue (348), and, similarly, lesions of the PVN have practically no effect on brown adipose tissue recruitment state (228, 703, 811, 880, 885). However, the NPY "hunger" signal, originating from the arcuate nucleus, inhibits brown adipose tissue through Y5 receptors in the PVN (53, 54, 192, 195, 409, 789, 849). CART inhibits this inhibition (407a, 851), i.e., it activates brown adipose tissue. The brown fat inhibitory effect of the hunger signal from the stomach in the form of ghrelin (877a) may also be mediated by NPY. The role of the hypothalamic peptide orexin is currently ambiguous.

Chronic neonatal treatment of animals with monosodium glutamate ("umami") leads to destruction of the arcuate nucleus. That such animals become hypophagic is expected because of the ensuing destruction of the NPY pathway (531); however, such animals also become obese, and the obesity is associated with inactivity and

atrophy of brown adipose tissue (531, 821, 881, 888, 889) (i.e., similar to genetic obesity and gold thioglucose obesity, but in contrast to recruiting-diet obesity). The expected effect on brown adipose tissue of destruction of the inhibitory NPY pathway should be increased recruitment, and no simple explanation for the observed atrophy can therefore be currently offered.

VII. UPTAKES AND CLEARANCES

The substrates for thermogenesis, i.e., lipids (derived mainly from triglycerides in chylomicrons) and glucose, represent major uptake processes by brown adipose tissue; both substrates may be used either directly or may be stored for later use. From a systemic point of view, an important question is whether the uptake is not only of significance for brown adipose tissue function but is also of such magnitude that it has systemic relevance, i.e., whether uptake in brown adipose tissue is of significance for clearance of these substances in the blood.

A. Lipid Clearance and Brown Adipose Tissue

1. Triglyceride clearance through lipoprotein lipase activity

The total food intake in experimental animals in the cold may be fourfold higher than at thermoneutrality, to supply the fourfold higher metabolism (Fig. 12). During nonshivering thermogenesis in cold-acclimated animals, practically all this extra food is combusted in brown adipose tissue.

Much of the food is available in the form of triglyceride in the blood. The major part of the triglyceride clearance of brown adipose tissue is due to "clearing factor," i.e., the enzyme lipoprotein lipase. Lipoprotein lipase is synthesized by the brown adipocytes themselves but is transferred to the capillaries and exerts its action on the circulating chylomicrons and lipoproteins (Fig. 20). In contrast to what is the case in white adipose tissue, in brown adipose tissue, norepinephrine stimulates lipoprotein lipase activity (108, 642). This makes physiological sense, because this channels circulating triglycerides into the tissue when it is thermogenic. The norepinephrine-induced increase in lipoprotein lipase activity is mainly due to an increased level of lipoprotein lipase mRNA, which in its turn is due to an increased gene transcription rate (513). The half-life of lipoprotein lipase protein is short (~2 h), whereas that of the mRNA is relatively long (≥20 h). The kinetics are thus such that activity would reflect mRNA half-life rather than protein half-life (513), and activity can thus be conveniently regulated by transcription rate.

2. Passive effect of increased lipoprotein lipase activation on fatty acid composition of triglycerides and phospholipids

In unstimulated brown adipose tissue, most of the triglyceride lipids originate within the brown adipose tissue itself (or from elsewhere in the animal), mainly from de novo synthesis from glucose cleared from the circulaton (see sect. VII*B*). Accordingly, a fatty acid pattern consisting mainly of saturated or monounsaturated fatty acids (palmitic, oleic) is observed in the triglycerides of nonactivated tissue.

When lipoprotein lipase is activated, the fatty acid composition of the trigycerides of the tissue alters to closely reflect that of the diet. This occurs rapidly for the stored triglycerides (110). With time, also the phospholipid pattern may change. The composition of the fatty acids of certain phospholipids, especially those directly involved in signaling processes (such as the phosphoinositides of the cell plasma membrane), is probably strictly regulated, but this is apparently not the case for most of the bulk of phospholipids found in both the cell membrane and in the mitochondrial membranes (the dominating phospholipid compartment in brown adipocytes). Thus the available fatty acids tend to be the ones incorporated into new phospholipids, but because the turnover of phospholipids is slow, the alterations would tend to be smaller and slower than those in the triglycerides. Nonetheless, there are a series of reports, similar to that noted above for triglycerides, demonstrating a change from a saturated towards an unsaturated phos-

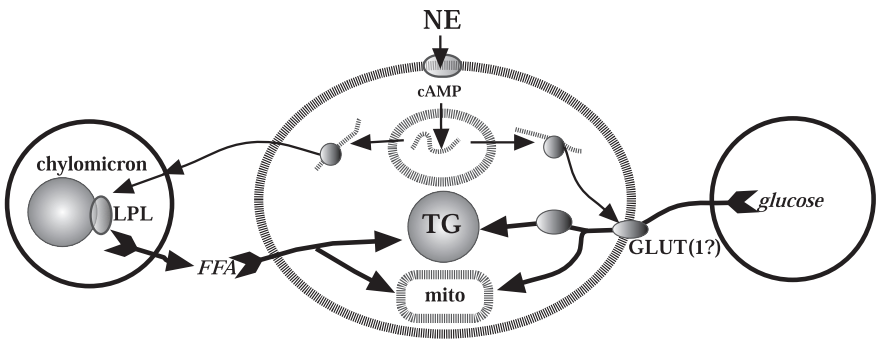

FIG. 20. Adrenergic stimulation of fatty acid and glucose uptake into the brown adipocyte. The left side depicts the ability of norepinephrine (NE) to increase the amount and thus activity of lipoprotein lipase (LPL) through a cAMP-mediated increase in gene expression, leading to increased uptake of free fatty acids (FFA) that are either combusted in the mitochondria or stored in the triglyceride (TG) droplets. Whereas this scenario is well-estabished, the pathway for norepinephrine-stimulated glucose uptake is not firmly established, but it may perhaps (right side) include an increase in the amount and activity of the glucose transporter GLUT1.

pholipid fatty acid composition under conditions of constantly activated tissue, such as chronic cold (661, 662, 664, 720; although this is not always seen, Ref. 104), chronic norepinephrine infusion (535), or experimental pheochromocytoma (663); similarly, a tendency is seen in the opposite direction under conditions of chronic inactivation of the tissue, such as maintenance at thermoneutral conditions (699), during fasting (698), and during postnatal development (601). These changes, which are thus in the direction of more unsaturated fatty acids in the activated state, will influence the physical characteristics of the lipids and membranes, principally by making them more fluid at lower temperatures, but there is no demonstrated functional significance of this, since the cells do not themselves encounter lower temperatures than 37°C. Thus, although often speculated that these types of changes are significant in the recruitment or thermogenic process, there is little if any evidence to support this tenet, and the phenomenon seems to be explainable merely by the increased lipoprotein lipase activity and utilization of dietary (i.e., more unsaturated) fatty acids.

B. Is Brown Adipose Tissue an Important Organ for Glucose Clearance?

Brown adipose tissue has a very high uptake of glucose per gram of tissue, which means that even though the total amount of brown adipose tissue in the body is not large, it can potentially be a significant glucose-clearing organ. Glucose uptake is stimulated in two opposite metabolic states: during active thermogenesis (stimulated by norepinephrine) and during active anabolic processes (stimulated by insulin).

1. Norepinephrine and GLUT(1?)-mediated glucose uptake

Glucose uptake is markedly stimulated by cold exposure (279, 603, 727, 736, 833). This occurs even in starved rats, which have very low insulin levels, indicating that insulin cannot be responsible (727), and implying a sympathetic pathway. In accordance with this, denervation prevents the cold-induced increase in uptake (736), and glucose uptake is stimulated both in vivo (145, 453, 474) and in vitro by norepinephrine and other adrenergic agents (187, 452, 479, 480, 606). The adrenergic response is mainly β-adrenergic and mediated by cAMP (130a, 480).

The mechanism of norepinephrine-induced glucose uptake is not clarified. Glucose uptake is generally mediated by protein transporters of the GLUT family, and the dominant isoform in brown adipose tissue is the muscle/fat-specific isoform GLUT4 (435b, 705). However, norepinephrine apparently fails to translocate GLUT4 to the plasma membrane. Concerning the alternative glucose transporter GLUT1, the situation is controversial, with some authors claiming that GLUT1 always resides in the plasma membrane, while others demonstrate translocation in response to certain hormone signals. It has been proposed that norepinephrine increases the functional activity of GLUT1 in a cAMP-dependent manner (735, 738), without increasing translocation.

The norepinephrine-induced glucose uptake may be important in different ways. It may be speculated that glucose has a direct role as a thermogenic substrate; indeed, the major part of the energy of ingested food comes in the form of carbohydrate, and it would be meaningful if it could be combusted in this form. However, most estimates support only a minor role (5–15%) for glucose as a direct oxidative substrate in brown adipose tissue, and most glucose taken up is converted to pyruvate and then to lactate and exported from the tissue (474). This glucose may function glycolytically to yield cytosolic ATP during thermogenesis (332, 474, 567). Some of the pyruvate may, however, enter the mitochondria to be converted to oxaloacetate by pyruvate carboxylase and by this anaplerotic reaction increase the capacity of the citric acid cycle (101). In agreement with this, norepinephrine-induced glucose uptake is eliminated when fatty acid oxidation is inhibited (479). Some of the glucose may be converted into fatty acids and triglyceride. Indeed, not only glucose uptake but also the enzymes involved in the synthesis of fatty acids and triglycerides are high or even increased under conditions of recruitment, probably through norepinephrine induction; these include fatty acid synthase (85, 157, 512, 897), which generates saturated fatty acids, with chain lengths of 16–18 carbons, as well as chain-elongating systems, including the Elovl3/Cig30 (823), Elovl6/LCE (525), fatty acid desaturase (182), and the lipogenesis-related "spot 14" (224); some of these are increased in a dramatic way. The cellular relevance of increasing the capacity for fatty acid synthesis, and presumably therefore the actual fatty acid synthesis, under conditions of ongoing thermogenesis is not immediately evident: the main substrate for thermogenesis is fatty acids, and the question is therefore why the cell would endeavor to synthesize fatty acid and then immediately break it down. One explanation could be that fatty acids are needed for the activation of thermogenesis through UCP1 (see sect. II*B4*), but the amount necessary for this could be obtained from uptake of fatty acids from the circulation (see sect. VII*A*). Alternatively, recruitment of the anabolic pathway could occur in anticipation of a later period of decreased thermogenic activity, allowing time for refilling of lipid stores.

The systemic significance of the norepinephrine-induced glucose uptake into brown adipocytes has not been directly quantitated, but in spite of the remarkable increases in glucose uptake after, e.g., β_3-adrenergic agonist stimulation, the quantitative role of the tissue in regulation of blood glucose concentration is probably minor (118).

2. Insulin and GLUT4-mediated glucose uptake

The alternative, and more white fat-like, regulation of glucose uptake in brown adipocytes is through an insulin-stimulated GLUT4 mechanism, probably mainly active under conditions of decreased thermogenic activity.

A) INSULIN SIGNALING IS SIMILAR TO PATHWAYS IN WHITE ADIPOSE TISSUE. Although the intracellular pathways of insulin signaling in brown adipose tissue have not been studied in full detail, the observations available would indicate that the pathways involved are similar to those in white adipose tissue. Most studies have been performed in fetal brown adipocytes, maintained in the absence of serum or growth factors, or in immortalized and/or transformed cell lines from fetal or newborn brown adipose tissue.

Insulin binding to insulin receptors (795) leads to receptor tyrosine kinase activation and to tyrosine autophosphorylation of the receptors (210). The signaling steps downstream of the receptor involve the insulin-receptor substrate proteins IRS 1–4 (Fig. 21).

The physiological effects of activation of these insulin pathways in brown adipose tissue are pleiotropic. As in other tissues, insulin has growth-promoting effects and can be mitogenic (622) and has anabolic effects, both on glucose uptake and metabolism (see sect. VII*B2*B) and on lipid accretion, both through stimulation of de novo fatty acid synthesis from glucose (see sect. VII*B2*B) and through stimulation of uptake of free fatty acids through lipoprotein lipase (109, 513) and triglyceride synthesis. Numerous reports indicate insulin effects on gene expression in these pathways (FAS, malic enzyme, glycerol 3-phosphate dehydrogenase, GLUT4, etc.; Refs. 211, 458, 840).

B) INSULIN AND UPTAKE OF GLUCOSE. Physiological conditions in which plasma insulin levels are elevated (refeeding) show increased glucose uptake into brown adipose tissue (750, 773), and conversely, states with low insulin levels (starvation or fasting) (773, 832) or where insulin resistance has developed (chronic high-fat diet, obesity) (86) demonstrate reduced glucose uptake. Accordingly, insulin directly stimulates glucose uptake both in vivo (145, 278, 368) and in isolated cell systems (155, 187, 479, 480, 606, 607). Brown adipose tissue is one of the most insulin-responsive tissues with respect to stimulation of glucose uptake (766).

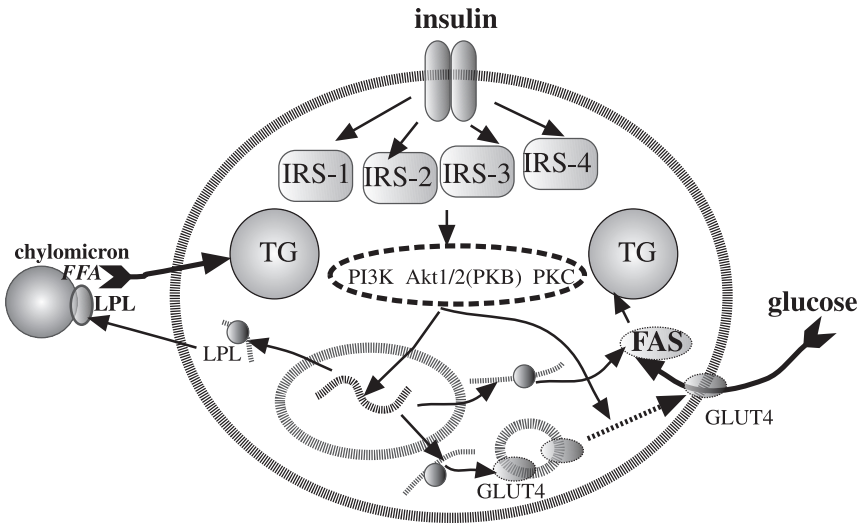

FIG. 21. Insulin signaling leading to glucose uptake and anabolism. A congruent picture cannot presently be made in any tissue, but the sketch depicts some of the pathways discussed in brown adipocytes. Insulin receptor substrate-1 (IRS-1) becomes tyrosine-phosphorylated and activated in response to insulin (837). Further signaling is probably through Akt activation, because immortalized brown adipocytes from mice with ablated IRS-1 have decreased Akt activation, lack GLUT4 expression, and possess essentially no insulin-stimulated glucose uptake (210), and the Akt inhibitor ML-9 inhibits glucose uptake and GLUT4 translocation (321). Insulin receptor substrate-2 (IRS-2) also becomes tyrosine-phosphorylated and activated in response to insulin (837). IRS-2 binds to the regulatory p85 subunit of phosphatidylinositol 3-kinase (PI3K), releasing and activating the p110 α-subunit (836). In immortalized brown adipocytes from IRS-2-ablated mice, Akt phosphorylation and activity are normal (211), but such cells still demonstrate a decreased insulin-stimulated glucose uptake, indicating another pathway for stimulation of glucose uptake than that through Akt. [Immortalized brown adipocytes from IRS-3-ablated mice also differentiate poorly, whereas such cells from IRS-4-ablated mice differentiate normally but have alterations in apoptosis control (399).] Further downstream effects of the different IRSs are not clearly distinguished as yet. IRS-1 (but probably also other IRSs) recruits SHC, Grb-2 and Sos. This probably activates Ras proteins, which then activate downstream mitogen-activated protein kinases (548, 621) (MEK1/2 and Erk1/2) which, in immortalized brown adipocytes, leads to DNA synthesis (622, 838) (not shown on figure). Insulin also activates the gene expression of lipoprotein lipase (LPL), of the fatty acid synthase complex (FAS), and of the glucose transporter GLUT4. Insulin also stimulates the transfer of GLUT4 to the plasma membrane, in this way increasing glucose uptake.

The mechanism for insulin-induced glucose uptake in brown adipocytes seems similar to general patterns from other tissues. Under basal conditions, 99% of the GLUT4 proteins may be found intracellularly, in the *trans*-Golgi reticulum and tubulovesicular structures (748). Insulin induces the translocation of GLUT4 (and GLUT1, Ref. 607) from intracellular stores to the plasma membrane, thus enhancing glucose uptake (607, 734, 748, 839).

In addition to stimulating GLUT4 redistribution, insulin also increases GLUT4 expression (799). Conversely, starvation leads to decreases in GLUT4 mRNA and protein (99, 294, 750), and another insulinopenic condition, diabetes, similarly decreases GLUT4 expression (18, 99, 605). Numerous studies in various animal models of obesity associated with insulin resistance, such as *ob/ob* (24), *fa/fa* Zucker (123), New Zealand obese (217), Long-Evans Tokushima Fatty (826), MSG-obese (531), GTG-obese (435b) and high-fat diet (86, 258) all show decreases in GLUT4 in brown adipose tissue.

The glucose taken up under the anabolic circumstances characterized by high insulin can be metabolized to provide glycerol phosphate for triglyceride synthesis or can itself provide 2-carbon units for de novo fatty acid synthesis (497, 774). The extent of de novo fatty acid synthesis perhaps depends on species (497). Alternatively, glucose can be stored as glycogen. Glycogen accumulates after transfer of animals to warm from cold (209), and glycogen present in brown adipose tissue at birth is utilized immediately after birth (248).

C) THE BATIRKO MOUSE. The extent to which the insulin-stimulated pathways are indispensible for brown adipose tissue function can in part be estimated from studies of a transgenic mouse lacking the insulin receptor specifically in brown adipose tissue (the BATIRKO mouse) (289). The weight of the brown adipose tissue is decreased in these mice, predominantly due to a decreased lipid accretion. Accordingly, the mRNA levels of enzymes of fatty acid synthesis are decreased. The cell number is, however, apparently not decreased. The results indicate that insulin action is necessary for lipid accretion in brown adipose tissue, but not for maintenance of cell number. The studies are as yet limited in that the mice have not been challenged to recruit brown adipose tissue in, e.g., cold acclimation, and the role of insulin under such circumstances thus not evaluated.

Conversely, the systemic significance of brown adipose tissue for insulin-induced glucose clearance is demonstrated by the BATIRKO mice (289). Such mice have fasting hyperglycemia and impaired glucose tolerance (without insulin resistance), indicating a major role of brown adipose tissue in glucose clearance, a role which apparently could not be compensated by glucose uptake elsewhere.

C. During Nonshivering Thermogenesis, Brown Adipose Tissue Is the Major Oxygen-Consuming Organ in the Body

In addition to triglycerides (and glucose) as oxidizable substrates for thermogenesis, oxygen is also needed for combustion. It is remarkable that brown adipose tissue, which at the most constitutes a few percent of the body mass of an animal, utilizes during peak thermogenesis practically all the extra oxygen consumed in the body. This means that in a small animal in the cold, more than one-half of all oxygen taken up (as well as more than one-half of all food eaten) is transferred to brown adipose tissue, and all this oxygen is then reduced there, certainly an impressive accomplishment for this minor tissue. In consequence, the organism has to direct the major part of its total cardiac output to brown adipose tissue; how this is accomplished is still not known. Basically, the increased blood flow can be centrally or locally regulated; if centrally, especially NPY from the sympathetic nerves could be involved (see sect. v*G7*), if local, nitric oxide could be involved (see sect. viii*B3*). Experiments where the oxygen-carrying capacity of blood was varied may be interpreted to indicate that blood flow is mainly locally controlled and altered based on demand for oxygen (471).

VIII. BROWN ADIPOSE TISSUE AS A SECRETORY ORGAN

Although adipose tissue in general has become recognized as a prominent endocrine organ, the relatively small size of brown adipose tissue indicates that a systemic endocrine role can only be postulated for substances that are produced and secreted in significant amounts. A local autocrine and/or paracrine role is therefore often more probable for most substances; physiologically this is mainly of interest in relation to the recruitment process. We have here divided secreted substances into those that have their effects foremost on brown adipocytes (or preadipocytes) in the tissue (autocrine), on other cell types of interest mainly for the brown adipocytes themselves (paracrine), and those which possibly represent a truly endocrine secretion (Fig. 22).

A. Autocrine

1. Basement membrane proteins

Basement membrane proteins secreted from brown adipose tissue include collagen IV, laminin, heparan sulfate proteoglycan, and fibronectin (297). Collagen IV (A2COL6) is a specific marker for the early steps in cell differentiation (152).

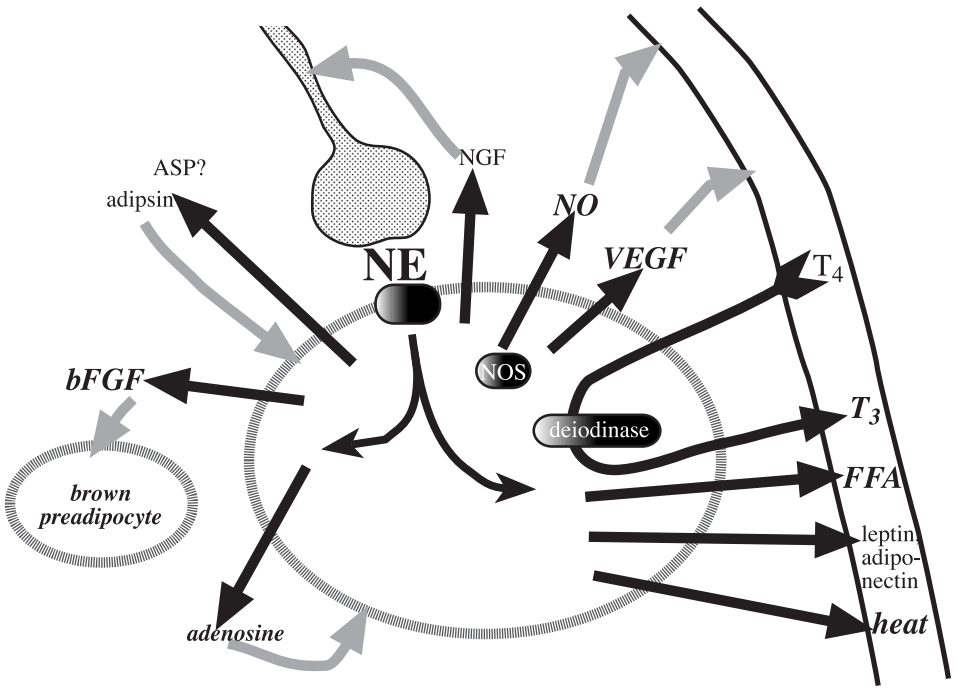

FIG. 22. Autocrine (*left*), paracrine (*top*), and endocrine (*right*) functions of brown adipocytes. Those factors, the secretion of which is stimulated during sympathetic stimulation, are shown with italics; the others are decreased during brown adipose tissue activity. Gray arrows indicate action targets. NE, norepinephrine; NGF, nerve growth factor; VEGF, vascular endothelial growth factor; FFA, free fatty acids; bFGF, basic fibroblast growth factor; ASP, acylation-stimulating protein; NOS, NO synthetase.

2. Adipsin

Adipsin (also known as complement factor D) is a serine protease synthesized in and secreted from adipose tissue, including brown adipose tissue (547). Expression of the adipsin gene is not stimulated by sympathetic stimulation, nor is it repressed by sympathectomy, but treatment by a β_3-adrenoreceptor agonist suppresses gene expression (547). Thus it is negatively associated with (or dissociated from) activation and recruitment. Adipsin can cleave complement protein C3 into C3b and C3a, the latter can be inactivated to C3adesArg, also known as acylation-stimulating protein (ASP), a small protein that stimulates triglyceride synthesis and glucose uptake at least in white adipocytes (132, 396). C3 is found at higher expression levels in preadipocytes from white than from brown adipose tissue (61, 62). The presence of ASP has not been demonstrated in brown adipose tissue, but an anabolic function of adipsin/ASP would fit with adipsin expression being a sign of thermogenically inactive brown adipose tissue, i.e., in an anabolic state.

3. Basic fibroblast growth factor

Basic fibroblast growth factor (bFGF or FGF-2) is synthesized in brown adipose tissue. Acute and chronic cold exposure lead to an increase in bFGF gene expression (29, 877), and this increase can be mimicked in cell culture by norepinephrine, acting at least in part through β-adrenergic receptors (451, 876).

The corresponding FGF-receptor 1 is expressed in the brown adipocytes themselves (451), and bFGF can apparently through this increase the density of brown adipocyte precursor cells in culture (236, 877), as can conditioned medium from brown adipocyte cultures; this effect is inhibited by anti-FGF. The increased cell density may be partly through an inhibition of apoptosis, via activation of MAP kinase Erk1/2 pathways (451).

As norepinephrine induces bFGF expression in brown adipocytes and as bFGF increases cell growth, the possibility exists that some of the growth-promoting effects of norepinephrine (see sect. III*A*) may be indirectly meditated, i.e., via bFGF. In accordance with this, the stimulatory effects of norepinephrine on cell density and on inhibition of apoptosis are partly inhibited by anti-FGF antibodies (451, 876). bFGF may thus be an indirect mediator of the prolonged growth phase of the tissue during recruitment processes. Two other members of the FGF family may be involved in brown adipose tissue recruitment: aFGF, which is a potent mitogen (236), and FGF-16, which is mainly expressed during embryonic development (514); its expression is decreased during cold acclimation. The mitogenic activity of FGF-16 on brown adipocytes from embryonic tissue is mediated by FGF-receptor 4; it may be a growth factor unique for embryonic brown adipose tissue (407).

4. Insulin-like growth factor I

Insulin-like growth factor I (IGF-I) mRNA levels increase in brown adipose tissue of rats during cold exposure (875). IGF-I receptors are highly expressed on the plasma membrane of brown adipocytes (459). IGF-I is mitogenic for fetal (but not for newborn, Ref. 318) brown

adipocytes (459, 835) and can prevent TNF-α-induced apoptosis (623), and anti-growth hormone antibody, expected to decrease IGF-I, inhibits cell proliferation in brown preadipocytes (875). Thus IGF-I may also be involved in the recruitment process.

5. Prostaglandins

Prostaglandins are proposed to have important autocrine functions in white adipose tissue (6, 571), but arachadonic acid metabolism has been poorly studied in brown adipose tissue to date: only prostaglandins E_2 and F_α have been tentatively identified (624, 625).

6. Adenosine

Brown adipocytes, just as most other cell types, may release adenosine, supposedly under conditions of low energy charge within the cells (high AMP). The brown adipocytes themselves possess adenosine A_1 receptors that are inhibitory for thermogenesis (695, 788, 828). To what extent they control thermogenesis in vivo is unknown.

B. Paracrine

1. Nerve growth factor

Nerve growth factor (NGF) is a neurotrophin, essential for survival and maintenance of sympathetic neurons. NGF is secreted from brown adipocytes (552, 582, 584). The secretion is high during perinatal development (552, 584). The further regulation of NGF secretion is not clear but may be self-inhibitory. The data available (74, 552, 582, 584) may be consistent with a view that NGF is mainly secreted from proliferative brown preadipocytes during phases of tissue growth. This will promote sympathetic innervation and thus permit increased norepinephrine stimulation of the cells; however, in the (mature) brown adipocytes, norepinephrine stimulation is inhibitory for NGF secretion, and a new steady-state innervation density may thus be achieved. The invasion of brown adipose tissue by sympathetic neurons is regulated by the release of NGF, attracting the neurons to the tissue: rats immunized to produce anti-NGF demonstrated atrophy of superior cervical ganglia and serious reduction in neuronal number, and norepinephrine content in brown adipose tissue is reduced by 90% (272).

2. VEGF

Vascular endothelial growth factor (VEGF) proteins are among the most important angiogenic factors recognized.

VEGF-A is highly expressed in brown adipose tissue (30), and it is well expressed in both proliferating and in mature brown adipocytes (225). Its expression is stimulated by cold exposure (30) and by norepinephrine stimulation (225, 812), through β-adrenergic pathways (225, 812) involving cAMP/protein kinase A and Src tyrosine kinase, but not the MAP kinase Erk1/2 pathway (225).

VEGF-B is also expressed in brown adipose tissue (29, 422, 423), but its expression is not altered by sympathetic stimulation (29). VEGF-C is also expressed, but its expression is suppressed by adrenergic agonists (28).

The VEGF receptors Flk-1 and Flk-4 are expressed in brown adipose tissue, but no VEGF receptors are expressed in primary cultures of brown adipocytes (225; unpublished observations). The receptors are therefore most likely expressed on the numerous endothelial cells in the tissue and could in theory be involved in the extensive angiogenesis occurring during cold-induced tissue recruitment; VEGF thus has a paracrine effect. However, sympathetically stimulated VEGF expression, both in vivo during cold exposure and in vitro during norepinephrine stimulation, peaks after only 2–4 h and then returns to basal levels, and this time course does not really support an angiogenic role (30, 225). Rather, VEGF may be involved in the maintenance of the constitutively high level of vascularization in the tissue but not in its growth. An alternative role, in accordance with one of the original names of the factor, vascular permeability factor, could be in bringing about the notable edema seen during the first hours of cold stress (108, 306, 719) (but the physiological significance of this phenomenon is obscure). In that case, another angiogenic factor must be responsible for inducing the marked increase in vascularization of the tissue during, e.g., cold acclimation.

3. Nitric oxide and blood flow

Nitric oxide (NO) is a widespread cell signaling molecule, earlier known as endothelial-derived relaxing factor, because of its potent vasodilator action. NO is produced from L-arginine by nitric oxide synthases (NOS), of which the inducible NOS (iNOS = NOS II) (583) and the constitutive endothelial NOS (eNOS = NOS III) (394) isoforms have been identified in brown adipose tissue; the constitutive brain NOS (bNOS = NOS I) is absent (394). The eNOS is not only present in endothelial cells in brown adipose tissue (372), but also in the brown adipocytes (394). Both iNOS and eNOS expression are increased by cold exposure, probably via β-adrenergic receptors (394, 583).

The brown adipocytes themselves thus have the possibility to generate NO. If NO is produced by iNOS, synthesis of this inducible enzyme is required, whereas NO may be immediately generated by the constitutive eNOS. Norepinephrine stimulation of isolated tissue pieces (696) or cultured brown adipocytes (583) induces NO production; this NO production is inhibited by actinomycin (583).

For NO, both autocrine and paracrine effects may be discussed. Within the brown adipocyte itself, NO may inhibit mitochondrial respiration, probably by a high-affinity competitive effect directly at the oxygen binding site in cytochrome-*c* oxidase (405). If this were a physiologically occurring phenomenon, it could lead to an inhibition of thermogenesis. It may, however, only become evident at extremely low oxygen tensions (404). Long-term treatment of cultured brown adipocytes with NO donors decreases cell proliferation and increases differentiation (580) through unknown mechanisms.

Within the tissue, NO could mediate the rapid and vast increase in blood flow occurring during increased brown adipose tissue activity (from 2 to 57 ml/min in cold-acclimated rats treated with norepinephrine, Ref. 222). The increase in blood flow is apparently secondary to the demand for extra oxygen, as an increase is not seen in UCP1-ablated mice (275a). Arteriovenous anastomomes have been described in the tissue (437, 588), but so far there is no functional study of the regulation of the blood flow through these and of a possible shunting of the blood from these to the tissue during thermogenesis. NO or NPY (see sect. v*G*) could be involved in the increase in blood flow since systemic inhibition of NO production by N^G-nitro-L-arginine methyl ester (L-NAME) decreases both resting (280, 281, 457) and norepinephrine-stimulated blood flow to brown adipose tissue (544). However, because L-NAME may acutely decrease sympathetic firing rate in the tissue (168) (with atrophy as a chronic effect, Ref. 697), the effect on blood flow may be indirect and a consequence of a lack of brown adipose tissue activation, and the significance of NO for the vast increase in blood flow seen in active tissue has thus not been unequivocally demonstrated. Because the increase in blood flow is a very rapid process, the NO cannot be produced by iNOS, but must come from the constitutive eNOS activity. However, a direct demonstration of eNOS-mediated NO production in brown adipocytes, e.g., in response to norepinephrine stimulation, is so far lacking.

4. Angiotensinogen

Angiotensinogen is an inactive hormone precursor that is converted to angiotensin I by renin and by subsequent cleavage by angiotensin converting enzyme (ACE) to the active form angiotensin II. Of the factors in this cascade, angiotensinogen is found in brown adipose tissue (98, 116, 117, 235, 264). Although renin activity has been found in brown adipose tissue (even after perfusion to remove blood), PCR determination failed to show expression of renin; consequently, renin protein is perhaps taken up from the circulation (724). The final product angiotensin II is found in the tissue (115).

Angiotensin II receptors (114) exist in the tissue, and through these angiotensin II may somehow (in a paracrine manner) increase norepinephrine release (115, 202). Although this would seem to indicate a reinforcing effect of the angiotensinogen system on thermogenesis, the expression of angiotensinogen does not follow the activity state of the tissue [increased both by cold exposure (114), and by genetic obesity (113)], and a role can therefore not presently be formulated.

C. Endocrine

For information about IL-1 and IL-6, see section v*E*.

1. Fatty acids

At least in vitro, brown adipocytes, when maximally stimulated by norepinephrine, produce more fatty acids due to stimulated lipolysis than they can combust (568), and fatty acids are indeed released from the tissue during maximal stimulation (474). Whether these released fatty acids are of physiological significance or merely represent an imbalance between the lipolytic and the thermogenic capacity in the brown adipocytes is not known.

2. Leptin, adiponectin, and resistin

Leptin is expressed in adipose tissues and is expected to monitor (among other things) the status of the energy reserves of the animal, more being secreted the larger (more fat filled) the cell is. Leptin is also expressed in brown adipocytes, but only under conditions of inactivity and atrophy. Conditions associated with activation of brown adipose tissue, such as cold, decrease leptin gene expression, often down to undetectable levels, whereas inactivating conditions increase expression. The expression is accordingly negatively regulated via β_3-adrenoceptors (95), cAMP, and protein kinase A activation, but further steps have not been defined. These conditions are, of course, also associated with a lower lipid content in the cells, i.e., smaller cells. Brown adipocytes are in themselves normally smaller than white fat cells, and as adrenergic stimulation further decreases the size of lipid droplets, a specific mechanism for adrenergic suppression of leptin expression in brown adipocytes may not be needed: the brown adipocytes may simply react as all fat cells and with the help of leptin secrete information on their lipid reserves.

Thus leptin secretion may be considered as a characteristic of poorly differentiated brown adipocytes (with respect to thermogenic capacity), perhaps as a vestige of the evolutionary history of the cells as "white" adipocytes, and leptin is probably therefore hardly of physiological significance within brown adipose tissue.

Adiponectin is also expressed in both white and brown adipose tissues and similarly to the case for leptin, its expression is diminished by adrenergic stimulation

(630a, 843a, 898a). While resistin is expressed in brown adipose tissue, regulation of its expression is unclear (630a, 843a).

3. T_3

T_3 is the physiologically active form of thyroid hormone and is formed by deiodination of thyroxine (T_4). One of the deiodinases, type II, is found in brown adipose tissue (743), and the activity of this deiodinase is increased in the cold (745) through a synergistic α- and β-adrenergic effect (637). The increase in activity is due to an increased expression of the gene (385, 744). The absence of this deiodinase has profound negative effects on brown adipose tissue function (163).

The T_3 formed may have both autocrine and endocrine functions. T_3 increases in the cells to saturate the thyroid hormone receptors (50), and it is in this way probably directly involved in the regulation of UCP1 gene expression (see sect. III*B3*). The T_3 produced should theoretically be sufficient to affect systemic levels; calculations imply that brown adipose tissue is responsible for about one-half the total systemic conversion of T_4 to T_3 (745). In agreement with this, a net release of T_3 occurs from brown adipose tissue, and this release is increased 10-fold in the cold and eliminated by starvation (214). The physiological significance of the released T_3 is not known; animals without the T_3-generating brown fat deiodinase have normal serum T_3 values, but this may be because of a compensatory increase in T_4 levels in the animals (163).

4. *Is an antiobesity factor secreted from brown adipose tissue?*

The inital observations that brown fat-deficient mice became obese on a stock diet (462), whereas UCP1-ablated did not (200), could be interpreted to indicate that brown adipose tissue functioned not only by combusting an excess of food but also in some way secreted a signal that would modulate obesity: an antiobesity factor (506). However, such a hypothesis, although appealing, is dependent on the difference between UCP1-ablated and brown fat-deficient mice being observable even when these modifications are studied on the same genetic background (see sect. VII*B*). Thus such an antiobesity factor remains hypothetical.

5. *Heat*

Heat is, of course, the main "product" released from brown adipose tissue. The heat-producing capacity of the tissue can be calculated to be some 300 W/kg when it is working at its highest intensity; this is about two orders of magnitude higher than the normal metabolic rate of a mammalian tissue. This also means that an amount of brown adipose tissue corresponding to only a few percent of the body weight can produce as much heat as all the rest of the body.

IX. SIGNIFICANCE OF BROWN ADIPOSE TISSUE FOR HUMANS AND OTHER MAMMALS

A. Brown Adipose Tissue and Humans

Because human beings are mammals, albeit fairly large, there is no a priori reason to expect that brown fat-related results obtained in experiments with other mammals (even acknowledging that these are mostly rodents) are not principally valid for humans as well.

At least as newborns, we have relatively large deposits of brown adipose tissue, and UCP1 is found in the tissue (430, 431). That brown adipose tissue has a functional significance for us as newborns is illustrated by the significance that development of the incubator had for keeping alive premature infants (with an incompletely developed brown adipose tissue, Ref. 508), reported in France already at the end of the 19th century (189). Thus, although it may be said that the heat production in brown adipose tissue is optional if other means are available, in reality, it is nonetheless probably essential for the survival of human newborns under "normal" conditions.

It is often stated that with age, but probably more correctly with size (and social culture), our relative functional capacity of brown adipose tissue decreases (as indeed it does in other larger mammals); this is because of the relatively higher ratio between heat production from basal metabolism and smaller surface area encountered in all adult animals, and because clothing and indoor life protect us from cold. There is no reason to think that we deviate from other mammals in these respects, and it is therefore also likely that, given the necessity, we could regain or maintain the thermogenic capacity we had when young. Thus exposed to the same absolute cold (in degrees C) as experimental animals, our need for extra heat is less manifest due to our large size; however, when exposed to an equally severe relative cold, there is no a priori reason to think that adult humans would not recruit brown adipose tissue, although the experimental evidence is weak. Definitely other primates show cold acclimation-induced recruitment of brown adipose tissue (119, 120, 242). A pathological situation of extremely high adrenergic activation in humans is pheochromocytoma, in which adult humans certainly regain UCP1-containing brown adipose tissue (69, 201, 665).

Thus, until recently, it has generally been assumed that healthy adult humans are practically devoid of functional brown adipose tissue (154, 387). However, this earlier notion is being revised. Within conventional "white" adipose tissue depots in adult humans, islets of

brown adipocytes may be found, and UCP1 mRNA is detectable in human "white" adipose tissue (133, 238, 593) and its levels can be elevated in vitro by norepinephrine (121). Thus, if adult humans can demonstrate adaptive nonshivering thermogenesis, it may indeed be through recruitment of brown adipocytes, even in conventional "white" adipose tissue depots.

1. Norepinephrine-induced thermogenesis in humans

In adult humans, norepinephrine injection causes a thermogenic response (418, 436) (as indeed it does in non-cold-acclimated animals, see sect. v*C1*). However, because this response is evoked by a systemic injection of norepinephrine, it is probable that it represents the result of the simultaneous stimulation of all adrenergic receptors in the body, a situation that is unlikely to occur physiologically and will not be utilized by an organism requiring thermogenesis. This implies that conclusions concerning the localization of "nonshivering thermogenesis" in, e.g., humans, with this methodology will necessarily include a large fraction of nonadaptive (and probably thermophysiologically irrelevant) thermogenesis.

The evidence for adaptive adrenergic nonshivering thermogenesis in humans is scarce. Concerning cold acclimation, at least two positive reports, one experimental (386) and one occupational (389), imply the recruitment of an adaptive adrenergic nonshivering thermogenesis in adult humans, but only to the level of ~15% above basal. It is unknown whether the norepinephrine treatment used was sufficient to elicit a maximal response, but this is probably unlikely, in view of the risk factors involved in the use of high systemic norepinephrine concentrations.

Conversely, to inhibit possible brown fat-derived heat production by propranolol (as it is presumably through β_3-adrenoceptors) would demand such high propranolol doses that these would not be feasible for human use. That propranolol is effective is normally verified by monitoring, e.g., effect on heart rate, but as this inhibition is through β_1-receptors, which are much more sensitive to propranolol, the dose that would be needed to inhibit β_3-mediated thermogenesis should be 100-fold higher.

Concerning adaptation to diet, the most ambitious study published to date failed to observe diet-recruited increases in norepinephrine-induced thermogenesis (or decreases following low-calorie diet) (390). It can thus be questioned whether any diet-recruited adrenergic nonshivering thermogenesis exists in humans. However, again, it should be noted that the testing dose of norepinephrine may not have been adequate to activate any recruited brown adipose tissue.

2. A cure for obesity?

Although no adaptive UCP1-independent nonshivering thermogenesis exists constitutively, it could be (and has been) hypothesized that genetic differences in the magnitude of nonadaptive UCP1-independent nonshivering thermogenesis between individuals could be of significance for the development of obesity.

Conversely, it may be hypothesized that recruitment of a larger amount of brown adipose tissue would counteract the development of obesity, with its accompaning morbidities, such as type 2 diabetes. Consequently, investigations in this direction have been ongoing for a long period. The outcome of such studies is, however, to date meager, for understandable reasons. Because the proliferatively competent brown preadipocytes do not possess β_3-receptors, β_1-stimulation is needed to increase the number of brown adipocytes, with unavoidable consequences on, e.g., heart function. Nonadrenergic activators of specific transcription factors would probably also be dependent on preexisting brown preadipocytes. Furthermore, even if an increased amount of brown adipose tissue could be achieved by a given treatment, it must be realized that it would also have to be constantly activated.

Based on physiological situations, in humans or in other mammals, of increased energy expenditure, such as exercise, gestation, and lactation, and based on the general concept of the existence of an adipostat or energystat, increased energy expenditure should not necessarily lead to weight loss (just as a low metabolic rate does not necessarily lead to obesity; Ref. 259). Against this theoretical opinion stand experimental observations that chronic treatment of animals with β_3-agonists does indeed lead to weight reduction (245). Whether this is due to the observed activation and recruitment of brown adipose tissue, or to effects of β_3-treatment on other organs, especially white adipose tissue, is not known.

An alternative to promotion of recruitment of brown adipose tissue for counteracting obesity would seem to be to direct the expression of UCP1 to other organs, e.g., white adipose tissue (408a, 408b) or muscle. If ectopic *over*expression results, an unregulated artefactual mitochondrial uncoupling may be the outcome (96, 771). Thus to *over*express UCP1 in other tissues is not principally different from using a chemical uncoupler (although overexpression can be directed to specific tissues); chemical uncouplers (such as DNP), again surprisingly considering expected adipostat effects, do induce weight reduction, but at the cost of available ATP. Even though muscle and heart can sustain high ATP production when UCP1 is ectopically expressed in these tissues, muscle mass and composition are altered (151a, 440). Thus recruitment and activation of brown adipose tissue would still seem to be a better avenue to combat obesity which, with its comorbidities, is rightfully considered a major and increasingly important health problem.

B. Benefits of Nonshivering Thermogenesis

The development of a mechanism for nonshivering thermogenesis in the form of a new tissue, brown adipose tissue, and a new protein, UCP1, would seem to coincide with the development of the new chordate group, the mammals; there is no indication that UCP1 is found in any nonmammalian species. The UCP-like protein found in birds (645, 843) is similar to UCP2 and UCP3 but less so to UCP1 (65, 659), and it is therefore doubtful that it has thermogenesis as its function.

The acquirement of a mechanism for nonshivering thermogenesis is not an obligatory means for survival when mammals are exposed to cold (260). In reality, animals would probably seldom encounter a situation in which they experience a very sudden and large drop in environmental temperature; rather, a successively colder environment would be expected, and under such conditions, mammals can develop sufficient shivering endurance to allow them to defend their body temperature (260). Although an ability to regulate body temperature through endothermic mechanisms in the form of shivering enables active life in the cold, the ability to perform nonshivering thermogenesis still allows for a more comfortable existence in moderate cold. It would also seem that constant shivering correlates with a significant reduction in life span (260). Furthermore, the total range of survival temperatures increases with the development of nonshivering thermogenesis, because when the nonshivering thermogenic capacity becomes insufficient, the animal is still able to further increase heat production by shivering. Therefore, the development of nonshivering thermogenesis opened new niches for the developing mammals, both at new geographical locations and in new functional niches (such as the cold night).

Because we have worked with brown adipose tissue for several decades, it has been exciting for us to see an increasing understanding of the physiological significance of brown adipose tissue in many areas of biology and medicine. It is clear that the current scientific and pharmaceutical interest in brown adipose tissue is related to possibilities that it can be recruited to allow for body weight maintenance or reduction. However, should it finally turn out that brown adipose tissue is of little therapeutic value in adult humans, we can be comforted that it has been of paramount significance both in the early days of life for all of us, and in the early days of development of our mammalian pedigree.

We thank colleagues and students for stimulating discussions over the years and referees and colleagues for comments on this review. This work is in honor of our late teacher, mentor, and friend Olov Lindberg.

Our research into the physiology of brown adipose tissue has been continuously supported by the Swedish Science Research Council.

Address for reprint requests and other correspondence: J. Nedergaard, The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, SE-106 91 Stockholm, Sweden (E-mail: jan@metabol.su.se).

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