deposits (interscapular and dorso-cervical) flows almost directly to the heart. The tissue may therefore have importance as local warming elements[26]. In the guinea-pig, thermosensitive elements have been discovered in the cervical CNS that suppress shivering thermogenesis, if kept above a (variable) threshold temperature[27]. Local warming is also of importance during arousal from hibernation, as the thoracic region warms first and only then is warm blood shunted peripherally[28–30]. It should be noted that conclusive evidence is now available that in the cold acclimated rat, the hamster and the human neonate, BAT exports free fatty acids in amounts that can cause plasma free fatty acid levels to rise[31–34]. In the hibernating hamster, BAT may export acetate[35]. These substrates will be delivered along with the heated blood to local target organs. The possible importance of substrate export from BAT for NST in the whole organism is as yet uninvestigated.

In conclusion, BAT is now known to be widely distributed in mammalian neonates, cold-stressed adults and in hibernators. This indicates a physiologically important role of the tissue for the efficient functioning of the organism in a cold environment. During the neonatal period, BAT can contribute half or more of nonshivering thermogenesis, but the tissue involutes as other thermoregulatory mechanisms (insulation, muscular activity) become effective. In adult animals in the cold, BAT is attributed a local warming function on the CNS and heart, and it also exports free fatty acids in the heated blood. Just as white fat produces a certain amount of heat in addition to free fatty acids, so brown fat produces free fatty acids as well as heat. The most important qualitative differences between the 2 tissues, apart from the ratio in which each releases its products, might then reside in their control systems, which cause an activation of BAT by cold and of white adipose tissue by starvation. BAT has also been hypothesized to have an endocrine function, but the evidence for this is conflicting. An important source of technical difficulties, and hence ambiguities in the interpretation of results, can be traced to the diffuse distribution of BAT within the small rodents generally used for research. The appreciation that neonates of larger animals also have brown adipose tissue, which is largely concentrated to the abdominal cavity, may aid future attempts to define more precisely the ways by which this tissue affects thermogenesis.

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Role of the plasma membrane in brown fat thermogenesis

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The plasma membrane is the site of several mechanisms involved in the cold-induced heat generation by brown adipocytes. On the one hand, membrane receptors recognize an extracellular signal that arrives at the brown adipocyte in the form of graded amounts of norepinephrine (NE) as released from nerve terminals near the cell. This incoming signal originates in a complex neural network involving thermoreceptors strategically situated to provide the central nervous system with inputs that are integrated and channelled over efferent sympathetic pathways. Following recognition by the membrane, the extracellular signal is converted to an intracellular message capable of activating the metabolic pathways that result in elevated rates of brown fat heat production. Finally, the plasma membrane appears to function, to a certain extent, as part of the thermogenic effector mechanism itself. Thus, there are at least 3 aspects of brown fat thermogenesis with which the cell membrane is directly associated – recognition of the extracellular signal, transduction of this signal to an intracellular message, and conversion of chemical and potential energy to heat. These 3 aspects are the focus of the following discussion.

Recognition of the extracellular signal

The brown adipocyte membrane has both α-and β-adrenergic receptors for detection of the extracellular information. The evidence that activation of brown fat thermogenesis involves β-adrenergic receptors and

the stimulation of the adenyl cyclase-cAMP system derives from a variety of in situ as well as in vitro studies[1–3]. Moreover, that α-adrenergic receptors are also associated with the enhanced rate of brown fat heat production is suggested by a number of recent experimental findings including the following: a) interscapular brown fat temperatures are elevated following administration of phenylephrine (an α-adrenergic agonist) to intact, anesthetized rats. (This response is abolished by doses of phentolamine [an α-blocking agent] that do not significantly affect the increase in temperature induced by isoproterenol [a β-adrenergic agonist]. On the other hand, the phenylephrine stimulation is not inhibited by doses of propranolol [a β-antagonist] that are sufficient to depress the isoproterenol-induced response[4].) b) Rates of oxygen consumption (heat production) are augmented after addition of phenylephrine to isolated hamster brown adipocytes[5,6]. This enhancement is inhibited by concentrations of phentolamine that do not alter the magnitude of the isoproterenol-induced increase in oxygen consumption (Horwitz, unpublished). Thus both α- and β-adrenergic agonists appear capable of independently stimulating brown fat heat production, an observation that supports the view that NE activation of the thermogenic response reflects interaction with both types of membrane receptors, although the contribution of the β-pathway may be quantitatively more significant[5,6].

Transduction of the extracellular signal to an intracellular message

Although there is general agreement that the membrane of the brown adipocyte responds to catecholamines such that there is generated within the interior of the cell a recoded signal that stimulates the conversion of chemical energy to heat, there is no consensus as to the identity of the intracellular signal responsible for orchestrating this metabolic activation. The lack of agreement regarding the nature of the intracellular messenger reflects a difference in opinion as to the metabolic basis of the enhanced brown fat heat production. The 2 mechanisms that are currently under consideration are indicated below in terms of the intracellular signal that would be required for their activation.

1. *Mitochondrial uncoupling*

One widely-held view is that increased brown fat thermogenesis is mediated by the uncoupling of mitochondrial oxygen consumption from ATP synthesis (vide Nicholls, this issue, for a detailed description of this alternative). But despite the attractiveness of this putative mechanism, the nature of the intracellular signal that would elicit physiological uncoupling in the activated brown adipocyte has as yet to be identified.

2. *Increased ATP turnover*

In contrast, an explicit intracellular signal can be described for an alternative explanation of brown fat thermogenesis. Viewed in general terms, this alternative mechanism postulates that NE activation of the brown adipocyte is accompanied by a greater demand for ATP, and that this demand is met by increased rates of mitochondrial ATP synthesis coupled to substrate oxidation[3,7,8]. According to this mechanism, the intracellular message most directly responsible for stimulation of mitochondrial respiration (and therefore heat production) would be the [ATP] / [ADP] [P_i] ratio.

Evidence currently available is consistent with the contention that such a signal is generated by at least 2 components of the plasma membrane – namely, the membrane-bound enzyme adenyl cyclase and the Na^+/K^+ membrane pump. In terms of the latter ATP-requiring process, the possibility that pump activity is elevated following NE interaction with the plasma membrane was first suggested by Girardier et al.[9] and is compatible with data now available from several laboratories. For example, among the earlier observations consistent with this proposal are the findings that: a) NE administration to in vivo[7,10] or in vitro[9,11,12] preparations of brown fat results in redistribution of ions across the membrane of the adipocyte as indicated by a transient membrane depolarization; b) in vivo stimulation of the nerves to the interscapular brown fat pad is followed by membrane depolarization that precedes the rise in brown fat temperature[7]; c) administration of NE to the intact rat elicits changes in membrane resistance[10]; and d) in vitro incubation of brown fat pieces with NE evokes faster K^+ efflux, elevated intracellular Na^+ concentrations, and decreased intracellular K^+ levels[13]. These observations, taken together, support the view that NE increases

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the permeability of the adipocyte membrane, thereby facilitating the movement of K^+ and Na^+ down their electrochemical gradients. This augmented ion leak would, in turn, stimulate the Na^+/K^+ membrane pump to restore the intracellular ion concentrations toward resting, steady-state levels and return the membrane potential to its prestimulus value. Thus, this postulated mechanism predicts enhanced Na^+/K^+ pump activity concomitant with NE activation of brown fat heat production.

The most critical experiments designed to-date to determine if indeed any significant increases in pump activity do occur have involved examination of the effects of pump blockade[8,14]. In experiments with isolated hamster brown adipocytes,the pump was inhibited by addition of ouabain or by replacement of extracellular NaCl with choline chloride. Regardless of the method utilized, about 60% of the NE-induced orygen consumption was abolished[8]. In view of the unlikelihood that this respiratory depression is seccondary to changes in intracellular levels of Na^+ and/or K^+ resulting from pump blockade[5], it appears that the membrane transport system – by virtue of its requirement for ATP – plays a significant role in generating an intracellular signal capable of stimulating mitochondrial substrate oxidation.

Adenyl cyclase also contributes to the generation of this intracellular signal as a result of the catalytic role of the enzyme in the synthesis of cAMP. This synthesis promotes ATP turnover not only because of the conversion of ATP to the cyclic nucleotide, but perhaps more importantly, because cAMP has a stimulatory effect on the Na^+/K^+ membrane pump[15]. Evidence currently is thus consistent with the view that NE stimulation of the Na^+/K^+ pump activity is mediated in part by an action of cAMP on the transport system and in part by changes in membrane permeability and attendant increases in passive ion fluxes.

Conversion of chemical and potential energy to heat

The view that brown fat thermogenesis involves conversion to heat of the chemical energy stored in fat vacuoles and in the molecular oxygen diffusing into the adipocytes is generally accepted. It is further agreed that mitochondria play a central role in this energy conversion. That is, on command of the appropriate intracellular signal, mitochondrial reaction rates are increased, and at each step in the reaction sequence, a fraction of the chemical energy of the reactants is directly dissipated as heat. An additional site of heat evolution is the plasma membrane where, as ions passively diffuse across, some electrochemical energy is converted to heat. Heat is also generated with each step in the biochemical reactions associated with the membrane pump. Thus, both the mitochondria and the plasma membrane are sites of heat dissipation although the contribution of the former is likely to be quantitatively greater than that of the latter.

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Control of brown fat thermogenesis by the sympathetic nervous system

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Heat production in brown adipose tissue is known to involve both the cell membrane and the mitochondria and to be under the control of the sympathetic nervous system shose activation induces the following phenomena: a) change in membrane potential of the adipocyte resulting from an alteration of the ionic permeability of the membrane[1–3]; b) activation of adenylate cyclase resulting in an increased intracellular concentration of cyclic AMP which causes, through the activation of a lipase, an increase in the lipolysis of storage fats and a consequent increase in the intracellular concentration of free fatty acids[4,5]; c) increase in free fatty acid oxidation and cell respiration.

Temporal sequence of responses

Since the precise temporal sequence of these phenomena had never been determined, in vitro experiments using fast recording systems were performed in order to do this[6]. In all of the experiments, a fragment of brown

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