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. 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 oxygen consumption was abolished. In view of the unlikelihood that this respiratory depression is secondary to changes in intracellular levels of $Na^+$ and/or $K^+$ resulting from pump blockade, 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. 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.

Control of brown fat thermogenesis by the sympathetic nervous system

by J. Seydoux and L. Girardier

Department of Physiology, Faculty of Medicine, University of Geneva, Geneva, Switzerland

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 whose activation induces the following phenomena: a) change in membrane potential of the adipocyte resulting from an alteration of the ionic permeability of the membrane; 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; 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. In all of the experiments, a fragment of brown fat 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 whose activation induces the following phenomena: a) change in membrane potential of the adipocyte resulting from an alteration of the ionic permeability of the membrane; 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; c) increase in free fatty acid oxidation and cell respiration.

adipose tissue of rat was excised along with its nerve supply and tonometered in a physiological solution with a 95% O2, 5% CO2 gas mixture at 30°C. The nerve supply was placed on a pair of electrodes and stimulated at various frequencies. Membrane potential measurements were made by impaling the adipocyte with a glass KCl microelectrode whose tip was less than 0.5 μm in diameter and using a silver-silver chloride reference electrode placed in the physiological solution. Changes in cell respiration were detected by means of a rapid response polarographic microelectrode (less than 50 msec) whose tip, which measured about 2 μm in diameter had been introduced into the tissue. It was possible to monitor fatty acid oxidation by observing the changes in surface fluorescence of the reduced NADH component of the NADH-NAD couple since this reduction of pyridine nucleotides (NAD(P)H) results from the oxidation of fatty acids and is reflected by increased emitted fluorescence when the tissue is submitted to an excitation light of 366 nm. Simultaneous and successive recordings of membrane potential, PO2 tension and NAD(P)H redox of brown adipose tissue whose nerve supply was under electrical stimulation, made it possible to establish a temporal sequence for these phenomena which is presented in figures 1 and 2. Figure 1 shows the simultaneous recording of changes in membrane potential and PO2 tension within the tissue when the nerve supply is stimulated at 10 Hz. After a latent period of 1 sec a depolarization can be seen to occur. This is followed by a decrease in PO2 tension usually beginning at about 700 msec after depolarization and reflecting an increase in respiration. At frequencies lower than 3 Hz however, no depolarization occurred but an increase in respiration was noted at frequencies even as low as 0.1 Hz. Figure 2 shows the successive recordings, with the same tissue preparation, of the changes in PO2 tension and surface fluorescence of pyridine nucleotides when the tissue nerve supply is stimulated at frequencies of 0.3 and 1 Hz. At all frequencies tested, up to and including 10 Hz, the increase in the concentration of reduced pyridine nucleotides can be observed to start before the increase in respiration. It can also be seen that the response curves for the 2 phenomena are almost congruent. Thus, from these data, a precise temporal sequence of the events induced by stimulation of the nerve supply of brown adipose tissue at frequencies above 3 Hz can be established; first, the depolarization of the cell membrane, then, the increase in NAD(P)H and finally, the increase in cell respiration. However, since it was found that at stimulation frequencies below 3 Hz, no depolarization whatsoever occurred, it can be concluded that depolarization is not necessarily a concomitant of the increased metabolic activity and that when it does occur, it cannot be considered a consequence of this increased activity since it precedes it. Furthermore, since a response of increased metabolic activity of brown adipose tissue was obtained at stimulation frequencies as low as 0.1 Hz which is considerably lower than the frequencies generally quoted for other tissues, it can be concluded that brown adipose tissue must be under the tonic control of the sympathetic nervous system.

Circulating norepinephrine versus that released by the nerve endings

This conclusion raises the obvious question, however, as to whether it is the influence of the circulating norepinephrine or that of the NE liberated directly from the sympathetic nerve endings that is predominant. In an attempt to answer this question, it was necessary to determine the steady-state NE concentrations during trains of stimulation at various frequencies and comparing them with average levels of circulating NE7,8. A close approximation of this

---