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.

14 P. A. Herd, R. P. Hammond and M. W. Hamolsky, Am. J. Physiol. *224*, 1300 (1973).
15 B. A. Horwitz and M. Eaton, Eur. J. Pharmac. *34*, 241 (1975).

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 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

1 L. Girardier, J. Seydoux and T. Clausen, J. gen. Physiol. *52*, 925 (1968).
2 B. A. Horwitz, J. M. Horowitz and R. Em. Smith, Proc. nat. Acad. Sci. *64*, 113 (1969).
3 J. M. Horowitz, B. A. Horwitz and R. Em. Smith, Experientia *27*, 1419 (1971).
4 J. Skala, P. Hahn and T. Braun, Life Sci. *9*, 1201 (1970).
5 A. Beviz, L. Lundholm and E. Mohme-Lundholm, Acta physiol. scand. *81*, 145 (1971).
6 J. Seydoux, M. Tsacopoulos and L. Girardier, Experientia, in press (1977).

adipose tissue of rat was excised along with its nerve supply and tonometered in a physiological solution with a 95% O_2, 5% CO_2 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, PO_2 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 PO_2 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 PO_2 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 PO_2 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.

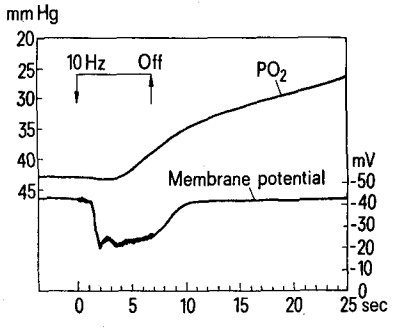

Fig. 1. Effect of electrical stimulation of BAT nerve supply in vitro at 10 Hz on PO_2 tension and membrane potential as shown by simultaneous recording.

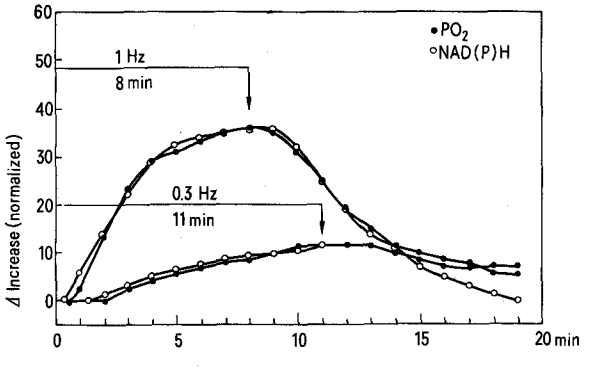

Fig. 2. Effect of electrical stimulation of BAT nerve supply in vitro at 2 different frequencies (1 Hz and 0.3 Hz) on PO_2 tension and redox level of pyridine nucleotides as shown by successive recordings made with the same preparation.

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 NE[7,8]. A close approximation of this

7 F. Depocas and W. A. Behrens, Can. J. Physiol. Pharmac. *55*, 212 (1977).

8 G. B. Picotti, H. U. Bühler and M. Da Prada, Experientia, in press (1977).

was achieved by the addition of NE in quantities sufficient to induce a response matching that of a given frequency stimulation as reflected by emitted surface fluorescence of the pyridine nucleotides.

This procedure was based on the assumption that the steady-state concentration of NE liberated by the nerve endings in the vicinity of the receptors during a train of electrical stimulation would be the same as the concentration of exogenous NE required to induce a response of the same magnitude if both complexation and oxidation of the NE added to the perifusion medium are avoided.

The frequency/response and dose/response curves could be satisfactorily fitted by means of a hyperbolic function which made it possible to deduce the half effect from the calculation of the maximum effect. This maximum effect was found to be the same whether the metabolic activity of the tissue was stimulated by NE liberated by the nerve endings or by NE added to the perifusion medium. The half effect values were found to be 0.4 Hz and 240 nM of NE (apparent km) respectively for the 2 curves, indicating that the apparent NE concentration in the vicinity of the receptors resulting from stimulation at frequencies within a range in which the regulation is most efficient, is 30–150 times greater than that of circulating NE[7,8].

It can be concluded, therefore, that since BAT metabolism can be activated by the sympathetic nervous system within seconds and at very low frequencies, and since in the frequency domaine in which the metabolic activity is most efficiently modulated by the nerve, the NE concentration is considerably higher than that of average plasma NE concentration, that the metabolic activity of BAT is predominantly under the tonic control of the sympathetic nervous system rather than that of circulating norepinephrine.

Cellular mechanisms in brown fat thermogenesis mitochondria

by D. G. Nicholls

Department of Psychiatry, University of Dundee, Ninewells Hospital, Dundee DD2 1UD (Great Britain)

In the chain of events beginning with the binding of noradrenaline to the plasma membrane receptor and terminating with the oxidation of released fatty acids by the mitochondria, it is this final stage which is by far the most exothermic and whose rate therefore defines the capacity of the tissue for thermogenesis. Classically, the dissipation of chemical energy as heat by mitochondria is minimized by the existence of a tight coupling of respiration to ATP production, respiration thus being limited neither by substrate supply nor by the oxidative capacity of the mitochondrion, but by the rate of extra-mitochondrial ATP utilization. However in brown adipose tissue no ATP hydrolyzing system has been described with sufficient capacity to account for the high rates of respiration of which the tissue is capable. This implies that in vivo brown adipose tissue mitochondria can uncouple the oxidation of fatty acids from the stoichiometric production of ATP, and a search for such a physiological uncoupling system has provided the main impetus for the study of isolated brown adipose tissue mitochondria.

The chemiosmotic theory of Mitchell[1] provides a conceptual framework for investigating possible sites for such uncoupling. In essence the chemiosmotic theory, for which there is a wealth of experimental evidence, proposes that the respiratory chain and the mitochondrial ATPase are linked by a circuit of protons, the proton electrochemical gradient generated by the expulsion of protons from the mitochondrial matrix being utilized to drive the synthesis of ATP. If the sole pathway for the protons to re-enter the matrix is via the ATPase, linked stoichiometrically to the production of ATP, then when ATP synthesis ceases proton re-entry in turn ceases. This in turn results in a build-up in the proton electrochemical gradient, stopping further proton release, and hence respiration, by the respiratory chain. This automatic respiratory control could in theory be bypassed in 2 ways, either if respiration could occur without the obligatory expulsion of protons, or if protons could re-enter the matrix by an alternative pathway not linked to the synthesis of ATP.

Much confusion existed in the early literature[2,3] dealing with isolated brown adipose tissue mitochondria as to whether respiration was coupled to the synthesis of ATP. The key to this complexity was the discovery by Rafael and co-workers[4] that certain purine nucleotides such as ADP, ATP or GDP were having unprecedented effects on these mitochondria. In the absence of such nucleotides in the medium, and even after exhaustive removal of endogenous

1 P. Mitchell, Trans. Biochem. Soc. *4*, 399 (1976).
2 T. Flatmark and J. I. Pedersen, Biochim. biophys. Acta *416*, 53 (1975).
3 D. G. Nicholls, FEBS Lett. *61*, 103 (1976).
4 J. Rafael, H. J. Ludolph and H. J. Hohorst, Hoppe-Seylers Z. Physiol. Chem. *350*, 1121 (1969).