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

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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

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fatty acids, the mitochondria are capable of rapid uncontrolled respiration without synthesizing ATP. Addition of these purine nucleotides to the medium in low concentrations produced a transformation into classic respiration-controlled mitochondria. The effect appears to be unique to these mitochondria and is unconnected with any classical roles of purine nucleotides on mitochondria[2,3].

The uncontrolled respiration in the absence of external nucleotides is not due to defective proton extrusion by the respiratory chain[5] and the alternative of an additional pathway for proton re-entry, independent of ATP synthesis, has been confirmed by osmotic swelling experiments[6] and by quantitation of the effective proton conductance of the inner membrane[5], which in the absence of purine nucleotides is some 20fold higher than for liver mitochondria. Addition of low concentrations of one of the purine nucleotides decreases the effective proton conductance of the membrane to levels similar to liver mitochondria[5]. The effect of this enhanced proton conductance is to allow the proton circuit to be completed by a means which does not involve ATP synthesis, and therefore provides a mechanism for physiological uncoupling. By the chemiosmotic theory[1], respiratory control occurs because the rate at which the respiratory chain is able to pump out protons, becomes limited as the back pressure from a high proton electrochemical gradient increase. The effect of the high proton conductance of the membrane in the absence of external purine nucleotide is to lower the proton gradient to levels at which no such respiratory control occurs.

The purine nucleotides act by binding to a specific site on the outer face the inner mitochondrial membrane[7] with dissociation constants as low as 10^{-5} M, suggesting the existence of a specific receptor protein on the inner membrane. The extent of nucleotide binding and of their influence on the membrane conductance appears to correlate for the guinea-pig with the physiological status of the tissue, being maximal at birth, and subsequently declining unless the animals are reared in the cold[2,3]. Interestingly, 2 hibernators, the hamster and the hedgehog, appear to retain nucleotide sensitivity regardless of developmental or adaptive status[2,3].

While this nucleotide-sensitive proton short-circuit provides a plausible in vitro model for the physiological uncoupling mechanism, it must be shown to be capable of being switched on at the induction of nonshivering thermogenesis, and equally of being switched off at its termination, as otherwise the high mitochondrial proton conductance would de-energize the cell when respiration returns to resting levels. This requires a messenger whose nature is not yet known. Purine nucleotides themselves do not appear to change extensively or rapidly enough in isolated brown adipocytes on addition of noradrenaline in order to modulate the proton conductance directly[8]. It has been suggested that fatty acids themselves serve the dual functions of substrate and 'uncoupling' messenger[9]. While this has the attraction of providing a simple means of self-regulation, it fails to account for the existence of the unique purine nucleotide-sensitive pathway (which is not affected by fatty acids) and does not correlate with measurements of cellular levels of fatty acids on noradrenaline addition[10], with the failure of all but extremely high level of added fatty acids to stimulate cell respiration[11], and with the high levels of fatty acids measured in resting cells[10].

The nature of the second (or third) messenger communicating between the plasma membrane noradrenaline receptor and the receptor located on the outer face of the mitochondrial inner membrane clearly requires further investigation. However the characteristics of the nucleotide-sensitive proton short-circuit are such as to leave little doubt that it represents the actual molecular site of nonshivering thermogenesis in brown adipose tissue.

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