THE REGULATION OF OXIDATIVE METABOLISM OF ISOLATED BROWN FAT CELLS[1]

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Earlier studies from this laboratory suggested that the mechanism of thermogenesis in brown fat involved a respiratory chain of poor phosphorylating efficiency. This hypothesis was based on the inability to obtain P/O ratios above 0.5 in brown fat mitochondria under normal assay conditions (Lepkovsky et al., 1959; Lindberg et al., 1966, 1967; Smith et al., 1966). Recently it has been possible to isolate mitochondria from this tissue that show P/O ratios of about 2 (Hohorst and Stratmann, 1967; Lindberg et al., 1967; Joel et al., 1967; Guillory and Racker, 1968; Hittelman, personal communication). These higher phosphorylating efficiencies, however, were obtained only under unusual conditions of preparation and assay. In this communication we report on the metabolism of isolated whole cells in order to avoid possible artifacts. The use of whole cells has the additional advantage of permitting the study of the overall cellular metabolism.

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[4]ABBREVIATIONS: NE = norepinephrine, αGP = α-glycerol phosphate, PN = pyridine nucleotide, fp = flavoprotein, TMPD = N,N,N',N'-tetramethyl-p-phenylenediamine, FCCP = Carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone, IAA = iodoacetate.

Fluorimetric studies of adult hamster brown adipose tissue *in vivo* demonstrated oxidation of PN upon NE^4 infusion (Prusiner et al., 1968), suggesting a coupled system. Similar observations have been made in isolated brown fat cells (Prusiner, Eisenhardt and Lindberg, unpublished observations). This interpretation was strengthened by the observation by Fain (1967) of a partial oxidation of cytochrome *b* following NE addition and further oxidation upon FCCP addition in isolated brown fat cells from adult hamsters and rats. In this communication we report on oxygen uptake studies which strongly suggest the presence of oxidative phosphorylation in brown fat cells isolated from adult hamsters, and the need for mitochondrial ATP as well as glycolysis for the NE-mediated release of thermal energy from triglyceride depots.

METHODS

Brown fat cells were isolated from hamsters more than 60 days old (Smalley and Smalley, 1967) by a method similar to that of Fain et al. (1967) and of Steiner (personal communication). The following modifications were found of importance: a) the hamsters were starved for 48 hours prior to sacrifice to reduce the size of the lipid globules within the cells in order to prevent rupture of the cellular membranes during centrifugation, and b) the cell volume was determined by a modified "microhematocrit" of the final suspension to permit standardization of the concentration. Oxygen uptake was measured at 23°C with a Clark electrode equipped with a sensitive electronic differentiator (Eisenhardt and Herr, manuscript in preparation).

RESULTS AND DISCUSSION

As shown in Fig. 1, the basal rate of oxygen uptake can be strongly stimulated by NE, as well as by succinate or αGP. Other substrates did not increase oxygen uptake measurably. The order of addition does not change the magnitude of the individual stimulations demonstrating that the respiratory chain is not rate limiting. The NE-mediated respiration is PN-linked, as seen by its rotenone sensitivity (Fig. 1a). Succinoxidase activity is inhibited over 90% by 5 mM malonate, whereas 3 mM malate represses it by about 20%. The entire system is sensitive to antimycin A and azide, and the antimycin A block can be bypassed by TMPD (Fig. 1b).

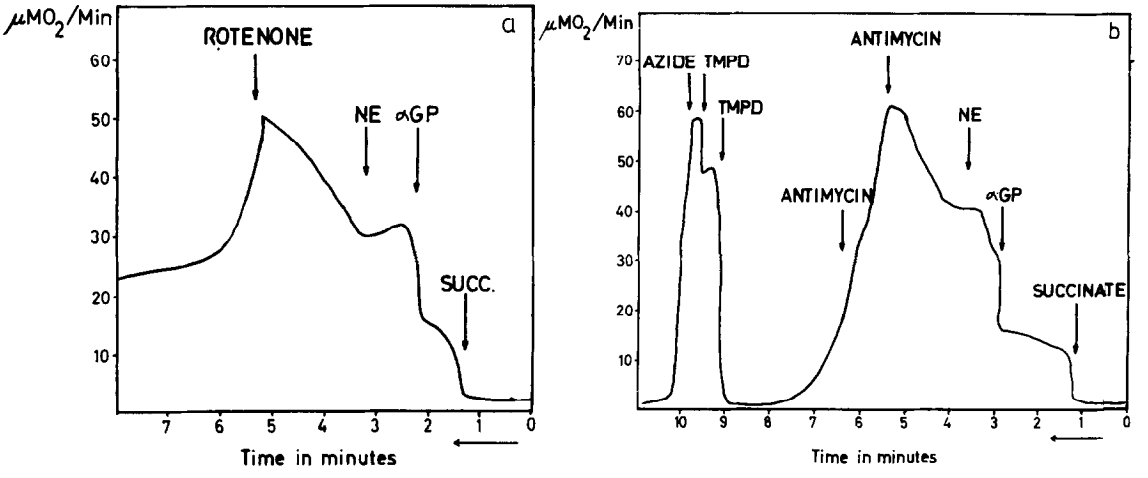

Fig. 1. Stimulation and inhibition of O_2 uptake in brown fat cells incubated in Krebs Ringer phosphate buffer pH 7.4 at 23° C. Final suspension contained 2% cells by volume and 0.4% Pentex Bovine Serum Albumin, Fraction V. Additions: 10 mM succinate, 12 mM αGP, 0.04 μg/ml NE, μM rotenone, 1 μg/ml antimycin, 100 μM TMPD, 1 mM azide.

In contrast to the fp-linked substrates, response to NE develops gradually following a delay period of up to one minute (Fig. 2a). This may be due to the time required for the formation of intermediates in the reaction sequence.

The development of the NE-mediated oxygen uptake shows sigmoid kinetics, suggestive of an autocatalytic activation process. The β-adrenergic blocking agent propanolol prevents the hormonal stimulation if added before NE. The hormonal effect is probably mediated via the adenyl cyclase system since dibutyryl-3',5'-cyclic AMP has been shown to mimick the effects of catecholamines on brown fat cells (Reed and Fain, 1968). 3',5'-cyclic AMP probably activates at least two enzymes in these cells: phosphofructokinase, which will be discussed later, and a lipase which initiates the release of fatty acids from triglycerides.

In contrast to the relatively small increase in oxygen uptake upon NE addition previously reported for experiments on tissue fragments in Warburg manometers (Joel, 1965; Lindberg et al., 1967), the cell preparations used in the present study show stimulations by a factor of 10 or more (Fig. 2). Part of this difference may arise from the inhibition of respiration that develops soon after the NE effect has reached its maximum. NE-mediated respiration is inhibited 80% by oligomycin (Fig. 2b); similar

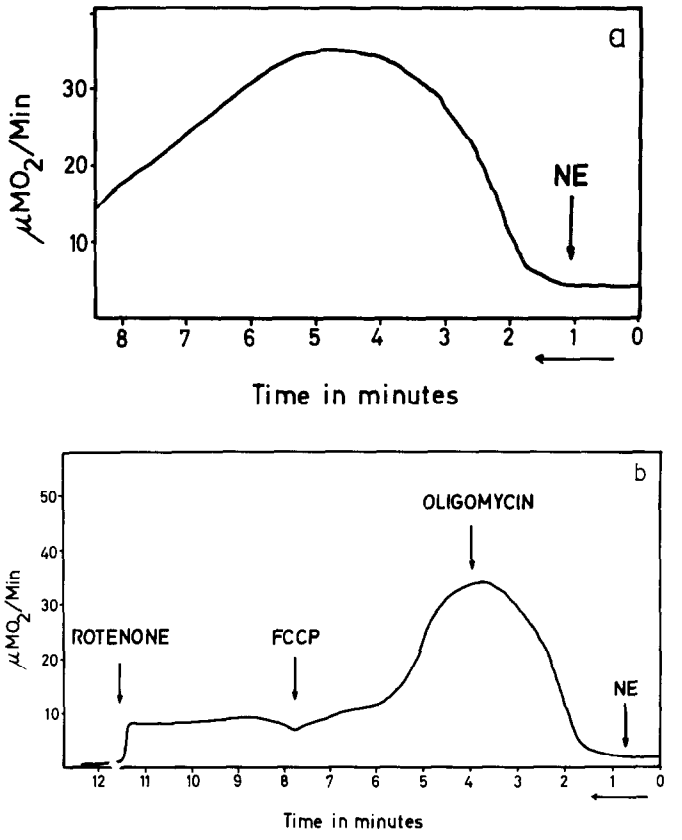

Fig. 2. NE stimulation. Additions: 4 μg/ml oligomycin, 1 μM FCCP. Other conditions as in Fig. 1.

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sensitivities in non-mitochondrial systems have been observed in ascites tumor cells (Dallner and Ernster, 1961; Minakami and Yoshiwaka, 1963) and in Chang liver cells (Prusiner and Eisenhardt, unpublished observations). But in contrast to these systems, uncoupling agents do not release the oligomycin inhibition in brown fat cells. Since in the latter tissue ATP is required for the activation of the physiological substrates, i.e., fatty acids, FCCP stimulation of oxygen uptake no longer can manifest itself in the presence of oligomycin, which blocks ATP synthesis.

Fig. 3a shows the transient stimulation and subsequent inhibition of respiration by the uncoupling agent FCCP in the presence of a suboptimal dose of NE (succinate and αGP stimulated respiration is unaffected by uncouplers). Here FCCP and oligomycin have similar effects because both interfere with the production of ATP which is needed to activate fatty

acids in mitochondrial oxidation. The decreased ATP levels lead to exhaustion of activated substrate and consequently inhibition of respiration. This is in agreement with previous findings from this laboratory where the effects of uncouplers and oligomycin on ATP levels were found to be additive (Lindberg et al., 1967). The interpretation is strengthened by the work of Siliprandi et al. (1965) which demonstrated that dinitrophenol decreased the respiration of rat liver mitochondria during oxidation of endogenous fatty acids. Fig. 3b shows the relationship between the respiration evoked by NE and that by an uncoupling agent. When NE is absent or present in very low concentrations, FCCP causes a sizeable transient stimulation of respiration. As the rate of O_2 uptake rises with increasing concentrations of NE, the FCCP transient stimulation falls. At maximal NE induced respiration, uncoupling agents no longer manifest any increase in oxygen uptake. Possible mechanisms to account for the observed maximal rates of fatty oxidation are discussed below.

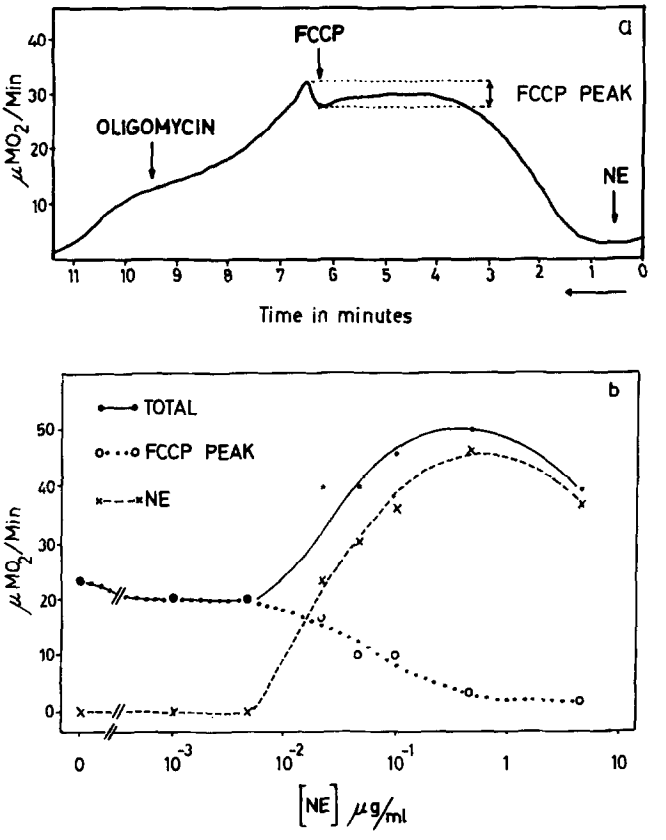

Fig. 3. Effect of 1 μM FCCP on NE-mediated respiration. Other conditions, including NE-concentration in "a", as in Fig. 1.

IAA, generally used to inhibit glycolysis, was previously shown to inhibit NE-mediated respiration (Lindberg et al., 1967). It is without effect on succinate and αGP evoked respiration. Mansour (1966) has suggested that catecholamines regulate glycolysis by activating phosphofructokinase via 3,5-cyclic AMP. In the present study IAA was found to inhibit NE-mediated oxygen uptake (Fig. 4a). If partially inhibiting concentrations of IAA are added before NE, the development of the response to NE are considerably retarded (Fig. 4b).

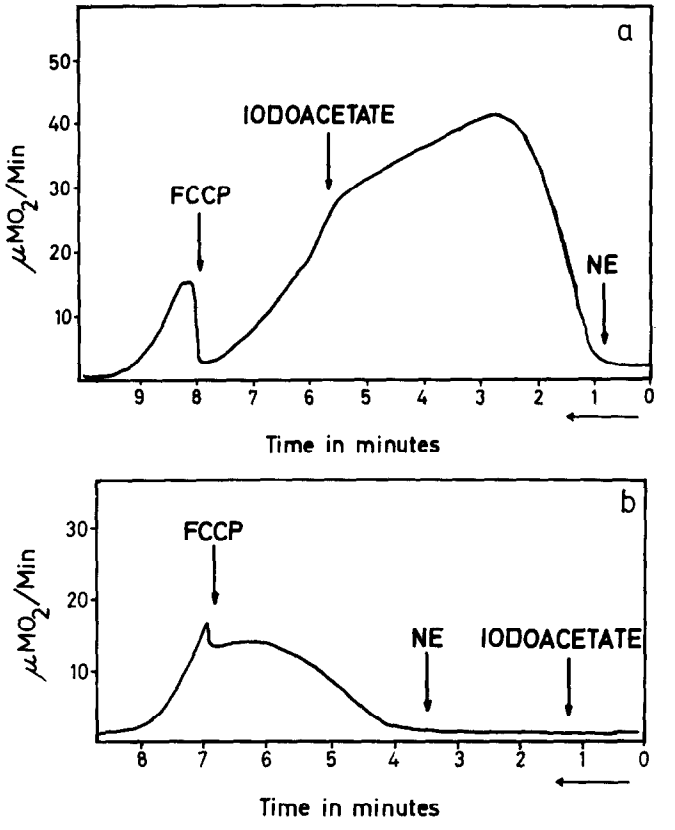

Fig. 4. Effect of 1.6 mM IAA. Other conditions as in Fig. 1.

In conclusion, we have demonstrated that the NE-mediated conversion of the triglyceride energy stores of brown fat into heat (Prusiner et al., 1968) utilizes a classical mitochondrial electron transport system as shown by its normal response to inhibitors. Hence, NE induced respiration requires both oxidative phosphorylation and glycolysis each of which has been shown to be necessary for NE stimulated lipolysis alone in white adipose tissue (Fassina et al., 1967).

The experiments presented above with FCCP and oligomycin show that

the mitochondria of brown fat are at least partially coupled both before and after NE stimulation. This finding is in agreement with the calcium uptake studies of Hittelman et al. (1967) and the cytochrome b changes described above. Since only a small amount of the ATP which theoretically can be obtained from oxidative phosphorylation accompanying fatty acid oxidation is required for the activation of fatty acids, an energy dissipating system must be postulated which permits electron transport to operate at the high rate observed after NE stimulation. We have earlier discussed the alternatives of uncoupling versus reesterification of liberated fatty acids (Lindberg et al., 1967). Indead, partial uncoupling by the detergent action of fatty acids from NE-mediated lipolysis is a distinct possibility for the dissipation of this energy. However, it is also possible to visualize a tightly coupled system where the ATP formed is degraded by the acyl CoA hydrolase via the formation of activated fatty acids (Srere et al., 1959; Bremer and Norum, 1967). Such a system might operate in a cyclic fashion as suggested in Fig. 5 and could even account in part for the increase in respiration seen during "fatty acid uncoupling" in mitochondria from other tissues. Thus, it appears that the metabolism of brown fat cells requires a critical level of ATP which is necessary for substrate activation and that the level of phosphate acceptor is adequate to maintain a high rate of respiration.

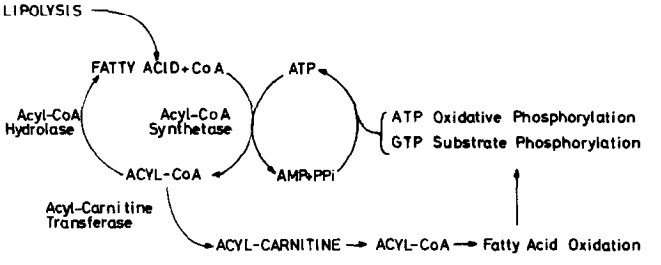

Fig. 5. Working hypothesis for the mechanism of metabolic control of thermogenesis in brown adipose tissue mitochondria.

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