DOES CYTOPLASMIC ALKALINIZATION TRIGGER MITOCHONDRIAL ENERGY DISSIPATION IN THE BROWN ADIPOCYTE ?

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Indirect calorimetry measurements showed that brown fat thermogenesis was very sensitive to modifications of intracellular pH induced by extracellular acid-base perturbations. Specific blockage of active Na-K transport by ouabain inhibited the thermogenic response only in acidosis and more efficiently when the glycoside was administered before the catecholamine stimulus than when it was added after the full calorigenic response had developed. It is suggested that the catecholamine stimulus might initiate a positive feed-back alkalinization of the cytoplasm, concomitant with activation of Na-K transport.

The hypothesis according to which brown adipose tissue (BAT) calorigenesis, under physiological stimulation of the metabolism by noradrenaline (NA), directly results from an increased rate of active Na-K transport across the plasma membranes arose from electrophysiology data (8) and metabolic rate measurements by various authors. Among them Yoshimura et al. (18) and Herd et al. (10) showed that respiratory response to NA of rat BAT slices incubated in Krebs-Ringer phosphate buffer containing ouabain (a specific blocker of active Na-K transport) was inhibited, as compared to controls which had not been preincubated with the glycoside. The same observation was made by Horwitz (12) on isolated hamster brown adipocytes incubated in a low bicarbonate buffered medium at pH 7.2. Using direct and indirect calorimetry techniques which allowed for the possibility to incubate the tissue slices at physiological PCO_2 and bicarbonate concentration (1 ; 3) we soon observed that ouabain inhibited the NA-induced calorigenesis by only 20 to 30 % at pH 7.4 (unpublished observation). Besides, this effect could

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only be quantified in a modified (high Mg^{++}, low Ca^{++}) bicarbonate medium, as in the standard one it was transitory (3). Indirect evidence that BAT calorigenesis was strongly influenced by intracellular pH (pHi) led us to examine the possibility that the active Na-K transport might essentially play an indirect role in the physiological response of BAT to NA (4). Studies on frog muscle (7), giant barnacle muscle (11) and mammalian muscle (9) have suggested that pHi is a regulated variable. This is but one of the multiple aspects of cellular homeostasis. Stimulation by catecholamines of the active sodium transport has been demonstrated by several authors, among which Horwitz and Eaton in BAT of cold adapted rat (13), Rogus et al in the extensor digitorum longus muscle of the rat (16) and Clausen and Flatman in rat soleus muscle (6). Finally, that there might be a link between pHi homeostasis and active Na-K transport is strongly suggested by the works of Williams et al (17), Clancy et al (5) and Moore (14). The question we ask is whether the active Na-K transport, which seems to play a part in pHi homeostasis in general, might also control energy dissipation in brown adipose tissue via its alkalinizing effect on the cytoplasm. Some evidence supporting this possibility is now presented.

The rate of oxygen uptake ($\dot{M}O_2$) by BAT slices was measured with O_2 electrodes over several hours in stop-flow respirometers (1) and the effect of ouabain on the NA-induced respiration reexamined under various conditions which entail at least some intracellular acidosis or alkalosis. These were either extreme extracellular acid-base conditions (pH 6.8 and 7.7, both respiratory and metabolic), or extreme CO_2 partial pressures (about 140 and 18 mmHg) at constant extracellular pH. The results of a series of experiments performed at medium pH 6.8, with a final stage at pH 7.7, are summarized in Fig. 1. At pH 6.8, the $\dot{M}O_2$ responses to NA (10^{-8} - 10^{-7} M), expressed as a fraction of their values at the moment ouabain (10^{-3} M) was added to the perifusion me-

dium, fell by about 60% in two hours of exposure to the glycoside. Since no difference was observed between respiratory

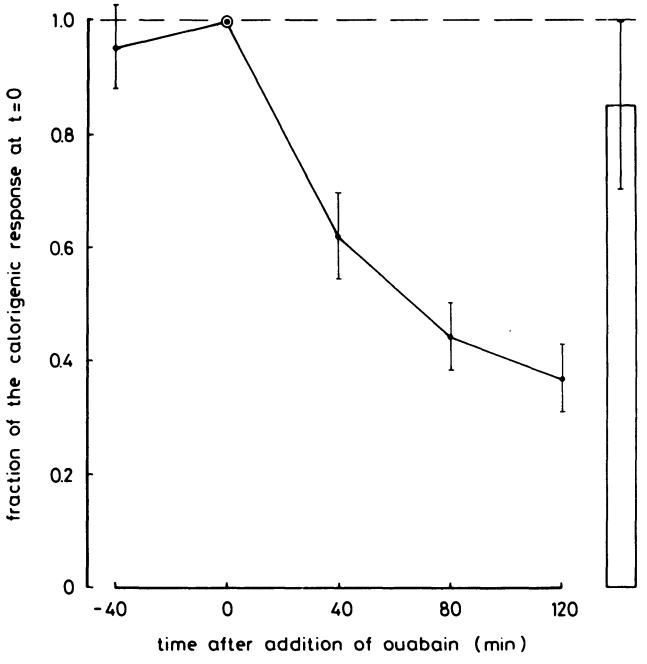

Fig. 1 The $\dot{M}O_2$ response of BAT to NA, relative to its value at t=0 (n=10, ± SEM). The response had practically reached a steady state 40 min before 1 mM ouabain was added to the perifusion medium at pH 6.8. At 120 min, pH was changed to 7.7. The $\dot{M}O_2$ response 40 min later was normalized (i.e. divided by 2.4, see text) and represented by the empty column, ± SEM.

acidosis (high PCO_2, n=6) and metabolic acidosis (low bicarbonate, n=4), the results were pooled. This 60% inhibitory effect is comparable to that described by Horwitz in isolated BAT cells from hamsters. In the last part of the experiments, medium pH was raised to 7.7, ouabain being still present. This was immediately followed by a large increase in $\dot{M}O_2$, up to a steady state value for as long as it was observed (i.e. 80 min). Since in previous experiments the $\dot{M}O_2$ response to NA had been found to be 140 (± 30) % larger at

medium pH 7.7 than at pH 6.8 (n=10), the value obtained 40 min after the change of pH was divided by 2.4 in order to normalize it with respect to the effect of pH in the absence of ouabain. The mean normalized value is represented by the empty column, ± SEM, on Fig. 1 which thus illustrates two facts : (a) with the sodium pump blocked after the $\dot{M}O_2$ response to NA had reached its steady state value, the inhibitory effect of ouabain on respiration developed slowly and (b) this inhibitory effect almost completely disappeared in alkalosis. Other experiments in which the preparations were submitted to acute PCO_2 changes at constant extracellular pH (7.4; PCO_2 and bicarbonate concentration changed simultaneously) showed that ouabain had no inhibitory effect at low PCO_2. This suggested that the insensitivity to ouabain at high pH demonstrated in Fig. 1 was related to a concomitant intracellular rather than to the extracellular alkalosis. These first experiments would indicate that active Na-K transport critically intervened in brown fat thermogenesis only under conditions which entail intracellular acidosis. This had already been suggested by the finding that, at low PCO_2, $\dot{M}O_2$ responses to NA could be obtained in media with no Na (Li substitution) or no Na nor K, pH 7.4 (4). Furthermore, the very slow rate of respiratory inhibition by ouabain in acidosis did not support the hypothesis of a direct relationship between Na-K transport and energy dissipation. In summary, our experiments strongly suggest that the Na-K transport system in thermogenesis is required mainly to counteract intracellular acidification.

As a further step, the idea that the sodium pump might trigger the thermogenic process was examined. Energy dissipation in mitochondria could indeed well be started by intracellular alkalinization, either directly by decreasing the affinity of GDP for the proton leak it controls (15) or indirectly by enhancing lipolysis and formation of acyl-CoA which, in turn, would induce further cytoplasmic alkaliniza-

tion by proton redistribution from the cytoplasm to the mitochondrial matrix. If such a positive feed-back exists, then the sodium pump should be more important for the initiation of thermogenesis than for its maintenance. To test this, the effects of ouabain administered before were compared to those obtained after the NA stimulus. Fig. 2 presents the results of 12 experiments at pH 6.8, in which $\dot{M}O_2$ was measured before and during stimulation by NA (starting at arrows), as a function of time of exposure to ouabain. In six experiments (closed circles) the glycoside was administered after the $\dot{M}O_2$ response to NA had developed, whereas in the paired experiments (open circles) it was administered before the NA stimulus. $\dot{M}O_2$ values of the two sets of experiments were significantly different at 90 min ($p < 0.0005$) and at 120 min ($p < 0.005$). This indicates that blocking of the sodium pump prevented more efficiently than it extinguished the thermogenic process. This finding is compatible with our hypothesis.

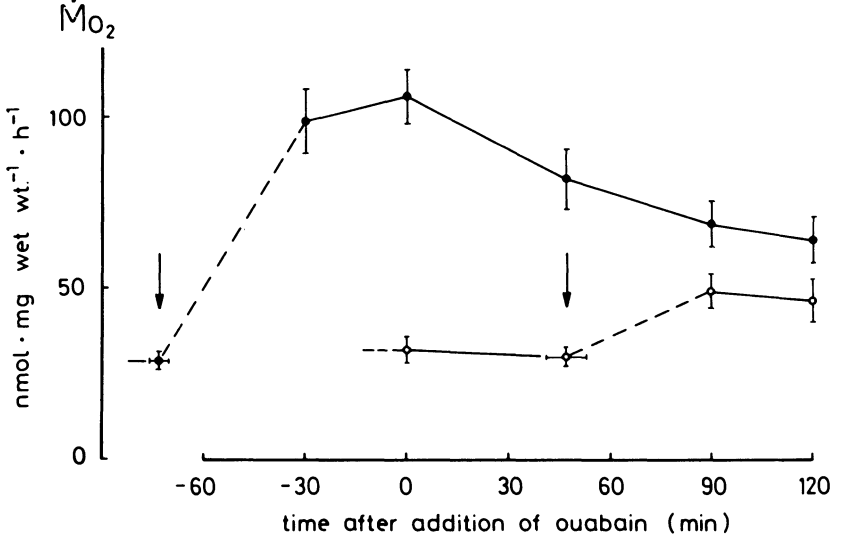

Fig. 2 Basal and NA-stimulated $\dot{M}O_2$, before and after exposure to 1 mM ouabain at t=0. Closed circles : sodium pump blocked after the $\dot{M}O_2$ response to NA (10^{-8} M, starting at arrow) had developed; open circles : sodium pump blocked before application of the NA stimulus at arrow. N=6, ± SEM for each point.

Direct evidence that NA stimulation actually induces a rise in pHi has not been obtained so far. Neither has it been proved that a small pH perturbation can quickly influence mitochondrial respiration in the intact cell. However, observation of the redox state of pyridine nucleotides in perifused BAT preparations with the surface fluorescence technique revealed that oxidation could occur immediately after a metabolic alkaline change in the medium. This preliminary result seems to indicate that a pHi perturbation can indeed quickly affect the proton motive force in mitochondria and, as a direct consequence, respiration (15). Finally, it should be stressed that the interpretation we have given of our results is based on the critical assumption that the intracellular pH regulatory process in powerful in BAT. Such a process might both minimize pHi perturbations originating from the extracellular medium, and initiate physiological changes in pHi when stimulated by NA.

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