Decreased guanine nucleotide binding and reduced equivalent production by brown adipose tissue in hypothalamic obesity

Recovery after cold acclimation

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1. INTRODUCTION

Brown adipose tissue (BAT) responds to an electrical stimulation of its sympathetic nervous supply by an increase in the level of reduced equivalents [1]. This response is decreased in rats made obese by a lesion of the ventromedial hypothalamus (VMH) [2]. BAT of VMH-lesioned rats also exhibits a decreased response to exogenous norepinephrine and to octanoate, indicating a decreased tissue capacity to oxidize fatty acids. In BAT, the ultimate fate of reduced equivalents produced by β-oxidation is heat production via a unique energy-dissipative mechanism consisting in a regulating shunting pathway of the protonmotive force across the inner mitochondrial membrane [3]. This energy-dissipative mechanism is inhibited by purine nucleotides which bind to a 32 000 M_r membrane polypeptide. The amount of purine nucleotide bound to isolated mitochondria is an index of the number of shunt pathways and thus of the thermogenic capacity of the tissue [3]. The mechanism controlling the number of shunt pathways is unknown. This number increases during cold exposure or acclimation [4], or spontaneous overfeeding [5].

Abbreviation: FCCP, carbonylcyanide-*p*-trifluormethoxy-phenyl-hydrazone

VMH-lesioned rats are hyperphagic, and the measurement of purine nucleotide binding was of interest in this context. The results show that VMH-lesioned rats have a decreased binding of purine nucleotides which correlates with the observed decreased capacity to produce reduced equivalents. Cold acclimation reverses these alterations. Food restricted VMH-lesioned rats do not show any changes in the metabolic response or in the purine nucleotide binding.

2. MATERIALS AND METHODS

Female SIVZ rats fed ad libitum (230–270 g body wt) were used. Bilateral electrolytic lesions of the ventromedial hypothalamic area (VMH) were performed as in [6]. On recovery from the anesthesia, ventromedial hypothalamic (VMH)-lesioned rats were fed ad libitum for 3 days to select successful lesions by the occurrence of hyperphagia and increased body weight. Following this, two groups of VMH-lesioned rats were fed ad libitum and kept either at 23°C or at 5°C for 3 weeks. A third group of VMH-lesioned rats was fed for 3 weeks the same amount of food as control animals (i.e., 15 g/day) using an automatic food distributor.

In each case, at the end of the 3-week period, a blood sample was taken from a tail vein to mea-

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sure plasma insulin levels according to [7]. The index of metabolic response to electrical nerve stimulation, norepinephrine or octanoate addition on neuro–adipose preparations in vitro was measured via continuous monitoring of NAD(P) redox state by surface-emitted fluorescence as in [1]. BAT plasma membranes were used for studies of (−)[^{3}H]dihydroalprenolol binding as detailed in [8].

Mitochondria were isolated by differential centrifugation [9]. Analysis of the polypeptide composition of mitochondria was performed by SDS–polyacrylamide gel electrophoresis. The proportion of protein of 32 000 M_r to total mitochondrial protein was determined by scanning the gels after staining with Coomassie blue R250 [10]. The guanosine-diphosphate (GDP) binding to isolated mitochondria was measured using equilibrium dialysis with [8-^{3}H]GDP (spec. act. 1130 mCi/μmol) [11]. Proteins were determined by the Coomassie brilliant blue method [12] (Bio-Rad protein assay, BioRad Lab.) with bovine albumin as standard.

3. RESULTS

3.1. *Effect of electrical nerve stimulation, norepinephrine or octanoate addition on reduced equivalents production*

Fig.1 illustrates the values of NAD(P) redox state in BAT of VMH-lesioned rats after nerve stimulation, norepinephrine, and octanoate addition. The data are expressed as percent of the values obtained in the non-lesioned (control) rats. BAT from VMH-lesioned rats fed ad libitum and kept at 23°C has a lower response to the 3 stimuli, whereas BAT from cold-acclimated or food-restricted VMH-lesioned rats show responses comparable to those of control rats in the same conditions (fig.1). These data show that VMH-lesioned rats respond normally when cold-acclimated or food-restricted.

Since the relative content of lipid in BAT of VMH-lesioned rats is higher than in control tissue [2] and the tissue volume explored by the spectrophotometric beam is the same for both preparations, it can be expected that a smaller mass of active tissue in VMH-lesioned rat is involved. To take this 'dilution' of active mass into account, the total metabolically mobilizable reducing equivalents were determined in an additional series and

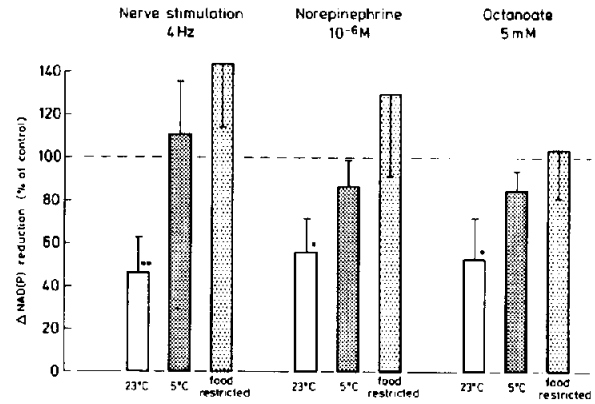

Fig.1. Effects of nerve stimulation, norepinephrine and free fatty acid additions on the NAD(P) steady redox state of isolated neuro–adipose preparations of VMH-lesioned rats. The results (mean ± SEM) are expressed as % of the response obtained on tissues from appropriate controls: $*p \leq 0.02$; $**p \leq 0.002$; $n = 9–12$.

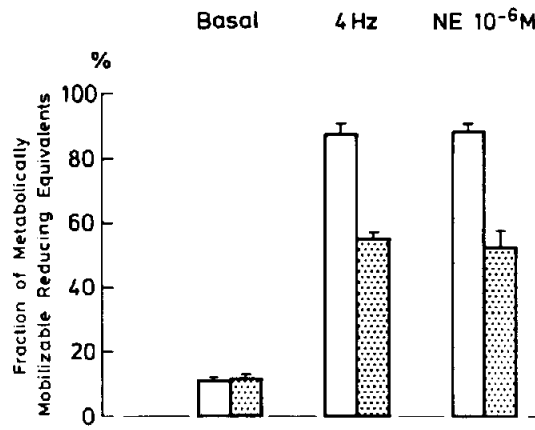

Fig.2. Fraction of metabolically mobilizable reducing equivalents obtained by maximal nerve and exogenous norepinephrine stimulations in brown adipose tissue of control (open column) and VMH-lesioned rat (dotted column), ($n = 4$). Total metabolically mobilizable reducing equivalents were determined by measuring the shift of steady-state emitted fluorescence when the preparation was first exposed to FCCP (10^{-5} M) in well-oxygenated medium and then to anoxi-induced by tonometering the medium with oxygen-free gas in absence of the uncoupler (pH 7.4) in both situations.

Table 1

Immunoreactive insulin (IRI) in plasma, GDP-binding sites and 32 000 M_r polypeptide in BAT mitochondria of control and VMH-lesioned rats (mean values ± SEM)

Condition	T (°C)	IRI (ng/ml)	GDP-binding sites (nmol/mg protein)	32 000 M_r polypeptide (%)
Control fed ad libitum (10–14)	23	2.3 ± 0.2	0.23 ± 0.03	5.8 ± 0.2
VMH fed ad libitum (10–16)	23	7.2 ± 1.4[b]	0.12 ± 0.01[b]	5.1 ± 0.3[a]
Control (5–9)	5	2.3 ± 0.4	0.68 ± 0.07[c]	8.1 ± 0.2[c]
VMH (6–12)	5	7.0 ± 1.0[b]	0.58 ± 0.05[a]	8.0 ± 0.4[c]
Control, food-restricted (5)	23	4.0 ± 0.6	0.25 ± 0.02[c]	5.8 ± 0.2
VMH, food restricted (6)	23	3.8 ± 0.8	0.26 ± 0.03[c]	5.4 ± 0.2

[a] $p \leq 0.025$
[b] $p \leq 0.005$ *vs* appropriate control
[c] $p \leq 0.01$ acclimated at 5°C *vs* 23°C or food-restricted *vs* ad libitum

are illustrated on fig.2. It can be seen that the BAT basal redox state value is similar, i.e., 12% of the total reducing capacity for control and VMH-lesioned rats, and that BAT from VMH-lesioned rat maximally stimulated by either nerve stimulation or norepinephrine addition shows responses representing only 55% ($p \leq 0.001$) of total capacity whereas the value for the control is 90% of maximum reduction. Thus there is a defective capacity to produce reduced equivalents to the respiratory chain in response to various stimuli in BAT of VMH-lesioned rats.

3.2. *β-Adrenergic receptors*

The decreased response to nerve stimulation or to norepinephrine could be the consequence of a modification of the number of β-receptors. The stereo-specific binding of (−)[^{3}H]dihydroalprenolol to plasma membranes was significantly higher in BAT from VMH-lesioned rats (0.631 ± 0.023 pmol/mg protein) than in BAT from control rats (0.481 ± 0.054 pmol/mg protein). K_d-Values were similar in both groups: controls, 3.81 ± 1.75 nM; VMH lesioned, 3.04 ± 0.51 nM.

3.3. *GDP binding to isolated mitochondria and percentage of 32 000* M_r *polypeptide to total mitochondrial proteins*

BAT of VMH-lesioned rats fed ad libitum shows a decreased binding of GDP to isolated mitochondria (table 1) and a small decrease in the percentage of polypeptide with 32 000 app. M_r known to be associated with thermogenesis [13,14]. When VMH-lesioned rats and control rats are cold-acclimated, the binding of GDP as well as the percentage of 32 000 M_r polypeptide increase and the same values are obtained in both groups (table 1) indicating that for these parameters, VMH-lesioned rats exhibit a normal response to cold acclimation. Food-restricted, VMH-lesioned rats do not show any change in GDP binding compared to their control, suggesting that the lesion itself is not sufficient for the changes in GDP binding observed in VMH-lesioned rats fed ad libitum.

Table 1 also shows the level of plasma immunoreactive insulin (IRI) in the 6 groups of rats studied. VMH-lesioned rats fed ad lib have a higher level of insulin, which is maintained after cold acclimation, suggesting that the high insulin per se is not responsible for the metabolic decreased GDP binding observed in VMH-lesioned rats at 23°C. Food restricted control and VMH-lesioned rats have similar levels of IRI at the end of the 3 weeks of food restriction. However, until 1 week after the lesion, food-restricted VMH-lesioned rats still have higher level of plasma immunoreactive insulin [6].

4. DISCUSSION

The study shows that in brown adipose tissue (BAT) of VMH-lesioned rats fed ad lib and kept at 23°C there is a decreased production of reduced equivalents, a decreased GDP binding to isolated mitochondria and a slight decrease in 32 000 M_r protein. These defects may be a consequence of the decreased sympathetic tone of the tissue suggested by:

(i) The increased number of β-receptors;
(ii) The decreased turnover of norepinephrine shown in [15];
(iii) The reversal of the defect after cold acclimation, a situation known to increase the sympathetic tone in BAT.

The opposite is observed in rats fed a cafeteria diet in which both norepinephrine turnover [16] and the number of GDP binding sites [5] are increased. The values of GDP binding and the changes in reduced equivalent production after various stimuli are similar in food-restricted control and VMH-lesioned rats. Food restriction may represent a stress to VMH-lesioned rats prone to overeat and therefore increase their sympathetic tone. In this situation, again an parallelism is maintained between the capacity to produce reduced equivalents and GDP binding suggesting a relationship between the substrate supply to the respiratory chain and the number of GDP binding sites.

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REFERENCES

[1] Seydoux, J., Constantinidis, J., Tsacopoulos, M. and Girardier, L. (1977) J. Physiol. (Paris) 73, 985–996.
[2] Seydoux, J., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F. and Jeanrenaud, B. (1981) Pflügers Arch. 390, 1–4.
[3] Nicholls, D.G. (1979) Biochim. Biophys. Acta 549, 1–29.
[4] Desautels, M. and Himms-Hagen, J. (1979) Can. J. Biochem. 57, 968–976.
[5] Brooks, S.L., Rothwell, N.J., Stock, M.J., Goodbody, A.E. and Trayhurn, P. (1980) Nature 286, 274–276.
[6] Rohner-Jeanrenaud, F. and Jeanrenaud, B. (1980) J. Clin. Invest. 65, 902–910.
[7] Herbert, V., Lau, K.S., Gottlieb, C.W. and Bleicher, S.J. (1965) J. Clin. Endocrinol. Metab. 25, 1375–1384.
[8] Seydoux, J., Giacobino, J.P. and Girardier, L. (1982) Mol. Cell. Endocrinol. 25, 213–226.
[9] Ricquier, D., Mory, G. and Hemon, P. (1975) FEBS Lett. 53, 342–346.
[10] Ricquier, D. and Kader, J.C. (1976) Biochem. Biophys. Res. Commun. 73, 577–583.
[11] Lin, C.S. and Klingenberg, M. (1980) FEBS Lett. 113, 299–303.
[12] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[13] Heaton, G.M., Wagenvoord, R.J., Kemp, A. and Nicholls, D.G. (1978) Eur. J. Biochem. 82, 515–521.
[14] Ricquier, D., Gervais, C., Kader, J.C. and Hémon, P. (1979) FEBS Lett. 101, 35–38.
[15] Vander, J.G., Knehans, A.W. and Romsos, D.L. (1982) Life Sci. 30, 913–920.
[16] Young, J.B., Saville, E., Rothwell, N.J., Stock, M.J. and Landsberg, L. (1982) J. Clin. Invest. 69, 1061–1071.