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## Noradrenergic control of ascorbic acid and glutathione concentrations in brown fat of cold-exposed rats

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*Summary.* Ascorbic acid and glutathione concentrations increase in brown fat of cold-exposed rats. This phenomenon can be reproduced by noradrenaline or isoproterenol administration, and thus seems to be under sympathetic control. Histological study shows ascorbic acid storage in brown adipocyte nuclei.
*Key words.* Brown fat; cold exposure; ascorbic acid; gluthatione; noradrenaline; α-agonist; β-agonist.

In several mammalian species, exposure to cold triggers redevelopment [1] of brown adipose tissue (BAT), a tissue specialized in thermogenesis [2]. This so-called 'trophic response' is the cumulative result of various events, including increases in cell number, in mitochondrial mass per cell, and in the mitochondrial concentration of uncoupling protein (UCP), the protein responsible for the thermogenic capacity of BAT [2].

BAT trophic response is also characterized by an increase in ascorbic acid (AA) concentration [3, 4], the exact role of which has not been demonstrated [5], and an increase in reduced glutathione (GSH) concentration, for which a 'protective mechanism' against the peroxidative effect of AA has been put forward [4]. It has been suggested that AA is stored in the tissue nerve supply [5]. If the 'protective' hypothesis is correct, AA must be stored, at least in part, directly in brown adipocytes. The preliminary part of this work was thus an investigation of AA localization in BAT using a histological approach.

In rats, most of the events involved in the trophic response to cold are triggered and maintained by an increased stimulation of the tissue by noradrenaline (NA), through increased sympathetic tone [1, 6]. The second aim of the present study was to determine whether the rise of AA and GSH concentrations in BAT of cold-exposed rats is also due to noradrenergic stimulation. We used the technique utilized to show the control of UCP level by NA, i.e. chronic administration of NA by implanted pumps in rats kept at room temperature [7]. Since BAT has both α- and β-adrenoceptors [8], α- and β-agonists were tested in addition to the natural sympathetic mediator.

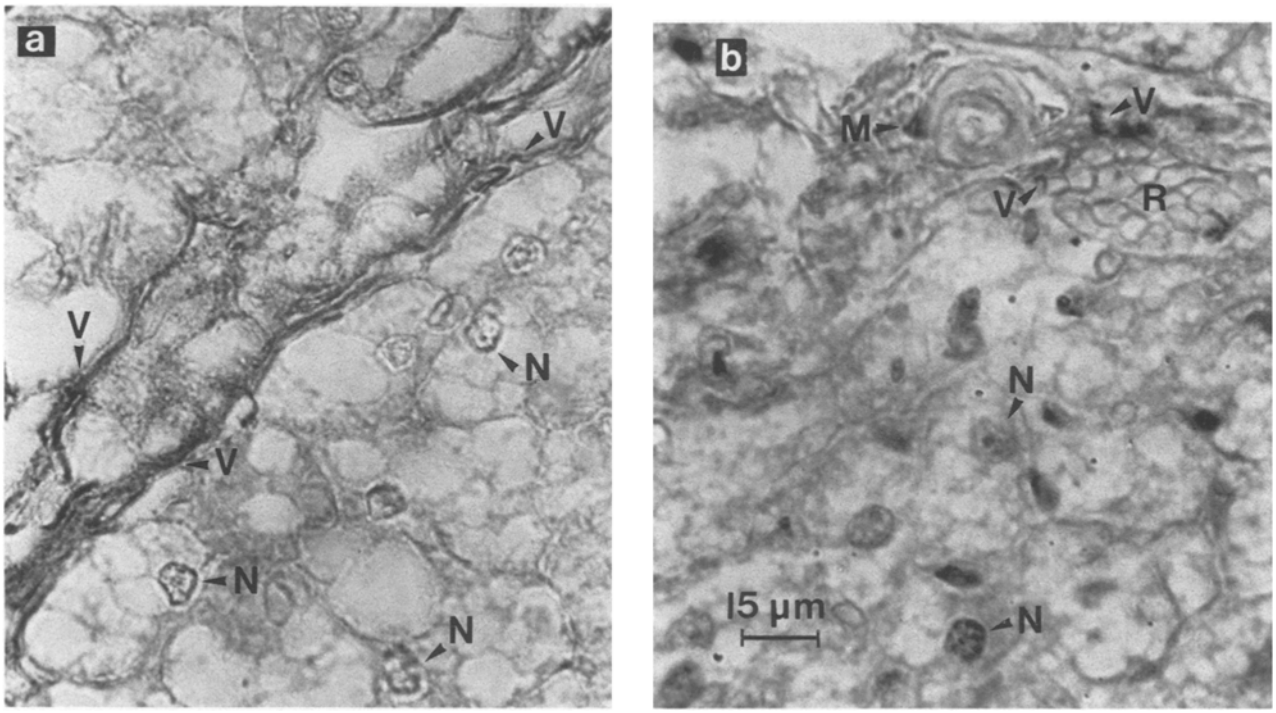
*Material and methods*

39-day-old male rats of the Wistar strain were either exposed to cold (5 °C) or kept at room temperature and implanted dorsally, exactly above the interscapular BAT, with an osmotic minipump (ALZETT model 2002). The pumps delivered amines dissolved in 0.1 M L-ascorbic acid and 0.02 M Tiron (SIGMA) medium which protects amines against oxidization[7]. (−) NA (arterenol bitartrate) and the β-agonist isoproterenol (L-isoproterenol bitartrate SIGMA) were delivered at $5 \cdot 10^{-8}$ mol · h$^{-1}$. The α-agonist phenylephrine (L-phenylephrine hydrochloride SIGMA) was delivered at a rate of $10^{-7}$ or $2 \cdot 10^{-7}$ mol · h$^{-1}$. Control rats kept at 25 °C were implanted or not with a pump containing only AA and Tiron. All rats were sacrificed at 48 days of age.

Localization of AA was performed according to Chinoy and Sanjeevan[9]: pieces of BAT from control rats without pumps as well as from cold-exposed animals were treated with a silver nitrate 1.15 mM/glacial acetic acid/ethanol mixture (3.2 V/0.5 V/6.3 V) at 3 °C. This reagent specifically stains AA dark brown. AA concentrations in interscapular BAT of all rat series were determined by the Zannoni method[10] and GSH concentrations were measured according to Beutler et al.[11]. It was verified in three separate experiments that AA and GSH concentrations in BAT are not affected by the AA and Tiron delivered by the minipumps (6 unimplanted rats and 6 rats receiving AA and Tiron in each experiment; see records of one such experiment in the table).

*Results and discussion*

*Histological localization of ascorbic acid.* Histological studies showed a double marking due to AA in the BAT from both control and cold-exposed rats (see fig.). Dark




Photomicrograph of brown fat from control (*a*) or cold-exposed (*b*) rat after staining for ascorbic acid detection. Fixation and staining were performed according to Chinoy and Sanjeevan[9]. N: stained nuclei of brown adipocytes, V: staining in vascular area, R: red blood cells in vessels, M: stained mast cells. Areas in nuclei which do not exhibit strong granular staining are coloured medium brown in the original, while cytoplasm is light brown. Moreover strong granular staining is not equally distributed in nuclei, so it is not possible to observe this staining on all nuclei present in a 5-µm section.

Effect of cold exposure or chronic administration of amines on ascorbic acid and reduced glutathione (GSH) concentrations in rat BAT.

| Treatment and number of determinations | Interscapular BAT (mg) | Ascorbic acid (µmol · g$^{-1}$) | GSH (µmol · g$^{-1}$) |
|---|---|---|---|
| Control without pumps (6) | 165 ± 13 | 0.16 ± 0.03 | 1.4 ± 0.05 |
| Control with pumps (6) | 211 ± 7 | 0.14 ± 0.03 | 1.25 ± 0.15 |
| NA $5 \cdot 10^{-8}$ mol · h$^{-1}$ (12) | 208 ± 12 | 0.29 ± 0.01 | 1.7 ± 0.1 |
| ISO $5 \cdot 10^{-8}$ mol · h$^{-1}$ (12) | 239 ± 17 | 0.31 ± 0.03 | 2.4 ± 0.2 |
| ISO (idem) + PHE $10^{-7}$ mol · h$^{-1}$ (4) | 286 ± 26 | 0.35 ± 0.03 | 2.6 ± 0.1 |
| exposure to 5 °C (6) | 395 ± 19 | 0.42 ± 0.07 | 2.6 ± 0.2 |

NA: noradrenaline; ISO: isoproterenol; PHE: phenylephrine. Values are means ± SEM. Statistics: statistical analysis of treatment effects is done using the non-parametric ANOVA of Kruskal-Wallis. $p \leq 0.05$ is considered to be statistically significant. Bars link groups of data which are not statistically different.

stained areas exhibiting a granular aspect caused by silber deposition were observed in vascular zones (in which nerve density is the largest) and in the nuclei of brown adipocytes. Some cells identified as mast cells by their typical granulation were also strongly stained. Thus, AA does not seem to be exclusively stored in BAT innervation. Furthermore, a nuclear localization of AA in BAT is not unexpected: Chinoy and Sanjeevan[9] also found AA in nuclei of liver, adrenal and epididymis cells. As these authors consider that their technique is qualitative and cannot be used for quantitative determination, we will not discuss the localization of the AA accumulation which occurs in the BAT of cold-exposed rats.

*Effects of amine administration.* Neither NA, nor ISO administration induced an increase in BAT weight (table), contrary to what was previously observed[7] (in the following discussion, data obtained with rats treated by amines are compared with those obtained with animals receiving solvent). This result could be due to a smaller amount of amine being delivered by the pumps than in our previous experiments and to a shorter duration of treatment. BAT weight of rats treated with an ISO + PHE combination was significantly larger than BAT weight of control animals, but not statistically different from tissue weight of rats treated with ISO alone. Nevertheless, amine treatments affected the AA concentration of the tissue. This concentration was increased to 107% in rats receiving NA, to 121% in animals treated with ISO, and to 150% in animals receiving both ISO and PHE. The same result was obtained with GSH concentration which was significantly increased by ISO or NA administration (NA: + 36%, ISO: + 92%, ISO + PHE: + 108%). Administration of PHE alone (three separate experiments using $10^{-7}$ or $2 \cdot 10^{-7}$ mol · $h^{-1}$ deliveries) did not modify AA and GSH concentrations (data not shown).

Statistical analysis of results (see table) shows that: 1) β-agonist ISO reproduced the effect of NA on AA and GSH concentrations, 2) ISO + α-agonist PHE did not induce a larger increase of AA and GSH levels than ISO alone, 3) ISO administration reproduced the effect of exposure to 5 °C on AA and GSH concentrations.

It can be concluded that the increase of NA release which occurs in the BAT of cold-exposed rats is most probably responsible for the rise of AA and GSH concentrations observed in this tissue. According to our results NA acts through a β-adrenoceptor and there is no argument for an α-adrenoreceptor role in the regulation of AA and GSH levels in BAT.

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## Possible existence of a presynaptic positive feedback mechanism enhancing dopamine transmission in the anterior cingulate cortex of the rat

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*Summary.* A series of microiontophoretic and VTA stimulation experiments, conducted in intact, GBR-12909-treated, α-methylparatyrosine-depleted or 6-hydroxydopamine-denervated rats, provide suggestive evidence for the existence of a presynaptic, positive feedback mechanism triggered by dopamine reuptake and favoring the release of this transmitter in the anterior cingulate cortex.
*Key words.* Dopamine; cingulate cortex; uptake; transmission; positive feedback.

Various lines of evidence suggest an involvement of the mesocortical dopamine (DA) system in higher cognitive processes and the regulation of emotional states. a) In the rat, the ventral tegmental area (VTA) from which this system originates is one of the brain sites which most consistently supports self-stimulation[2]. b) The mesocortical DA system is metabolically activated by electric footshock stress[3]. c) Experimental lesioning of the VTA