of dopamine receptors in kidneys of the Kyoto rats relative to those of normotensive animals[9]. Such observations could be related to changes in renal responses to catecholamines, or to changes of catecholamine release or turnover. The present results do not provide information on the kinetics of the situation, but they do indicate that, at least in the Otago strain, there is no obvious abnormality of tissue storage for either NA or DA.

Cuche and Liard[6] reported that bilateral denervation of the kidneys in juvenile SHR rats caused substantial reduction in tissue levels of all catecholamines, although precise values for DA were not given. In the present experiments, unilateral renal denervation in a 2nd series of 6 normotensive and 3 hypertensive adult rats affected tissue contents of NA and DA to very similar extents, the mean amounts remaining after denervation being respectively $8.1 \pm 1.9\%$ (NA) and $8.1 \pm 2.0\%$ (DA) of the amounts in the contralateral control kidneys. In 1 additional normotensive animal, NA content was reduced by 87% but DA remained similar to that in the control kidney. Amine levels in the control kidneys of this series were similar to those of unoperated animals.

The correlations observed in both man and animals between sodium balance and renal clearance of DA have led to suggestions that DA may constitute an intrarenal natriuretic factor[3,13,14]. Several recent studies have been concerned with differentiation between DA extracted by the kidney from arterial plasma and that produced within the kidney[14,18]. Intrarenally produced DA could represent the local metabolism of l-DOPA[15,16], or release from intrarenal chromaffin cells[21]. Such multiple possible sources for urinary and venous effluent DA complicate any analysis of intrarenal DA handling. The present results indicate that in the rat kidney virtually all of the endogenous DA is associated with the extrinsic renal nerve supply. Significant DA release from intrarenal chromaffin cells is therefore unlikely, and any DA of intrarenal origin which is not due to tubular metabolism of l-DOPA is likely to be due to release from the renal nerves. While this study was being prepared for publication, another report appeared in the literature in which similar results were obtained following surgical denervation in normotensive, Sprague-Dawley rats[22].

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## Parallel increase of ascorbic acid and glutathione contents in brown adipose tissue during chronic cold exposure

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*Summary.* Spontaneous lipid peroxidation rate was found unchanged in the brown adipose tissue of rats chronically exposed to cold, although oxidative metabolism, ascorbic acid and poly-unsaturated phospholipid amounts increased. It is suggested that the concomitant increase in glutathione concentration may protect the tissue from a possible peroxidative process.

Brown adipose tissue (BAT) is a tissue specialized for thermogenesis. In rats, chronic cold exposure induces several metabolic and biochemical changes in BAT (review in Barnard et al.[1]). Some of these suggest a role for glutathione in BAT response to cold: the increase of the oxidative metabolism in order to produce heat, the development of the tissue which requires an intense protein synthesis and the increase of unsaturation in the fatty acids of its total and mitochondrial phospholipids.

Glutathione is a component of the aminoacid transport system across membranes[2]. This peptide, through the activation of the glutathione peroxidase, also protects cells against the peroxidation of unsaturated lipids which can occur in tissue exhibiting an active oxidative metabolism[3]. It is known that a decrease of the glutathione level in a tissue leads to an increase of lipid peroxidation and thus to membrane damage and cell lysis[4,5].

Furthermore, the ascorbic acid concentration is increased

Effect of chronic cold exposure on the fatty acid composition of BAT lipids, the spontaneous lipid peroxidation rate and ascorbic acid and GSH concentrations in BAT

| | | Control | Cold-exposed |
|---|---|---|---|
| Fatty acids of PL (% of total) | Saturated | 46.7 ± 2.9 | 42.5 ± 1.5 |
| | Mono-unsaturated | 30.0 ± 1.4 | 21.6 ± 1.1* |
| | Poly-unsaturated | 23.3 ± 1.4 | 35.9 ± 1.8* |
| Fatty acids of TG (% of total) | Saturated | 44.95 ± 2.3 | 32.1 ± 1.4* |
| | Mono-unsaturated | 48.35 ± 2.9 | 60.2 ± 1.9* |
| | Poly-unsaturated | 6.7 ± 0.3 | 7.7 ± 0.6 |
| Spontaneous lipid peroxidation (nmoles MDA · $h^{-1}$ · g $tissue^{-1}$) | | 153.6 ± 22.8 | 158.8 ± 34.6 |
| Ascorbic acid (μmoles · g · $tissue^{-1}$) | | 0.195 ± 0.025 | 0.38 ± 0.04* |
| GSH (μmoles · g · $tissue^{-1}$) | | 1.2 ± 0.1 | 2.0 ± 0.1* |
| GSH/ascorbic acid ratio | | 6.1 ± 0.1 | 5.3 ± 0.1 |

BAT, brown adipose tissue; PL, phosphlipids; TG, triglycerides; MDA, malondialdehyde; GSH, reduced glutathione. Values are means ± SEM. *$p \leq 0.01$ according to the U-test of Mann-Whitney.

in BAT by chronic cold exposure[6] and this compound is known to induce peroxidation in unsaturated lipids[7]. Mc Cay et al.[3] showed that reduced glutathione (GSH) was also effective in vitro in inhibiting lipid peroxidation triggered by ascorbic acid.

In the present study the GSH concentration in BAT was measured to determine whether this parameter is modified by cold adaptation. We also determined the degree of unsaturation of the fatty acids of phospholipids and triglycerides, the level of spontaneous lipid peroxidation and the ascorbic acid content in the interscapular BAT of rats bred at room temperature or exposed to cold during 2 weeks.

*Materials and methods.* 22-day-old Wistar rats were exposed at 5 °C for 2 weeks and control animals kept at 25 °C. Then, some of the cold exposed rats were re-exposed to 25 °C during 24 or 72 h (disadapted animals). Spontaneous lipid peroxidation was determined in BAT total homogenates by measuring the amount of malondialdehyde (MDA) formed with the thiobarbituric test of Placer et al.[8]. Ascorbic acid content was determined according to Zannoni et al.[9] and GSH content according to Beutler et al.[10]. Lipids were extracted, separated by TLC and analyzed by GLC as in Ricquier et al.[11].

*Results and discussion.* The increase of BAT weight observed in cold-exposed rats (225 ± 12 mg vs 158 ± 19 mg, n = 6) was associated with an increase of the total phospholipid concentration in the tissue (1.17 ± 0.10% fresh tissue weight vs 0.48 ± 0.03%) and changes in the phospholipid fatty acid composition as previously described[11]. As shown in the table these changes led to a decrease in the proportion of mono-unsaturated fatty acids in BAT total phospholipids and an increase in the proportion of poly-unsaturated fatty acids. Thus, these membrane lipids should be a better substrate for peroxidation in the BAT of cold adapted rats than in rats kept at room temperature. Such a phenomenon was not found in the fatty acids of triglycerides; however, their degree of unsaturation was also increased since about 25% of their saturated fatty acids were replaced with mono-unsaturated fatty acids (table).

Pagé and Babineau[6] found a 5-fold increase in the ascorbic acid concentration in BAT from rats exposed to cold for 20 weeks. The present work shows that this concentration had already doubled after 2 weeks at 5 °C (table). In spite of all these modifications, cold adaptation did not affect the level of spontaneous lipid peroxidation measured in BAT total homogenates.

GSH concentration was increased by cold adaptation so that the GSH/ascorbic acid ratio was not significantly modified (table). In disadapted rats the ascorbic acid and GSH concentrations were as in cold adapted rats 24 h after re-exposure to 25 °C (ascorbic acid: 0.35 ± 0.01 μmoles · g $tissue^{-1}$, GSH: 2.2 ± 0.1 μmoles · g $tissue^{-1}$). Between 24 and 72 h, both ascorbic acid and GSH contents decreased and reached the values observed in control animals (ascorbic acid: 0.2 ± 0.02 μmoles · g $tissue^{-1}$, GSH: 0.95 ± 0.08 μmoles · g $tissue^{-1}$). We can note that the GSH/ascorbic acid ratio found in BAT, regardless of the temperature of exposure (see table), was the same as that found by Mc Cay et al.[3] to be necessary to obtain in vitro an inhibition of the ascorbic acid peroxidative effect by addition of GSH in isolated mitochondria (5.45 moles/mole).

Although membranes of BAT cells seem to be exposed to peroxidizing conditions in cold-adapted rats (higher oxidative metabolic rate, higher ascorbic acid concentration, increased unsaturation in lipids), an increased peroxidation rate could not be demonstrated by the present study or by previous ones (for instance, no histological study on BAT of cold-exposed animals has shown any increased cell lysis or appearance of intracellular damage in the tissue). Thus, the development of mechanisms preventing peroxidation and protecting the unsaturated lipids of membranes in BAT of cold-adapted rats can be expected, and the increase of GSH concentration described by the present study could be one of them. However, GSH also has other metabolic functions (i.e. control of aminoacid transport and prostaglandin synthesis) which will be affected by the increased GSH concentration, and which may also be important in the regulation of BAT metabolism in cold-exposed animals.

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