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Cadmium Chloride Prevents the Rise in Rat Brown Adipose Tissue Mitochondrial Respiration in Response to Acute Cold Stress

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Cold exposure induces a sharp rise in brown adipose tissue (BAT) thermogenesis in rats and other mammals. This effect is largely dependent on the increase in BAT noradrenaline concentration and on the acceleration of thyroxine (T_4) to triiodothyronine (T_3) conversion, which synergistically stimulate the synthesis of several proteins essential for the production and dissipation of heat (see reviews Nichols and Locke 1984, Rothwell and Stock 1984a, Himms-Hagen 1990).

In previous work we reported that cadmium inhibited the enzyme 5'-deiodinase in rat liver (Paier et al. 1993), the hypophysis (Pavia et al. 1997) and BAT (Paier et al. 1997) and depressed the hypothalamus-hypophyseal-thyroidal function (Pavia et al. 1997). Other workers (Miccadei and Floride 1993) have shown that cadmium inhibits liver mitochondrial oxidations. The effects of cadmium on BAT mitochondria have not been elucidated.

The present work was designed to examine the effects of cadmium on BAT mitochondrial O_2 consumption in response to cold in normal and T_3-treated or untreated hypothyroid rats. Furthermore, the activities of BAT mitochondrial enzymes cytochrome c oxidase, α-glycerophosphate dehydrogenase (α-GPD) and succinate dehydrogenase were determined.

MATERIAL AND METHODS

Male Wistar rats of 250 g body weight (BW) were used. Purina rat chow and tap water were available *ad libitum*. Groups of rats were made hypothyroid by a single i.p. injection of ^{131}I. After thirty days, groups of hypothyroid rats were treated with T_3 150 ng/100 g BW s.c., twice daily for 10 days before the experiments were initiated. This dosage replaces the daily production rate of T_3 and normalizes serum T_3 concentration (Bianco and Silva, 1987). Other hypothyroid rats remained untreated. Normal and untreated hypothyroid rats were injected with the vehicle alone.

Groups of normal, T_3-treated or untreated hypothyroid rats were injected with a single dose of cadmium chloride 220 ug/100 g BW, i.p. This dose carried 107.8 ug

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cadmium/l00 g BW. Control animals were injected with an equal volume of saline. One-half of each group was housed in individual cages in a cold room at 4°C for 24 h and the other half was kept at 22°C with the same 1ight:darkness cycle and free access to food and water. The untreated hypothyroid rats were exposed to 4°C for 8 h due to their low survival in the cold. Thereafter, animals were killed by cervical dislocation, the interscapular BAT was removed, weighed, and the mitochondrial O_2 consumption was determined in the manner described below. Mitochondrial enzyme activities were measured only in cold-exposed normal rats.

Groups of cadmium-free normal, T_3-treated or untreated hypothyroid rats were placed in a cold room for 24 h. The respective controls remained at 22°C. After this period, animals were killed by cervical dislocation and BAT mitochondria was obtained as described below. Aliquots of mitochondria from each group were added a 20 ul solution containing 2.5 or 5 mM cadmium chloride. These dosages carried 1.22 or 2.45 mM cadmium, respectively.

BAT was removed and homogenized under ice in buffer containing sucrose (0.25 M), K-TES (5 mM), EDTA (2 mM) and bovine serum albumin (2%) pH 7.2. Mitochondria were isolated by differential centrifugation as described by Cannon and Lindberg (1979). The final pellet was suspended in sucrose buffer (0.24 M) and the protein content was measured by the method of Gornall et al. (1949). O_2 consumption was measured in an oxygraph (Gilson Medical Electronic, Wisconsin) as described previously (Cageao et al. 1995). The medium employed (0.9 ml) contained potassium chloride (100 mM), K-TES (20 mM), magnesium chloride (2 mM), EDTA (1 mM) and 2% bovine serum albumin pH 7.2. a-glycerophosphate was used as substrate due to the high rate of oxidation of this compound in BAT catalysed by a highly active a-GPD enzyme (Cannon and Lindberg 1979).

Mitochondrial a-GPD activity was measured spectrophotometrically at 500 nm and 37°C, as described by Gardner (1974). Mitochondrial cytochrome c oxidase activity was measured spectrophotometrically at 550 nm and 25°C as described by Yonetani and Ray (1965) using cytochrome c (15 µM) at least 95% reduced (Wharton and Tzagoloff 1967) and dialyzed against 0.01 M potassium phosphate (pH 7.0) overnight.. Succinate dehydrogenase activity was measured by the method of Arrigoni and Singer (1962). To determine whether cadmium-induced low serum T_3 concentration had affected the activity of T_3-dependent a-GPD, normal rats were injected with T_3 500 ng/100 g BW s.c., twice daily, for two days prior to a-GPD measurement. One half of these rats received a single dose of cadmium chloride, i.p., 24 h before the experiments were initiated.

BAT cadmium concentration was measured by atomic absorption espectrometry (Shimadzu Mod.6501, Japan). Results were expressed in µg cadmium/g of BAT. Serum T_4 and T_3 concentrations were measured in duplicates by RIA as described before (Cageao et al. 1995). Statistical analyses were performed by the analysis of variance and the Duncan's test. Results throughout the text represent mean ± S.D.

RESULTS AND DISCUSSION

BAT weight in rats at 22°C ranged between 0.91 to 1.06 g/kg rat BW. The mitochondrial protein content averaged 10.6 ± 1.5 mg/ml. After 24 h in the cold, no significant changes were observed. Changes in BAT weight and protein content occur only after weeks of cold exposure (Rothwell and Stock, 1984b)

Figure 1 shows that the untreated, cold-exposed normal rats had a 2-fold rise in O_2 consumption. In the T_3-treated rats O_2 consumption at room temperature was significantly higher than normal, but the response to cold exposure was moderate and non-significant. Increases in BAT O_2 consumption after cold exposure were severely diminished after the administration of cadmium. BAT from the untreated hypothyroid rats kept at 22°C had normal O_2 consumption, but these rats were unable to increase consumption in response to cold. The results from hypothyroid rats did not change after cadmium administration.

When cadmium was added to BAT mitochondria *in vitro* (Fig. 2) it decreased the O_2 consumption in rats kept at room temperature or in the cold.

Normal rats (n=8) kept at 22°C and injected i.p. with cadmium chloride 24 h before the experiments, had a mean BAT cadmium concentration of 1.7 ± 0.92 (SD) µg/g of tissue. In another group of 8 normal rats injected i.p. with cadmium chloride and thereafter exposed to 4°C for 24 h, cadmium concentration increased to 15.6 ± 6.1 ($P<0.01$).

Normal rats (n=10) kept at room temperature had a mean serum T_4 concentration of 46.7 ± 12.3 nM and a serum T_3 of 1.58 ± 0.21 nM. Similar values were found in rats exposed to 4°C. After the *in vivo* administration of cadmium chloride n=8), serum T_4 declined to 26.1 ± 8.6 ($P<0.0l$) and serum T_3 to 1.08 ± 0.14 ($P<0.01$) nM. Mean serum T_3 concentration in T_3-treated hypothyroid rats (n=9) was 1.78 ± 0.24 nM and serum T_4 was undetected. After cadmium administration (n=8) T_3 decreased slightly to 1.62 ± 0.28 nM (NS). Cold exposure did not change the results. T_4 and T_3 were not detected in sera from untreated hypothyroid rats.

Table 1 shows the values of enzyme activities in cold-exposed control and cadmium-treated normal rats. Cytochrome c oxidase and α-GPD activities were both significantly decreased by the administration of cadmium. In normal rats receiving supplemental amounts of T_3, cadmium did not alter he activity of α–GPD. Cadmium administration had no effect on the activity of succinate dehydrogenase enzyme.

The data show that the administration of cadmium to normal rats inhibited the cold-induced increase in BAT mitochondrial O_2 consumption. This effect correlates with a 9-fold increase in BAT cadmium content in the cold, which, in

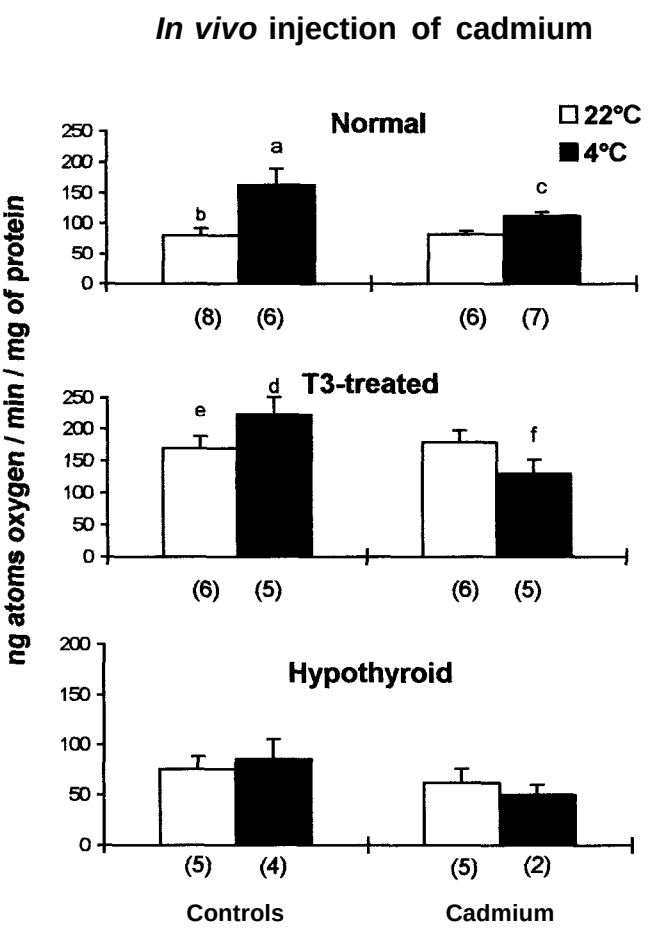

Figure 1. BAT mitochondrial O_2 consumption in rats injected with cadmium chloride 220 µg/100 g BW, i.p., 24 h before interscapular BAT was removed and processed. Cadmium was not added *in vitro*. Number in parenthesis indicates the number of experiments (BATfrom one rat/exp.). *a* versus *b*, *b* versus *e*, P<0.0l; *a* versus *c*, d versus *f*, P<0.02 (analysis of variance and Duncan's test).

turn, may result from a sharp rise in blood supply occurring in BAT after a few hours of exposure (Foster 1986).

The steps at which cadmium blocks the adaptative response of BAT to cold may be multiple. Conversion of T_4 to T_3 in BAT has been considered to be a critical step in the response to cold, since T_3 optimizes the noradrenaline stimulation of the uncoupling protein (UCP) synthesis. UCP is essential for the uncoupling of mitochondrial respiration and the production of heat. However, the present data show that serum T_3 concentration decreased in euthyroid rats treated with cadmium, but not in T_3-treated, hypothyroid rats treated with cadmium.. We

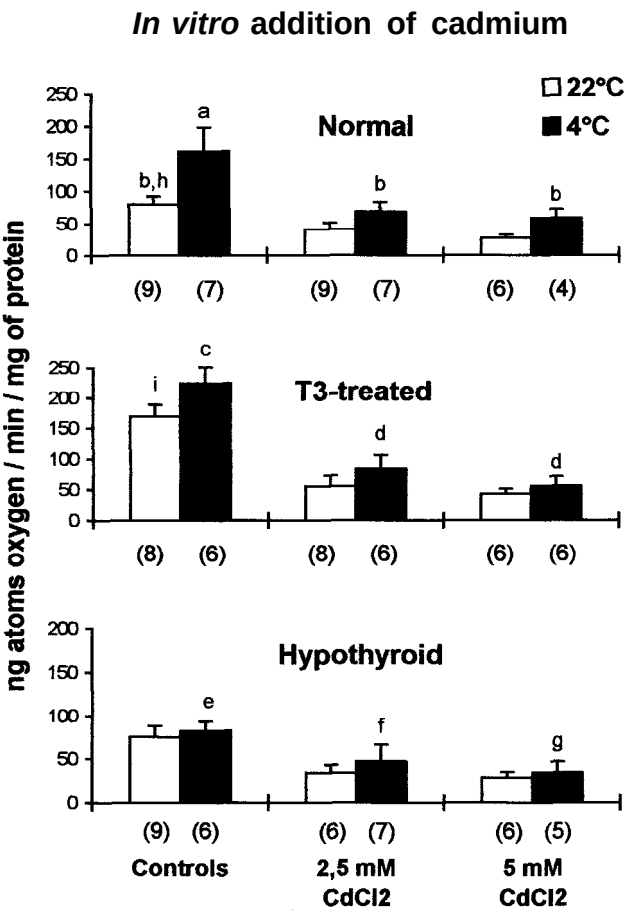

Figure 2. Mitochondrial O_2 consumption in BAT after cadmium chloride was added in vitro. Number in parenthesis indicates number of experiments (BAT from two rats/exp.). BAT mitochondria thus obtained was separated in aliquots, to which cadmium chloride in varied concentrations was added. *a* versus *b*, *c* versus *d*, $P<0.01$; *e* versus *f*, $P<0.05$; *e* versus *g*, $P<0.02$; *h* versus *i*, $P<0.0l$ (analysis of variance and Duncan's test).

recently observed that the *in vitro* T_3 production in BAT from cold-exposed normal rats decreased significantly after addition of cadmium (Paier et al. 1997). Further studies in rats subjected to BAT sympathectomy and thereafter implanted with slow-release noradrenaline tablets, showed that cadmium blocked the adrenergic activation of T_4 to T_3 conversion in BAT (Hofer et al. 1997). It appears, therefore, that cadmium blocked T_3 production in BAT.

The present results suggest that one mechanism by which cadmium alters O_2 consumption is through inhibition of BAT T_4 to T_3 conversion. This mechanism

Table 1. Effect of cadmium on BAT mitochondrial enzyme activities in cold-exposed normal rats and in normal rats receiving supplemental amounts of T_3.

		Cytochrome c oxidase	α-GPD
		μM cytochrome c oxidized/min/mg prot	ΔDO 500/min/mg prot
Control	(4)	12.4 ± 1.0	4.23 ± 0.62
Cadmium chloride	(4)	8 3 ± 0.9	2.20 ± 0.31
P value		< 0.01	< 0.01
Control + T_3	(3)		5.30 ± 0.72
Control+T_3+cadmium	(4)		4.34 ± 0.48

Number in parenthesis indicates number of determinations (BAT from two rats/determination). Cadmium chloride and T_3 were injected as described in the text. Values are means ± S.D. Probability values were determined by analysis of variance (only significant differences are shown).

however, can not account for the decreased O_2 consumption in cadmium-treated, cold-exposed rats whose T_3 derived solely from exogenous administration and not from endogenous T_4 deiodination, i.e.: the T_3-treated group. Even though enzyme activities were measured only in normal rats, it seems reasonable to assume that cadmium had also affected the activities of cytochrome c oxidase and α-GPD in the T_3-treated group, and that this effect reduced the consumption of O_2. α-GPD activity is T_3-dependent and therefore its decline in cadmium-treated normal rats could have been caused by the decreased serum T_3 concentration rather than by a direct effect of cadmium on the enzyme. This view is supported by the normal α–GPD activity in cadmium-treated normal rats injected with supplemental T_3.

In brief, the data show that cadmium-treated normal rats had decreased BAT mitochondrial oxidations in response to acute cold stress and a reduced activity of two enzymes of the respiratory chain. In addition, cadmium produced low serum T_3 and probably low BAT T_3 in normal rats, which had likely altered the uncoupling of the mitochondria.

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