Bull. Environ. Contam. Toxicol. (1997) 59: 164- 170

Bulletin of Environmental Contamination and Toxicology

Cadmium Inhibits the *in* vitro Conversion of Thyroxine to Triiodothyronine in Rat Brown Adipose Tissue

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Received: 12 December 1996/Accepted: 5 March 1997

Brown adipose tissue (BAT) thermogenesis during cold exposure in the rat is stimulated by noradrenaline (NA) activation of the synthesis of several enzymes essential for heat production and dissipation (see reviews Nichols and Locke 1984; Himms-Hagen 1990). One of the enzymes is 5'-deiodinase, whose activity in the cold increases by several-fold resulting in an increased T_4 to T_3 deiodination in BAT (Silva and Larsen 1985). Augmented T_3 production has been considered an important step in BAT response to cold, since deiodination of T_4 to T_3 optimizes NA activation of gene expression of the uncoupling protein (UCP), a protein crucial for uncoupling mitochondrial respiration to produce heat (Bianco and Silva 1987). In a previous study (Cageao et al. 1995) we observed that hypothyroid rats had a pronounced decrease of (^{3}H)guanosine-5'-diphosphate (GDP) binding to BAT mitochondrial proteins, This change reflected a diminished UCP concentration which follows the lack of thyroid hormones (Bianco and Silva 1987).

Cadmium has been shown to inhibit the *in vitro* conversion of T_4 to T_3 in several tissues (Yoshida et al. 1987; Paier et al. 1993; Pavia et al. 1997). Given the significance of BAT T_3 production in thermogenesis, the present study set out to investigate the effects of cadmium on the *in vitro* conversion of T_4 to T_3 in BAT from cold-exposed and control rats.

MATERIALS AND METHODS

Male Wistar rats (Dept. of Zoology, Univ. of Graz) of 220-250 g body weight were studied. They had free access to tap water and Purina chow in a room lighted between 06:00 and 20:00 h. A group of animals were placed in individual cages in a cold room at 4°C 24 h before the experiments were initiated. Double-labelled (3',5'-^{125}I)T_4 (Amersham, England, specific activity 1280 µCi/µg) was used. It was 95 % pure on arrival and it was used within a week. Its purity was checked during each experiment by chromatographic runs of the standard solution as received from the commercial source. Dithio-threitol (DTT) and cadmium chloride were

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purchased from Sigma Chemical Co., St. Louis, MO.

Control and cold-exposed rats were killed by cervical dislocation and the interscapular BAT was removed, weighed and individually homogenized under ice in buffer containing sucrose (320 mmol/l) and HEPES (10 mmol/l) in a proportion of 1 g BAT to 4 ml buffer, pH 7.4. The mixture was centrifuged at 500 g for 10 min at 4°C. This resulted in a creamy supernatant, a cloudy infranatant and a sediment. The infranatant contained the deiodinating activity and it was used for the study of T_4 deiodination. Two hundred µl aliquots of infranatant were separated and added cadmium chloride in doses of 0 (controls), 1, 50, 100 µmol/l, 1,5 or 10 mmol/l. Each aliquot was added, in addition, 0, 5, 10 or 25 mmol/l DTT (final concentration) and 1 µCi (^{125}I)T_4 containing 0.8 ng T_4. Tissue-free tubes containing reagents in concentrations similar to those added to homogenates, plus labelled T_4 were also prepared. All aliquots and blanks were incubated for 60 min in a water bath at 37°C under continuous shaking. Deiodination was stopped with an equal volume of methanol:ammonia (99:1).

Samples of each homogenate and tissue-free controls were subjected to paper chromatography in tertiary amyl alcohol:hexane:ammonia (TTA) (10:1:12) and butanol:dioxane:ammonia (4:1:5) solvents for approximately 18 h. Chromatograms were cut into 0.5 cm segments and counted. The counted radioactivity was corrected for the proportion of radioactive compounds other than (^{125}I)T_4 that were present in the chromatographic runs of the commercial solution of (^{125}I)T_4 and of the tissue-free preparations in the manner described previously (Ceppi and Zaninovich 1989).

The absolute amount of T_3 produced by deiodination of (^{125}I)T_4 was calculated from the product of the percentage of (^{125}I)T_3 present in BAT homogenates, as indicated in Table 1, and the amount of T_4 (0.8 ng) carried by the dose of (^{125}I)T_4. This value was corrected per mg of protein in the homogenate. The results thus obtained refer only to T_3 derived from added T_4 and does not represent the total pool of T_3 in BAT. A similar procedure was employed to calculate absolute T_4

Protein concentration in infranatants was measured by the method of Lowry et al. (1951). Statistical analyses were made by analysis of variance and the Dunnett's test.

RESULTS AND DISCUSSION

BAT weight in control groups ranged from 1020 to 1234 mg/kg body weight. In cadmium-treated rats the range was 964 to 1180 mg/kg body weight. Cold exposure did not change weight values although BAT became visibly darker due to a sharp increase in blood supply. Pronounced increases in BAT weight were only observed after several weeks of cold-exposure (Rothwell et al. 1984). In rats at 22°C protein concentrations in infranatants averaged 6.2 ± 1.1 (SD) mg/ml

whereas in cold-exposed rats the mean protein value was 6.9 ± 0.9 mg/ml. The difference was not significant.

Following the incubation of tissue-free systems and chromatographic runs of the standard (^{125}I)T_4 solution, no (^{125}I)T_3 radioactivity was detected. Therefore the (^{125}I)T_3 measured in the homogenates was considered to derive from tissue deiodination of (^{125}1)T_4. Table 1 shows that infranatants from control rats at 22°C deiodinated 29.1 ± 11.1 % of added labelled T_4 in the presence of 5 mmol/l DTT. This value represented 185.9 ± 66.8 pg T_4/h/mg protein. Of this amount, about 39 % or 72.7 ± 40.9 pg were converted to T_3, and the rest was mostly iodine. Only traces of radioactivity were detected in the chromatographic area of 3,3'-T_2. Addition to the homogenates of 0.001, 0.050 or 0.1 mmol/l cadmium chloride did

Table 1. Effect of cadmium chloride on T_4 to T_3 conversion in rat BAT in the presence of 5 mmol/l DTT.

		(^{125}I)T_4 degraded		(^{125}I)T_3 generated *	
		as % of injected dose	pg/mg protein/h	as % of injected dose	pg/mg protein/h
22°C					
Controls	(6)	29.1 ± 11.1	185.9 ± 66.8	11.4 ± 6.7	72.7 ± 40.9
Cadmium Chloride					
1 mmol/l	(9)	19.7 ± 5.7	126.5 ± 39.0	5.5 ± 2.8	36.2 ± 18.2
5 mmol/l	(9)	10.7 ± 4.5 [a]	67.3 ± 6.3 [a]	1.7 ± 1.1 [a]	11.9 ± 7.4 [a]
4°C					
Controls	(9)	97.8 ± 0.47	567 ± 3.0	46.7 ± 0.9	271 ± 5.4
Cadmium Chloride					
1 mmol/l	(5)	95.5 ± 2.0	555 ± 11	47.1 ± 7.6	272 ± 35
5 mmol/l	(8)	32.6 ± 19 [a]	188 ± 92 [a]	4.3 ± 1.9 [a]	25 ± 11 [a]
10 mmol/l	(8)	15.6 ± 2.8 [a]	90 ± 22 [a]	1.8 ± 1.6 [a]	10 ± 8.0 [a]

BAT homogenates were combined with 1 µCi (^{125}I)T_4, 5 mmol/l DTT and the indicated dose of cadmium chloride. Number in parenthesis indicates number of experiments, * T3 derived only from deiodination of added (^{125}I)T_4; these values do not represent the total BAT T_3 pool. For calculations see text. Other products of (^{125}I)T_4 deiodination were mostly iodine and traces of 3,3'- T_2. Values are means ± SD. P values versus respective control groups: a < 0.01 (analysis of variance).

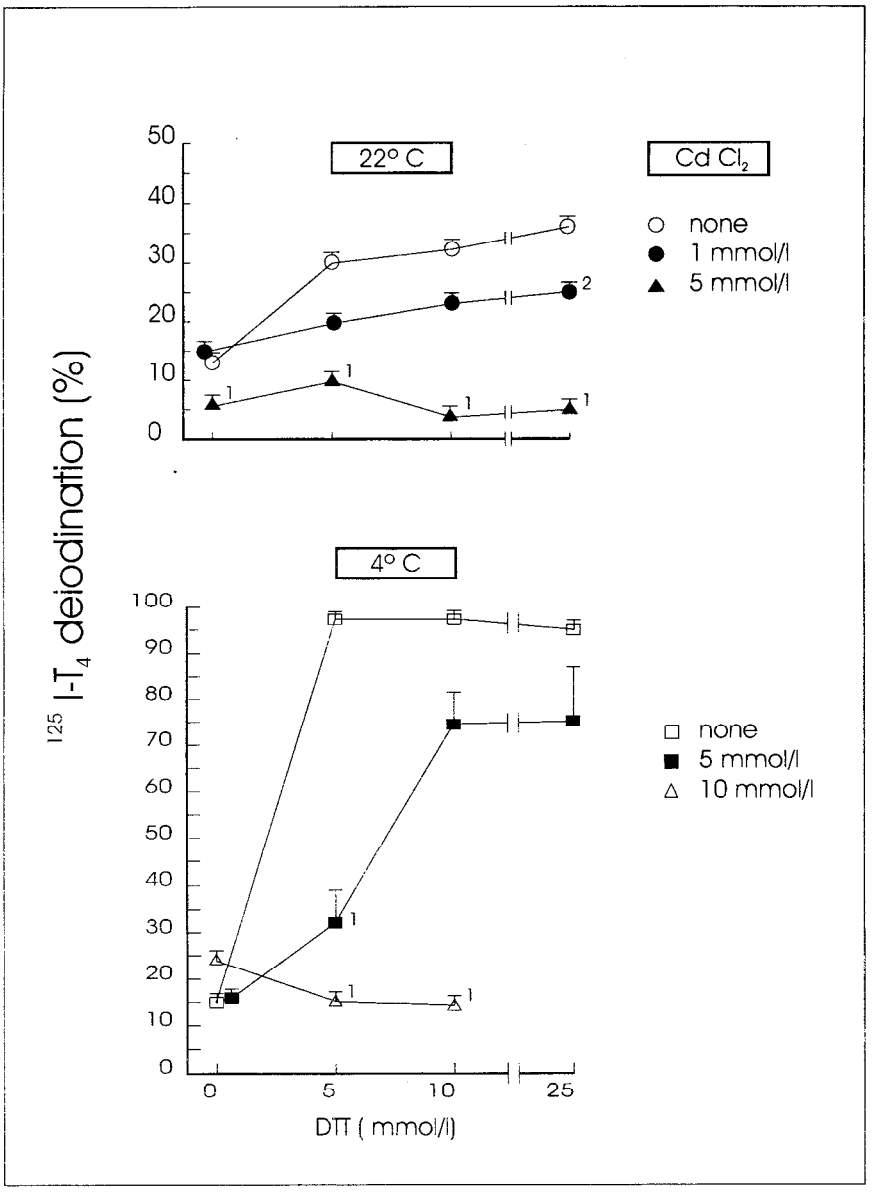

Figure 1. Effects of cadmium on BAT T4 deiodination in the presence of varied concentrations of dithiothreitol (DTT). Six to 10 experiments were performed in each cadmium treated or control groups. Means ± SD. P values versus control experiments: [1] < 0.01, [2] < 0.05 (analysis of variance).

not alter T_4 deiodination. With 1 mmol/l cadmium the conversion of T_4 declined to 19.7 % of the injected radioactivity. Despite a pronounced decrease in T_3

generation, the results were not statistically significant due to a wide distribution of values. When 5 mmol/l cadmium chloride was used, the decrease in deiodination and in T_3 generation were highly significant ($P<0.01$).
Cold exposure markedly increased T_4 deiodination as seen in Table 1. In the control group, about one-half of the reaction products was T_3 and the rest iodine. One mmol/l cadmium did not alter (^{125}I)T_4 deiodination. Five and 10 mmol/l doses significantly ($P<0.01$) inhibited T_4 degradation and the resultant T_3 generation ($P<0.01$).

Figure 1 depicts the influence of DTT concentration on T_4 deiodination. At every DTT concentration cadmium-treated homogenates deiodinated less T_4 than control homogenates.

The present findings indicate that the addition of cadmium to BAT homogenates blocked the *in vitro* T_4 to T_3 conversion. This study used cadmium concentrations similar to those which in liver (Yoshida et al. 1987; Paier et al. 1993) and the pituitary gland (Pavia et al. 1997) significantly inhibited T_4 deiodination.

Cadmium is a toxic metal that rapidly accumulates in liver and kidney following its administration to animals (Nordberg 1984). The toxic effect depends largely on the metal concentration in tissues. Foulkes (1990) reported a critical level of 200 µg cadmium/g of kidney tissue to observe toxicity. A peculiar aspect in brown fat physiology is that it activates several-fold after a few hours of exposure to cold. This leads to 7 to 10-fold enhancement of the synthesis and activity of 5'-deiodinase (Silva and Larsen 1985) and, therefore, larger concentrations of cadmium are necessary to block the enzyme. Thus, 1 mmol/l cadmium chloride blocked T_4 conversion in BAT homogenates from 22°C-adapted rats, but it was ineffective in blocking deiodination in homogenates from cold-exposed animals.

The probable mechanism of cadmium effect on 5'-deiodinase may be related to the strong affinity of cadmium for the sulfhydryl groups of the enzyme (Vallee and Ullmer 1972). The activity of 5'-deiodinase is largely dependent on the presence of sulfhydryl groups (Visser et al. 1976; Chopra 1978). Vallee and Ullmer (1972) demonstrated that cadmium can inhibit many enzymes through binding to sulfhydryl groups on the active site of the enzyme or by interference with the formation of enzyme-substrate complexes. This view was supported by the findings of Chan and Cherian (1992) that hepatic toxicity of cadmium may be due to its binding to intracellular sulfhydryl groups and that glutathione, a thiol carrier, provides protection against cadmium toxicity. We also observed that the *in vivo* administration of DTT to cadmium-treated rats restored hepatic concentrations of nonprotein sulfhydryl groups and activity of 5'-deiodinase to normal (Paier et al, 1993). The supply of thiol groups can protect enzymes from toxic effects of metals other than cadmium (Susuki and Osaki 1984). However, this and previous data suggest that factors other than thiol groups may be involved in T_4 deiodination, since the addition of DTT to cadmium-treated homogenates did not fully restore

the levels of deiodination observed in cadmium-free homogenates in this and previous work (Paier et al. 1993; Yoshida et al. 1987; Pavia et al. 1997). Similar conclusions were suggested by Harris et al. (1979) and Chopra (1980) after studying the role of sulfhydryl groups on T_4 to T_3 conversion in rat liver homogenates.

Even though the data demonstrate that cadmium blocks BAT T_4 to T_3 conversion *in vitro,* as it does in similar concentrations in other organs, one can not extrapolate these findings to the more complex situation of BAT calorigenesis *in vivo,* where a toxic effect will depend on the cadmium dose and its tissue concentration.

Acknowledgments. Supported by grant 4308 from der Österreichischen Nationalbank, Vienna, Austria.

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