Bull. Environ. Contam. Toxicol. (2001) 67:641–648

DOI: 10.1007/s00128-001-0172-1

Bulletin of Environmental Contamination and Toxicology

Effects of Zinc on Brown Fat Thermal Response to Cold in Normal and Triiodothyronine-Treated Hypothyroid Rats

I. Rebagliati,[1] M. Raíces,[1] C. Ricci,[1] A. Weisstaub,[2] K. Hagmüller,[3] A. A. Zaninovich[1,3]

[1] Hospital de Clínicas and INGEBI, CONICET and University of Buenos Aires, Argentina
[2] Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina
[3] Faculty of Natural Sciences, Department of Zoology, University of Graz, Austria

Received: 12 January 2001/Accepted: 26 June 2001

The cold-induced rise in brown adipose tissue (BAT) thermogenesis is largely dependent on the activity of noradrenaline (NE) and the thyroid hormones, which synergistically stimulate the synthesis of the uncoupling protein (UCP1) (Rothwell & Stock 1984, Himms-Hagen 1985, Bianco & Silva 1987, Silva 1988). Recently, activation of BAT by T3 with minimal presence of NE was reported (Branco et al. 1999). The activity of BAT 5′-deiodinase type II (5′-DII) is critical for thermogenesis because it supplies triiodothyronine (T3) needed for the synthesis of UCP1. Reciprocally, the activity of 5′-DII is dependent on NE supply to BAT (Hofer et al. 2000). Heavy metals, such as cadmium, have been shown to inhibit type I (Yoshida et al. 1987, Paier et al. 1993) and type II (Pavia et al. 1997, Paier et al. 1997) 5′-deiodinases. Zinc (Zn), another heavy metal, also blocked type I (Gonzalez Pondal et al. 1995) and type II (Pavia et al. 1999) 5'-deiodinases.

In view of the important rol of T3 in BAT thermogenesis, we examined whether Zn, in addition to its depressive effects on thyroxine (T4) to T3 conversion, alters the action of T3 on the thermogenic process.

MATERIALS AND METHODS

Male Wistar rats of 240-280 g of body weight (BW) were used. They had free access to water and food (Purina chow). Groups of rats were made hypothyroid by the i.p. injection of ^{131}I. After 4 weeks, groups of hypothyroid rats were treated with T3, dissolved in alkalinized saline, 300 or 1500 ng per 100 g of BW, s.c., in divided doses, daily for 2 days. Normal rats received the vehicle alone. One-half of all groups were injected, in addition, with Zn sulphate dissolved in saline, 10 or 20 mg per kg, i.p., given once daily for 2 days. Treatments continued in a cold room for 48 h. Thereafter, animals were killed by cervical dislocation and interscapular BAT and blood were obtained and processed as described below.

Correspondence to: A. A. Zaninovich, Hospital de Clínicas, C.C.29 Oficina Facultad, 1453 Buenos Aires, Argentina

Oxygen consumption was measured in BAT homogenized under ice in buffer containing sucrose (0.25 M), N-tris-methyl-2-aminoethanesulfonic acid (K-TES) (5 mM), disodium ethylenediamine tetraacetate (EDTA) (2 mM) and bovine serum albumin (BSA) (2%) pH 7.2. Mitochondria were isolated by differential centrifugation as described by Cannon & Lindberg (1979). The final pellet was suspended in sucrose buffer (0.24 M) and protein content was determined. 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% BSA pH 7.2. Alpha-glycerophosphate was used as substrate due to the presence in BAT of a highly active alpha-GPD enzyme (Cannon & Lindberg 1979).

Isolation of mitochondria and measurement of (^{3}H)GDP binding was performed by techniques described earlier (Scarpace et al. 1992, Cageao et al. 1995). Briefly, BAT mitochondria were incubated with 0.75 µM (8,5′-^{3}H)GDP (specific activity adjusted to 1.06 Ci/mM) in the presence or absence of unlabelled 1.5 mM GDP at 37°C for 15 min in a total volume of 250 µl of the buffer used for the final suspension, with the addition of 0.1 mg fatty acid-free BSA/ml and 2 mM rotenone. The reaction was terminated by dilution with buffer and the bound (^{3}H)GDP was separated from free (^{3}H)GDP by filtration over glass fiber filters (GD/B; Whatman, Clifton, NJ, USA). Specific binding was determined from the difference in binding with and without 1.5 mM unlabeled GDP.

BAT Zn concentration was determined by atomic absorption spectrometry, using a Varian Spectrophotometer SpectrAA-20 at a wave length of 213.9 mm air. Serum T4 and T3 concentrations were measured by RIA (Diagnostic Products Corporation, Los Angeles, CA, USA). Limits of sensitivity were 3 nmol/l for T4 and 0.10 nmol/l for T3. Statistical analysis was performed by variance analysis.

RESULTS AND DISCUSSION

Table 1 shows the effects of Zn on BAT weight and protein content. In normal rats, Zn 10 mg induced a significant decrease in BAT weight and protein content, whereas hypothyroid rats with normal T3 replacement were not affected. Hypothyroid rats treated with high T3 doses had a marked fall in BAT weight and protein content after the administration of Zn 20 mg. In normal rats without Zn treatment, BAT Zn concentration averaged 11.8±5.2 µg/g of tissue. Comparable figures were obtained in T3-treated, hypothyroid groups. Following Zn treatment, these values did not change. Zn induced a decrease in serum T3 levels in normal rats, whereas serum T4 was unchanged.

Table 1. Brown adipose tissue parameters in cold-exposed rats*.

Groups	BAT weight	Mitochondrial protein	Zn content	Serum T3
	mg	mg/ml	ug/g tissue	nmol/l
Normal	282±0.7[a]	5.6±0.22[e]	11.8±5.2	0.74±0.06[i]
Normal+Zn	222±10[b]	4.9±0.29[f]	12.5±2.8	0.61±0.04[j]
Hypothyroid				
+T3 300 ng	243±12	5.37±0.22	11.1±2.3	0.66±0.05
+T3 300+Zn 10 mg	238±11	5.28±0.12	11.4±2.5	0.64±0.05
+T3 1500 ng	260±10[c]	5.68±0.13[g]		1.13±0.11
+T3 1500+Zn 10 mg	247±12	5.50±0.20	12.1±3.1	1.09±0.10
+T3 1500+Zn 20 mg	135±09[d]	3.88±0.13[h]	14.1±3.6	1.16±0.15

Data are the means ± S.D. of eight rats per group. *Data from the groups of rats whose O_2 consumption values are presented in figure 1 and in the text. T3 doses are expressed as ng/100 g of BW/day and were injected s.c., in divided doses, every 12 h for 2 days. Zn doses are expressed as mg/kg of BW/day, given i.p., once daily for 2 days. T3 and Zn were injected simultaneously and animals were immediately placed in a cold room at 4°C for 48 h. Probability values: *a* versus *b*, *c* versus *d* and *g* versus *h*, $P<0.001$; *e* versus *f*, $P<0.01$; *i* versus *j*, $P<0.02$; (analysis of variance and Duncan's test).

Data from mitochondrial O_2 consumption are shown in figure 1. Zn significantly reduced O_2 consumption in normal and in T3-treated rats. High doses of T3 prevented the decrease in O_2 consumption in rats treated with the 10 mg Zn dose, but not in rats receiving the 20 mg dose of this metal.

Figure 2 shows a 65% reduction in GDP binding to BAT mitochondrial proteins in Zn-treated, normal rats. Before Zn administration, T3-treated, hypothyroid rats had lower binding values than untreated normal rats. This effect was seen before in T3-treated, cold-exposed, hypothyroid rats (Cageao et al. 1995; Zaninovich et al. 2000) and is presumably due to reduced UCP1 synthesis caused by lack of T4-derived T3 (Bianco et al. 1987). Injection of Zn into T3-treated, hypothyroid rats, induced a marked fall in GDP binding, an effect not prevented by treatment with high T3 doses.

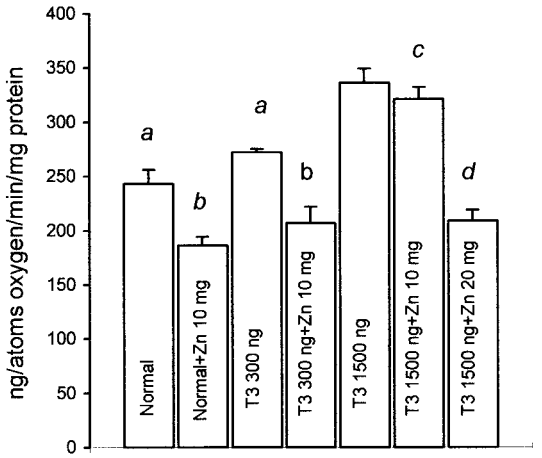

Figure 1. Brown adipose tissue (BAT) mitochondrial O_2 consumption after 48 h exposure to 4°C in normal or hypothyroid rats injected with T3 and/or zinc sulphate, daily, for 48 h before interscapular BAT was removed and processed. Data are the means ±S.D. of eight rats per group. T3 and Zn doses, time course and route of administration are described in Table 1. Probability values: *a* versus *b* and *c* versus *d*, $P<0.002$ (analysis of variance and Duncan's test).

Zn is a physiological element found in most biological systems and is an essential component of many enzymes involved in multiple metabolic processes (see review Vallee & Falchuk 1993). Zn deficiency affects numerous organs, including the endocrine system and the thyroid gland (Morley et al. 1980, Lukaski et al 1992). The adverse effects of excessive Zn intake has received increasing attention in recent years (Fosmire 1990). Its effects on BAT function, however, are largely unknown. In isolated brown adipocytes from genetically obese mice, doses up to 200 µM Zn sulphate decreased GDP binding, but this effect was not seen in lean mice (Chen et al. 1997). Paier et al. (1997), found that 100 µM Zn sulphate added to rat BAT homogenates decreased T4 deiodination. Comparable effects were seen in rat liver after the i.p. injection of a single dose of Zn sulphate 2 mg/kg of BW (Gonzalez Pondal et al. 1995).

In the present study, the *in vivo* administration of Zn inhibited mitochondrial O_2 consumption and GDP binding. Because large doses of Zn have caused generalized tissue damage in humans (Bennet et al. 1997) and animals (Meuers et al 1991), it is critical to determine whether the injected dose was sufficiently high as to cause unspecific tissue damage. The 10 mg Zn sulphate dose carried 3.5 µM Zn/100 g BW which makes an average of 8.8 µM/day for a 240 g rat. This dose represents after body distribution, a much lower load than the µM doses used in *in vitro* homogenates described above or in the

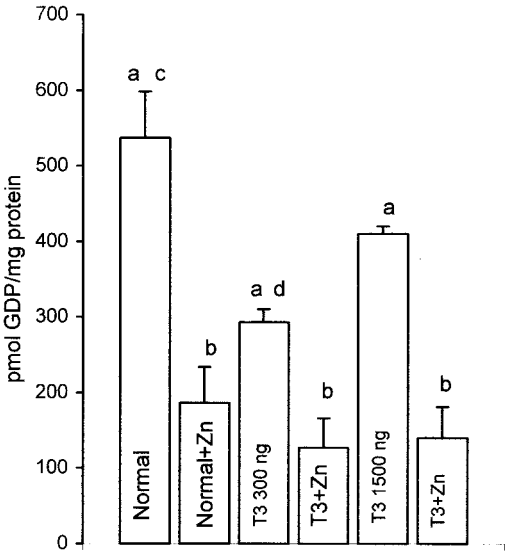

Figure 2. GDP binding to BAT mitochondrial proteins in normal and T3-treated hypothyroid rats exposed to 4°C for 48 h. Means±S.D. Five rats per group were studied. T3 doses represent ng/100 g BW /day, s.c., in divided doses, for 2 days. Zn sulphate was injected in a dose of 10 mg/kg BW/day, i.p., once daily for 2 days. *a* versus *b* and c versus d, $P<0.001$ (analysis of variance and Duncan's test).

study of Brown et al. (2000) adding up to 6.4 μM Zn to the Oxygraph chamber (1.7 ml) containing about one drop of liver mitochondria.

It was surprising to find normal Zn concentration in BAT from Zn-treated rats. This may reflect a low dose, low BAT uptake or, alternatively, a rapid Zn turnover. Parameters of Zn kinetics in BAT, which would enable a better assessment of these data, are not available. Nevertheless, the administered Zn dose induced marked reductions in BAT weight and mitochondrial protein content. These changes may have originated in an inhibitory action of Zn on protein or RNA synthesis. The *in vitro* exposure of rat and human lung cells to 120 to 150 μM Zn blocked RNA and protein synthesis (Walther et al. 1998). Similarly, 20 μM lead, another toxic metal, completely inhibited the DNA binding mechanism of Zn finger protein transcription factor IIIA (Hanas *et al.* 1999).

The mechanism by which Zn toxicity blocked the adaptative response of BAT to cold may be multiple. A reduced BAT T4 to T3 conversion or lack of T3 decreases T3 supply to BAT. This leads to a fall in UCP1 synthesis (Bianco & Silva 1987), mitochondrial GDP binding (Cageao et al. 1995, Zaninovich et al. 2000) or O_2

consumption (Noli et al. 1998). This mechanism could account for the findings in normal rats but it would not suffice to explain the results in T3-treated rats, whose T3 derived solely from exogenous administration and not from endogenous T4 deiodination. The observation that high T3 doses prevented the depressive effects of Zn on O_2 consumption and that the high Zn dose returned O_2 consumption to the range observed in BAT mitochondria from normal ratas, suggests a dose-related inhibition of Zn on T3 action. However, inhibition of mitochondrial respiration may have been produced by other pathways, as seen in liver mitochondria of rats adapted to room temperature (Brown et al. 2000).

Previous work demonstrated that Zn interacts with sites of the T3 receptor and induces reversible cross-linking in a concentration-dependent manner (Surks et al. 1989, Lin & Cheng 1991). Any step from the binding of T3 to its receptor, to the binding of the T3-receptor complex to a specific DNA segment and RNA polymerase could have been blocked by Zn.

In summary, the data suggest that Zn induced a depression of BAT thermogenesis mainly by blocking the participation of T3 at some step of the biosynthetic pathways. The depression of mitochondrial respiration through other mechanisms, as seen in liver via α-ketoglutarate dehydrogenase pathway (Brown et al. 2000), can not be ruled out.

Acknowledgements. This study was supported by grants PIP 4002 from CONICET and PICT 1678 from SECYT, Argentina, and from the University of Graz, Austria.

REFERENCES

Bennett DR, Baird CJ, Chan KM, Crookes PF, Bremmer C, Gottlieb MM, Naritoku WY (1997) Zinc toxicity following massive coin ingestion. American J Foren Med Path 18: 148-153.

Bianco AC & Silva JE (1987) Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. J Clin Invest 79: 295-300.

Branco M, Ribeiro M, Negrao N, Bianco AC (1999) 3,5,3′-Triiodothyronine actively stimulates UCP in brown fat under minimal sympathetic activity. American J Physiol 39: E179-E187.

Brown AM, Kristal BS, Effron MS, Shestopalov AI, Ullucci PA, Rex Sheu, Blass JP, Cooper AJL (2000) Z^{2+} inhibits α-ketoglutarate-stimulated mitochondrial respiration and the isolated α-ketoglutarate dehydrogenase complex. J Biol Chem

270: 25001-25006.
Cageao LF, Noli MI, Mignone IR, Farber M, Ricci CR, Hagmüller K & Zaninovich AA (1995) Relative roles of the thyroid hormones and noradrenaline on the thermogenic activity of brown adipose tissue in the rat. J Endocrinol 145: 579-584.
Cannon B & Lindberg O (1979) Mitochondria from brown adipose tissue: isolation and properties. Methods Enzymol 55: 65-71.
Chen MD, Lin PY, Chen PS, Cheg V & Lin WH 1997 Zinc attenuation of GDP binding to brown adipocytes mitochondria in genetically obese (ob/ob) mice. Biol Trace Elem Res 57: 139-145.
Fosmire GJ (1990) Zinc toxicity. American J Clin Nutr 51: 225-227.
Gonzalez Pondal M, Paier B, Noli MI, Tudino NB, Stripeikis JD, Hagmüller K & Zaninovich AA (1995) Changes induced by zinc on thyroxine deiodination by rat liver in vivo and in vitro. Acta Physiol Pharmacol Ther Latinoam 45: 34-41.
Hanas JS, Rodgers JS, Bantle JA & Cheng YG (1999) Lead inhibition of DNA-binding mechanism of cys(2)His(2) zinc finger proteins. Mol Pharmacol 56: 982-988.
Himms-Hagen J (1985) Brown adipose tissue metabolism and thermogenesis. Ann Rev Nutr 5: 69-94.
Hofer D, Raíces M, Schauenstein K, Porta S, Korsatko W, Hagmüller K & Zaninovich AA (2000) The in vivo effects of beta-3-receptor agonist CGP-12177 on thyroxine deiodination in cold-exposed, sympathectomized rat brown fat. European J Endocrinol 143: 273-277.
Lin KH & Cheng SY (1991) Zn^{+2} induces reversible cross-linking of human placental thyroid hormone nuclear receptor with no effect on hormone binding. Biochem Biophys Res Commun 175: 250- 255.
Lukaski HC, Hall CB & Marcello MJ (1992) Impaired thyroid hormone status and thermoregulation during cold exposure of zinc-deficient rats. Horm Metab Res 24: 363-366.
Meurs KM, Breitschwerdt EB, Baty CJ & Young MA (1991) Postsurgical mortality secondary to zinc toxicity in dogs. Veterin Human Toxicol 33: 579-583.
Morley JE, Gordon J & Hershman JM (1980) Zinc deficiency, chronic starvation and hypothalamic-pituitary-thyroid function. American J Clin Nutr 33: 1767-1770.
Noli MI, Pavia MA Jr, Mignone IR, Brignone JA, Hagmüller K & Zaninovich AA (1998) Cadmium chloride prevents the rise in rat brown adipose tissue mitochondrial respiration in response to acute cold stress. Bull Environ Contam Toxicol 61: 31-37.
Paier B, Hagmüller K, Noli MI, Gonzalez Pondal M, Stiegler C & Zaninovich AA (1993) Changes induced by cadmium administration on thyroxine deiodination and sulfhydryl groups in rat liver. J Endocrinol 138: 219-224.
Paier B, Pavia MA Jr, Hansi C, Noli MI, Hagmüller K & Zaninovich

AA (1997) Cadmium inhibits the in vitro conversion of thyroxine to triiodothyronine in rat brown adipose tissue. Bull Environ Contam Toxicol 59: 164-170.

Pavia MA Jr, Paier B, Hagmüller K & Zaninovich AA (1999) Zinc inhibits the in vitro conversion of thyroxine to triiodothyronine in brown adipose tissue. Medicina (B Aires) 59:265-268. (Article in Spanish).

Pavia MA Jr, Paier B, Noli MI, Hagmüller K & Zaninovich AA (1997) Evidence suggesting that cadmium induces a non-thyroidal illness syndrome in the rat. J Endocrinol 54: 113-117.

Rothwell N J & Stock MJ (1984) Brown adipose tissue. In: Backer PF (ed) Recent Advances in Physiology, vol 10. Churchill-Levingstone, Edinburgh, p 349.

Scarpace PJ, Matheny M & Borst SE (1992) Thermogenesis and mitochondrial GDP binding with age in response to the novel agonist CGP-12177A. American J Physiol 262: E185S-E-190.

Silva EJ (1988) Full expression of uncoupling protein protein gene requires the concurrence of norepinephrine and triiodothyronine. Mol Endocrinol 2: 706-713.

Surks MI, Ramirez IJ, Shapiro LE &Kumara-Siri M (1989) Effects of Zinc (II)and other divalent cations on binding of 3,5,3′-triiodo-L-thyronine to nuclear receptors from cultured GC cells. J Biol Chem 264: 9820-9826.

Vallee B & Falchuk KH (1993) The biochemical basis of zinc physiology. Physiol Rev 73: 79-119.

Walther UI, Schulze J & Forth W (1998) Inhibition of protein synthesis by zinc: Comparison between protein synthesis and RNA synthesis. Hum Exp Toxicol 17: 661-667.

Yoshida K, Sugihira N, Suzuki M,, Sakurada T, Saito S, Yoshinaga K & Saito H (1987) Effect of cadmium on T4 outer ring monodeiodination by rat liver. Environ Res 42: 400-405.

Zaninovich AA, Rebagliati I, Raices M, Ricci K & Hagmüller K (2000) Effects of thyroxine on rat brown fat and muscle thermogenesis in the cold. Endocr Res 26: 231-245.