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Effect of reserpine pretreatment on brown fat iodothyronine 5′-deiodinase of mouse

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Abstract—The effect of pretreatment with reserpine (1 mg kg^{-1} i.p. daily for 7 days) on the regulation of iodothyronine 5′-deiodinase (5′D) in mouse brown adipose tissue (BAT) has been examined. 5′D activity of BAT homogenate was assessed by the in-vitro formation of 3,5,3′-triiodothyronine from thyroxine and 3,3′-diiodothyronine from 3,3′,5′-triiodothyronine in the presence of 20 mM dithiothreitol. Reserpine treatment decreased the stimulation of BAT 5′D induced by acute cold exposure (4°C, 2 h) without a significant decrease in the basal 5′D activity, whereas stimulation of BAT 5′D elicited by noradrenaline (0·4 and 0·8 mg kg^{-1} s.c. 2 h previously) was not augmented after reserpine treatment. Although both noradrenaline and acute cold exposure increase BAT 5′D through α_1-adrenoceptors, our results show that chronic reserpine treatment prevents the effect of cold, but does not induce α_1-adrenoceptor supersensitivity in BAT.

Brown adipose tissue (BAT) is the main site of cold-induced thermogenesis in rodents, and the effect of cold exposure is mediated through activation of the sympathetic nervous system followed by β-adrenoceptor stimulation (Bukowiecki 1984; Himms-Hagen 1986). The intracellular conversion of thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3) is required for the optimal thermogenic function of BAT (Bianco & Silva 1987). The enzyme iodothyronine 5′-deiodinase (5′D) converts T_4 to physiologically active T_3 and also inactive 3,3′,5′-triiodothyronine (rT_3) to 3,3′-diiodothyronine ($3,3'T_2$). BAT contains a propylthiouracil-insensitive 'type II' 5′D (Leonard et al 1983), and a large increase in BAT 5′D activity by noradrenaline administration and by cold exposure was reported (Silva & Larsen 1983). Both noradrenaline and cold exposure increase BAT 5′D through α_1-adrenoceptors, whereas inhibition of catecholamine synthesis with α-methyl-*p*-tyrosine prevented the effect of cold but not that of noradrenaline. The latter result suggests that stimulation of the sympathetic nervous system may be responsible for the cold effect. It is known that chronic reserpine treatment causes an increase in the sensitivity of both vas deferens and caudal artery to a variety of contractile agents including α_1-agonists (Nasseri et al 1985). On the other hand, catecholamine depletion with reserpine induced an increase in β-adrenoceptor sensitivity in rat heart; however, cardiac α_1-adrenoceptors did not exhibit supersensitivity (Chess-Williams et al 1987). Therefore, chronic reserpine treatment does not necessarily induce α_1-adrenoceptor supersensitivity in all the organs which bear α_1-adrenoceptors. Recently, it was reported that atria and brown adipocyte exhibited β-adrenoceptor supersensitivity after reserpine pretreatment in rats (Grassby et al 1987). However, the change in α_1-adrenoceptor-mediated responses in BAT was not investigated. The purpose of the present study is to examine how reserpine treatment affects the α_1-adrenoceptor-mediated changes in the mouse BAT 5′D activity.

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Materials and methods

Reagents. [^{125}I]rT_3 and [^{125}I]T_4, labelled in the outer rings, were purchased from New England Nuclear (Boston, MA). Apoplon injection (0·1% reserpine) was purchased from Daiichi Pharmaceut. Co. (Tokyo) and Noradrenalin (0·1% (±)-noradrenaline) from Sankyo Co. (Tokyo). The drug solutions were diluted with sterile 0·9% saline immediately before use. All other drugs used were obtained from commercial sources.

Animals. Female mice aged between 8–10 weeks-old were maintained in an air-conditioned room (21–23°C) with lights on between 0600–2000 h. They were fed Labo MR Breeder chow (Nihonnosan Co., Yokohama) and had free access to water. For reserpinization, mice were given i.p. injections with 1 mg kg^{-1} reserpine once a day for 7 days. Control mice received 0·9% saline (10 ml kg^{-1}) i.p. instead of reserpine. The mean body weight ± s.e. of control mice was 29·9 ± 0·5 g (n = 25) and that of reserpinized mice was 22·1 ± 0·3 g (n = 25). Cold exposure and noradrenaline challenge were performed on the next day of the last reserpine injection. Cold exposure was done in a lighted walk-in refrigerated room at 4°C for 2 h. Noradrenaline was injected s.c. at the dose of 0·4 and 0·8 mg kg^{-1} in a volume of 10 mL kg^{-1}. Control (0 mg kg^{-1}) mice received s.c. injections of the same volume of 0·9% saline. Animals were killed by cervical dislocation 2 h after injection of noradrenaline or saline or at the end of 2 h cold exposure. Two h was chosen because a previous study examined the effect of noradrenaline in the rat BAT at that time (Silva & Larsen 1983).

Analytical procedure. BAT was taken from the interscapular region, trimmed free of white fat and weighed. The mean weight of BAT ± s.e. from control mice was 118 ± 6 mg (n = 25) and that from reserpinized mice was 95 ± 6 mg (n = 25). The tissues were homogenized in 9 volumes of ice-cold Krebs-Ringer phosphate buffer, pH 7·4 (KRP). The homogenate was centrifuged at 800 *g* for 10 min at 4°C and the liquid layer below the fat layer was used immediately for deiodinase measurement as the enzyme. 5′-Deiodination was assayed as described previously (Maeda & Ingbar 1982; Tsukahara et al 1989). Briefly, the incubation mixture contained the enzyme (0·5–1·5 mg protein), [^{125}I]T_4 or [^{125}I]rT_3 (0·5–1·5 μCi, or 2–3 pmol), dithiothreitol (4 μmol) and KRP buffer to make a final volume of 200 μL. After incubation at 37°C for 2 h, the reaction was terminated by addition of 200 μL methanol-2M ammonia (99 : 1 v/v) to the incubation medium. Each experiment contained two types of controls: zero time control and tissue-free control. Ten μL aliquots of the incubation mixture were spotted on Whatman No. 1 paper strips with carrier iodide, T_4 and T_3 as markers. Reaction products of the incubation mixture were analysed by descending paper chromatography in a hexane-tertiary amyl alcohol-2M ammonia (1 : 10 : 11) solvent system for 20–24 h. The spots of iodine-labelled compounds were located by staining and autoradiography, cut out, and counted in a gamma-scintillation counter (ARC 950, Aloca, Tokyo). Protein was determined by the method of Lowry et al (1951).

Calculations. The percent generation of labelled T_3 from [^{125}I]T_4 or labelled 3,3′T_2 from [^{125}I]rT_3 was calculated after correction for the enzyme protein as 1 mg per tube.

Statistical analysis. Results were analysed by ANOVA followed by Duncan's multiple range test, Student's *t*-test or by the Aspin-Welch test.

Results and discussion

In the control mice acclimatized to room temperature, cold exposure increased the T_4 to T_3 conversion by 3·8 fold and the rT_3 to 3,3′T_2 conversion by 4·3 fold (Fig. 1). There was no significant difference in 5′D activity between reserpine-treated mice and controls maintained under room temperature. Exposure to 4°C for 2 h resulted in a much smaller increase in both the T_4 to T_3 and the rT_3 to 3,3′T_2 conversions in the reserpine-treated mice. Thus we found that reserpine treatment for 7 days suppressed the stimulatory effect of cold exposure on 5′D activity in mice.

We further examined whether the stimulatory effect of noradrenaline on the BAT 5′D activity is increased by reserpine treatment. Administration of 0·4 and 0·8 mg kg^{-1} doses of noradrenaline increased the T_4 to T_3 conversion and the 3,3′T_2 formation from rT_3 in both the control and reserpinized mice, although the effect of 0·4 mg kg^{-1} noradrenaline on 3,3′T_2 formation in control mice was not statistically significant (Fig. 2). However, there was no significant difference between the control and reserpine-treated mice in the stimulatory effect of either dose of noradrenaline on BAT 5′D.

We showed that reserpine treatment attenuated the cold-induced increase in 5′D as assessed by both T_3 and 3,3′T_2 formation without affecting the basal 5′D of mouse BAT. This is consistent with the previous result using α-methyl-*p*-tyrosine in rats (Silva & Larsen 1983) and supports the conclusion that reserpine-induced depletion of catecholamines in BAT may prevent the effect of cold exposure, although we did not

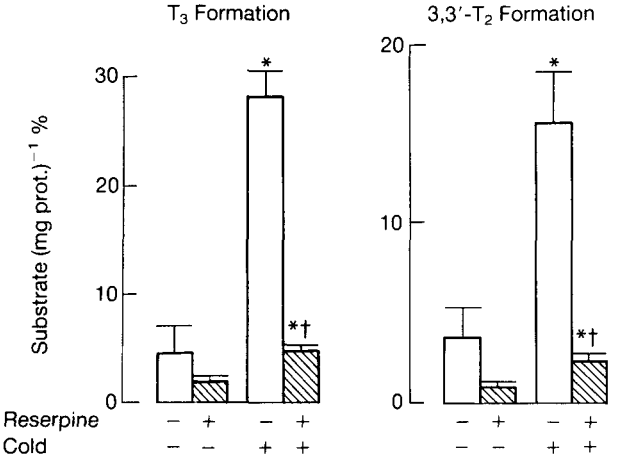

FIG. 1. Effects of reserpine treatment on cold exposure-induced increase in BAT 5′D activity. Columns and bars represent mean ± s.e. of 5 experiments. 2 Way ANOVA revealed a significant effect of reserpine (F(1/16) = 47·4, $P < 0·01$), a significant effect of cold (F(1/16) = 49·2, $P < 0·01$) and a significant reserpine × cold interaction (F(1/16) = 30·3, $P < 0·01$) for T_3 formation; a significant effect of reserpine (F(1/16) = 22·5, $P < 0·01$), a significant effect of cold (F(1/16) = 16·2, $P < 0·01$) and a significant reserpine × cold interaction (F(1/16) = 9·34, $P < 0·01$) for 3,3′T_2 formation. * $P < 0·05$ vs without cold exposure (Student's *t*-test) † $P < 0·05$ vs without reserpine treatment (Aspin-Welch test).

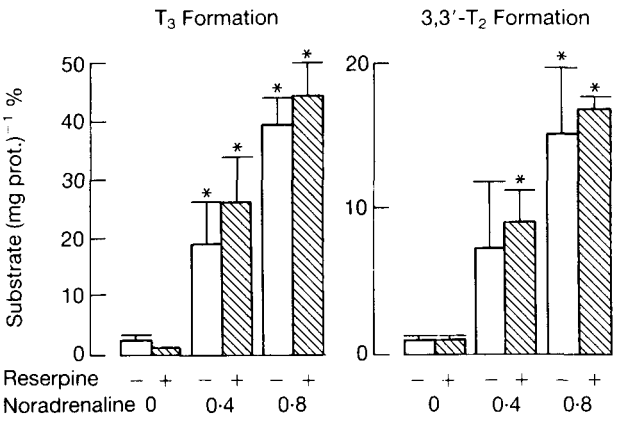

FIG. 2. Effects of reserpine treatment on norepinephrine-induced increase in BAT 5′D activity. Columns and bars represent mean ± s.e. of 5 experiments. 2 Way ANOVA revealed a significant effect of dose of noradrenaline for T_3 formation (F(2/24) = 28·2, $P < 0·01$) and for 3,3′T_2 formation (F(2/24) = 15·0, $P < 0·01$). The effect of reserpine and interactions were not significant. * $P < 0·05$ vs 0 mg kg^{-1} dose of noradrenaline (Duncan multiple range test)

determine the catecholamine content of the BAT. The reserpine treatment used in this study decreased the body weight of the mouse, probably due to suppression of food intake. Food restriction, by itself, lowers and cold exposure elevates noradrenaline turnover, an estimator of the sympathetic nervous system activity in the BAT of rats and mice (Young et al 1982; Knehans & Romsos 1983). Actually, Silva & Larsen (1986) found that the effects of all stimulators on BAT 5′D are blunted by fasting. Therefore, reserpine most probably decreases the response of BAT 5′D to cold mainly by the depletion of catecholamines. However, the decreased food intake in the reserpinized mice may contribute to the defective response to the cold exposure through lowering the sympathetic nerve activity in the BAT.

Although we used the same schedule of reserpine treatment as that demonstrating β-adrenoceptor supersensitivity of rat BAT (Grassby et al 1987), we could not find α_1-adrenoceptor supersensitivity in mouse BAT as assessed by the noradrenaline-induced increase in 5′D activity. This is in agreement with the report on the cardiac α-adrenoceptors in rats (Chess-Williams et al 1987), but is in contrast to other reports which demonstrated supersensitivity to α-agonists in other organs of rats without changes in α_1-adrenoceptor binding (Cowan et al 1985; Nasseri et al 1985). The cause of the different effects of chronic reserpine treatment on the sensitivity of α- and β-adrenoceptors is not clear. The failure of reserpine to elicit α_1-adrenoceptor supersensitivity in BAT may be due to the fact that the α-adrenoceptor of BAT is not under the direct tonic effect of sympathetic innervation as was suggested for the cardiac α-adrenoceptors (Chess-Williams et al 1987). Although less likely, it also remains a possibility that reserpine may modify the 5′D response to noradrenaline through reducing the food intake.

Exposure of the genetically obese (ob/ob) mouse to cold results in the subnormal rise in BAT type II 5′D; however, administration of noradrenaline caused similar elevations of BAT 5′D in both the ob/ob and lean mice (Kates & Himms-Hagen 1985; Kaplan & Young 1987). It is speculated that the impaired deiodination response in the obese mouse might be caused by defective sympathetic stimulation and result in low intracellular T_3 in BAT, thereby contributing to the diminished thermogenesis. Although the pathophysiology of the obese mouse is different from that of the reserpinized mice, our results showed that chronically reserpinized mouse is similar to the ob/

ob mouse as far as the response of BAT 5'D to cold exposure and to noradrenaline is concerned.

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Letters to the Editor

The application of circular dichroism (CD) to a binding study of latamoxef and β-lactamase

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β-lactam antibiotics (BLA) exert their action on two main groups of bacterial enzymes. One group, the cell wall synthesizing enzymes, penicillin binding proteins (PBPs), are inactivated by BLA. The second group, the β-lactamases, inactivate BLA and thereby protect the cell against BLA attack.

The difference in binding ability of BLA to these two groups of enzymes is closely correlated with BLA action.

In the present study, the antibiotic latamoxef has been subjected to a binding study with β-lactamase because latamoxef undergoes little hydrolysis by β-lactamase but has a high affinity for the enzyme. A significant spectral change in circular dichroism (CD) was observed after mixing it with β-lactamase. Our findings are given herein.

Method

Latamoxef was kindly donated from Shionogi Ph. Co. Ltd., Osaka, Japan. β-lactamase was prepared according to the method of Minami et al (1980). Briefly, *Enterobacter cloacae* NUH10 isolated clinically from Nagasaki University Hospital were harvested by centrifugation and washed twice with 0·05 M phosphate buffer (pH 7·0). The cells, suspended in 200 mL of the same buffer, were disrupted by an ultrasonic oscillator (Branson sonifier cell disrupter 185) in an ice bath. The sonicate was centrifuged at 1500 *g* for 30 min at 4°C, to give the crude β-lactamase in the supernatant fraction. The crude β-lactamase was purified with column chromatography using Carboxymethyl-Sephadex C-50 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden).The β-lactamase used in CD measurement was the enzyme showing an absorbance 2·98 at 278 nm. CD measurements were using a JASCO Model J-500A spectropolarimeter in cells of pathlength 10 mm. The dynode voltage was kept below 600 V and measurement was made at room temperature (22°C). CD studies of latamoxef-β-lactamase interaction were carried out between 280 and 350 nm and the following conditions: sensitivity; 1 milli degree cm^{-1}, time constant; 8 s, wavelength expansion; 10 nm cm^{-1}. The observed ellipticities were the actual CD of the latamoxef-β-lactamase complex, while the

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