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HYPERPLASIA OF BROWN ADIPOSE TISSUE AFTER CHRONIC STIMULATION OF β3-ADRENERGIC RECEPTOR IN RATS

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ABSTRACT

When mammals are exposed to a cold environment for a long time, the capacity of nonshivering thermogenesis by brown adipose tissue (BAT) increases in association with the increased expression of some specific proteins and tissue hyperplasia, which are totally dependent on sympathetic innervation to this tissue. To clarify roles of the β-adrenergic mechanism in BAT hyperplasia, the effects of chronic administration of various β-adrenergic agonists on BAT were examined in rats, especially focusing on some agonists to the β3-adrenoceptor which is present specifically in adipocytes. Chronic administration of noradrenaline or isoproterenol for 7–10 days produced a marked increase in the tissue contents of DNA, total protein, mitochondrial uncoupling protein, and insulin-regulatable glucose transporter protein. The trophic effects of noradrenaline and isoproterenol were mimicked by chronic administration of β3-adrenergic agonists, such as CL316,243, BRL 26830A, and ICI D7114. These results suggest that the β3-adrenoceptor plays important roles for hyperplasia of BAT, and thereby increasing in the capacity of thermogenesis.

Key Words : β3-adrenergic receptor, β3-agonist, Brown adipose tissue, Glucose transporter, Uncoupling protein

INTRODUCTION

In mammals, there are two types of adipose tissue, white (WAT) and brown (BAT) adipose tissues. WAT plays a central role of storing energy as triglyceride, while BAT is the major site of dissipating energy as heat. BAT thermogenesis is important, particularly in rodents and newborns, for the maintenance of body tempera-

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ture during exposure to cold environments. When rats are kept at 4°C, for example, BAT thermogenesis is much increased as a result of increased mitochondrial oxidation of fatty acids derived from triglyceride in this tissue and also from blood lipoprotein. In BAT mitochondria, substrate oxidation is poorly coupled to ATP synthesis on account of the presence of a specific protein named as uncoupling protein (UCP), thereby leading to energy dissipation, i. e., heat production[12,18]. In parallel with heat production, glucose utilization and blood flow in BAT are also enhanced[7,22,24]. In addition to the acute effects, prolonged cold exposure results in increased expression of various specific genes such as UCP, lipoprotein lipase and insulin-regulatable glucose transporter (GLUT4), as well as mitochondriogenesis, and finally tissue hyperplasia[4,16,21,23].

It is now established that most of these events associated with BAT thermogenesis, especially those seen after acute cold exposure, are directly controlled by sympathetic nerves distributed to this tissue, principally through the β-adrenergic action of noradrenaline[5]. In adipocytes, there are at least three distinct isoforms of β-adrenoceptor, β1-, β2- and β3-adrenoceptors[14]. The β1- and β2-adrenoceptors are well-known classical receptors, being present in the heart, lung, blood vessels and others. The presence of an atypical adrenoceptor was suggested first by the pharmacological studies on BAT in 1984[1], and its molecule was actually cloned recently as β3-adrenoceptor[6]. It is now confirmed that this new isoform is expressed largely in both brown and white adipocytes[17]. Furthermore, some agonists relatively specific to the β3-adrenoceptor have been synthesized in an attempt to explore an anti-obesity drug[2,25]. Although there have been a lot of literature on the acute effects of β3-agonists on BAT, limited informations are available on its chronic effects, particularly on BAT hyperplasia.

In the present study, we examined in rats the effects of chronic administration of some β3-agonists on BAT hyperplasia, and compared the results with those obtained after cold exposure or conventional β-agonists.

MATERIAL AND METHODS

Animals — Female Wistar rats weighting 150–220g were housed in plastic cages at 24±1°C with a 12h light-dark cycle (lights on at 07:00–19:00) and given free access to laboratory chow and water, unless otherwise stated. They were starved for 20–24h before being killed.

Materials — Noradrenaline and isoproterenol were purchased from Sigma as a hydrochloride from. CL316,243, disodium (R, R)-5[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino] propyl]-1, 3-benzodioxole-2, 2-dicarboxylate was provided from Lederle Laboratories, American Cyanamid Co., (Pearl River, N. Y., USA), BRL 26830A, (R*, R*)-(±)-methyl-4-[2-[(2-hydroxy-2-phenylethyl) amino] propyl]-benzoate from Smithkline Beecham Pharmaceuticals (Epsom, UK), ICI D7114, [(S)-4-(2-

hydroxy-3-phenoxypropylaminoethoxy)-N-(2-methoxyethyl) phenoxyacetamide] from ICI Pharmaceuticals (Macclesfield, UK), and arotinolol, 5-[2-[(3-tert-butylamino-2-hydroxypropyl) thiol]-4-thiazolyl]-2-thiophenecarboxamide from Sumitomo Pharmaceuticals (Osaka, Japan).

Treatments — Under pentobarbital anesthesia, unilateral sympathetic denervation of the interscapular BAT was performed by severing surgically five branches of the intercostal nerves to the right side of the BAT. Seven days after the operation, the rats were used for the following 3 series of experiment.

Experiment 1 ; The rats were transferred to a cold room (4°C), or kept at 24°C and chronically given noradrenaline. For chronic administration of noradrenaline, the rats were implanted subcutaneously in the lumbar region with an osmotic minipump (Alzet 1001, Palo Alto, CA, USA) filled with 0.3 M noradrenaline dissolved in a vehicle solution (0.1 M ascorbic acid and 20 mM 4, 5-dihydroxy-1, 3-benzene disulfonic acid disodium salt). For controls, some rats were implanted with a pump containing the vehicle alone. The pump delivered 0.5 μl/h resulting in a dose of about 3mg noradrenaline/day/kg body weight. After 10 days under these conditions, all rats were decapitated and the right (denervated) and left (intact) pads of interscapular BAT were taken and stored at −80°C until assays.

Experiment 2 ; The rats were kept at 24°C and chronically given 3 or 5 mg/day/kg isoproterenol as in Experiment 1. After 7 days, the denervated BAT pad was taken and stored at −80°C.

Experiment 3 ; The rats were kept at 24°C and given CL316,243 (1 mg/kg), BRL 26830A (1 mg/kg), ICI D7114 (1 mg/kg), or arotinolol (10 mg/kg). Each drug was dissolved in 5% arabic gum and given through oral gavage once a day. After 2 weeks, the denervated BAT pad was taken and stored at −80°C.

Sample preparation — The BAT pad was homogenized in 5–10 vol of a solution containing 10mM Tris-HCl and 1mM EDTA (pH7.4), for 30sec with Polytron. After centrifugation at 1,500xg for 5min, the fat cakes were discarded, and the infranants (fat-free extract) were used for assays of protein[15], DNA[13] and cytochrome C oxidase[20], and Western blot analysis.

Western blot analysis — UCP and GLUT4 proteins were measured by Western blot analysis using respective rabbit antisera raised against purified rat UCP and a synthetic GLUT4 peptide[23]. Tissue samples (10–20 μg protein) were diluted in solubilizing buffer (2% SDS, 0.25M Tris-HCl, 5% 2-mercapto-ethanol, 10% glycerol, final concentrations), heated for 2min at 100°C with 0.01% bromophenol blue, and run on a 12.5% or 10% SDS-PAGE with a 1.5% polyacrylamide as stacking. After transfer to a nitrocellulose membrane (A045A304D, Advantec Toyo, Japan), the blots were incubated with blocking buffer (5% non-fat dry milk in 0.02M Tris-HCl 0.9% NaCl and 0.5% Tween 20, pH7.3) for 2h at room temperature, then overnight at 4°C with antisera to UCP and GLUT4 diluted in blocking buffer at 1:1000 and 1:400,

respectively. After five washes (15min) in 0.02M Tris-HCl containing 0.9% NaCl and 0.5% Tween 20 (pH7.3), the membranes were incubated for 2h at room temperature with [^{125}I]-Protein A (1.85 kBq/ml, ICN, USA) and washed in 0.02M Tris-HCl 0.9% NaCl and 1% Triton X-100 (pH7.3). The dried blots were exposed to a X-ray film (X-Omat-AR, Kodak, Japan) for autoradiography and to the imaging plate of a bioimaging analyzer (BAS1000, Fuji Photo Film, Japan) for quantitative analysis.

Data analysis — All results are presented as means ± SEM, the number of values being indicated in the legends to Figure or Table. Statistical significance was assessed by Dunnet's multiple range test.

RESULTS AND DISCUSSION

It is well known that cold exposure not only activates BAT thermogenesis acutely but also increases the capacity of thermogenesis through chronic stimulation of protein synthesis and cell proliferation in this tissue[4,8]. Most of these effects depend on the action of noradrenaline released from sympathetic nerves in BAT[9]. To confirm this, in Experiment 1, we examined chronic effects of cold exposure or noradrenaline administration on BAT: that is, rats were either kept at 4°C or kept at 24°C but given noradrenaline continuously. After 10 days, interscapular BAT was compared with that of control rats kept at 24°C. As summarized in Table 1, 10 days of cold exposure produced a significant increase in DNA and protein contents, reflecting

Table 1. Tissue weight, protein and DNA contents, and cytochrome c oxidase activity of the interscapular BAT. Rats underwent unilateral sympathetic denervation of the interscapular BAT, and were exposed to cold or given noradrenaline for 10 days.
Values are means ±SEM for 5 rats.
*Significantly different from the controls at $P<0.05$.

	24℃		4℃	
	Control	Noradrenaline	Denervated	Intact
Tissue weight (mg)	80.8±10.8	74.4±8.2	77.0±7.2	85.0±10.2
Protein content (mg/tissue)	7.0±1.4	12.5±1.2*	7.2±2.2	13.4±2.2*
DNA content (μg/tissue)	299±25	570±38*	329±34	567±31*
Cytochrome c oxidase activity (min^{-1}/tissue)	65±4	274±38*	118±10*	502±28*

increased cell number and active cell components, respectively. Cytochrome C oxidase activity, which is a marker of mitochondrion number, was also increased by cold exposure. These results thus indicate that prolonged cold exposure produces hyperplasia of BAT. The tissue weight did not show any significant change despite of the apparent tissue hyperplasia. This would be due to a decreased triglyceride content as the result of thermogenesis-associated degradation of this energy substrate. Table 1 also shows that the trophic effect of cold was abolished or much attenuated when the sympathetic nerves to BAT were surgically served, but it was mimicked even at 24°C when noradrenaline was given continuously for 10 days. These results confirm that noradrenaline released from sympathetic nerves plays a critical role in cold-induced hyperplasia of BAT[8,9].

BAT consists not only of matured brown adipocytes but also preadipocytes, interstitial cells and vascular endothelial cells. Thus, the results of Table 1 do not necessarily show the increase in brown adipocyte number and the capacity of thermogenesis. Therefore, we measured the tissue content of some proteins specific to brown adipocyte, i. e., UCP and GLUT4. UCP is a proton channel protein and exists only in brown adipocyte mitochondria. GLUT4 is an isoform of glucose transporters specifically expressed in insulin-sensitive cells, such as adipocyte and skeletal muscle cell. It has been reported that the protein and mRNA levels of UCP and GLUT4 were increased after cold exposure[19,21,23]. We examined the protein contents of UCP and GLUT4 in BAT samples obtained from Experiment 1 by Western blot analysis. Fig. 1 shows typical examples of the autoradiogram, confirming two bands of about 32kDa and 48kDa, which correspond to UCP and GLUT4, respectively. Quantitative analysis of each band by a bioimaging analyzer revealed that the amounts of UCP and GLUT4 were increased by 150 and 140%, respectively, after cold exposure. It was also obvious that the effects of cold exposure were abolished by sympathetic denervation, but mimicked by continuous administration of noradrenaline. Thus, noradrenaline can stimulate the synthesis of some proteins specific to brown adipocytes, and thereby increase the capacity of thermogenesis.

To determine whether the chronic effect of noradrenaline is also based on β-adrenergic stimulation, next, we examined the effect of continuous administration of a β-adrenergic agonist, isoproterenol (Experiment 2). As shown in Fig. 2, when isoproterenol was given continuously for 7 days, the tissue contents of protein, DNA, UCP and GLUT4 increased significantly as after noradrenaline administration. A clear dose dependency was observed for the changes in DNA and UCP contents. These results are quite consistent with the view that the β-adrenergic mechanism is responsible for trophic effect of noradrenaline on BAT.

In brown adipocyte, there are 3 isoforms of β-adrenergic receptor, β1-, β2- and β3-adrenoceptors. β1- and β2- isoforms are well-known β-adrenergic receptors, present in heart, lung, blood vessels and others. In contrast, β3- isoform is

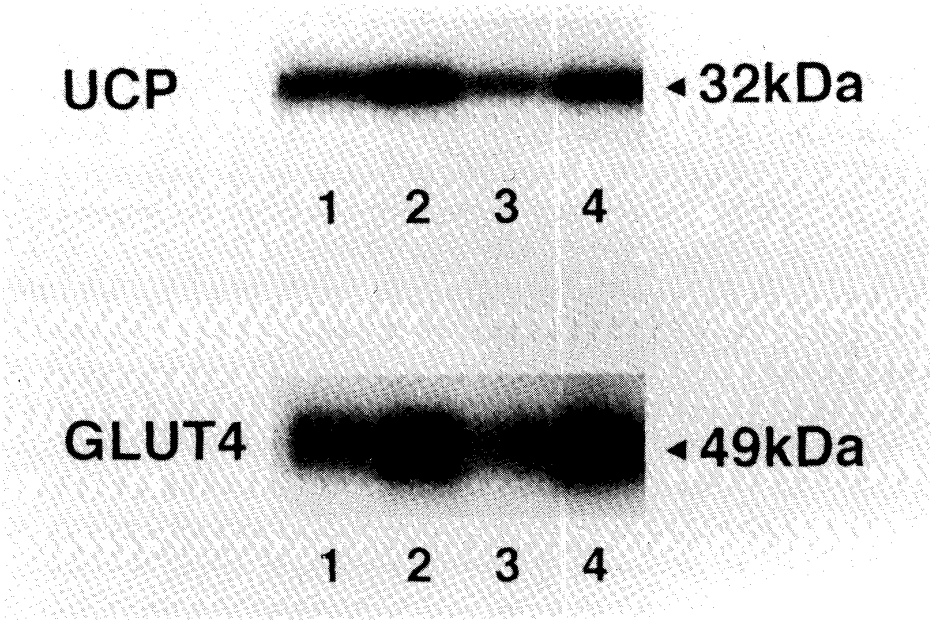

Fig. 1 Western blot analysis of UCP and GLUT4 proteins in BAT. Lane 1, control; Lane 2, noradrenaline; Lane 3, cold exposure (denervated); Lane 4, cold exposure (intact)

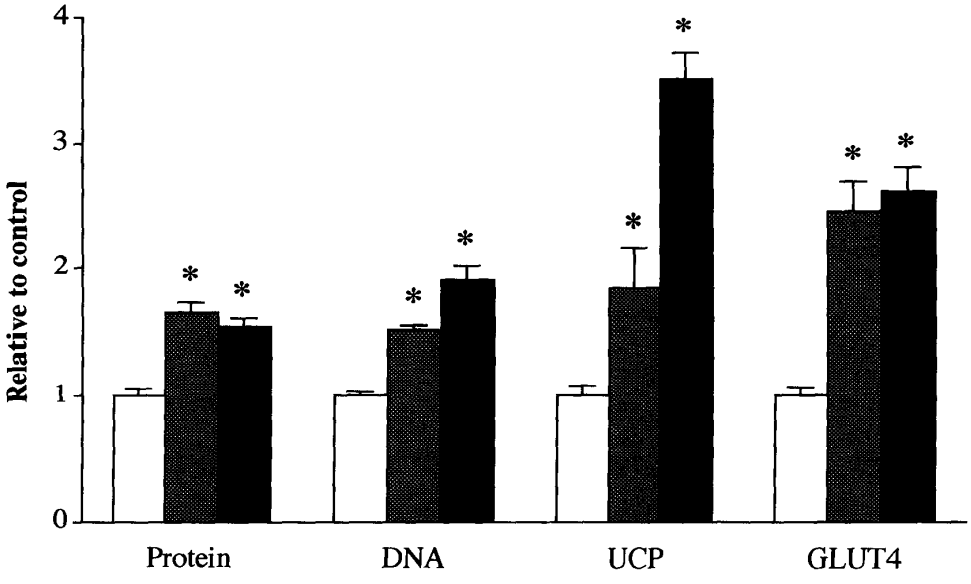

Fig. 2 Effects of chronic administration of isoproterenol on BAT. Open columns, vehicle control (n=7); Shadow columns, isoproterenol (3mg/day/kg, n=6); Solid columns, isoproterenol (5mg/day/kg, n=5). Values are means ± SEM. *Significantly different from the controls at $P<0.05$.

found specifically in adipocytes. Therefore, it might be expected that an agonist specific to this receptor can activate BAT thermogenesis without effects on other organs such as the cardiovascular system. Several companies have synthesized such agonists. In this study, we could obtain some of them, CL316,243[3,10], BRL 26830A[2] and ICI D7114[11]. Particularly, CL316,243 is reported as the most specific β3-agonist with relative potencies of β1 : β2 : β3=0:1:100,000[3]. In Experiment 3, we gave rats β3-agonists for 2 weeks through oral gavage. As shown in Fig. 3, all these agonists were effective in producing a marked increase in protein, UCP and GLUT4 contents in BAT, thus mimicking the trophic effects of noradrenaline and isoproterenol.

In Experiment 3, we also examined the effects of arotinolol, a non-selective α/β-antagonist. Surprisingly, chronic administration of arotinolol increased total protein content in BAT. Although no significant change was found in UCP and GLUT4 contents, this observation suggests that classical β-antagonist could act, at least partially, as an agonist of β3-adrenergic receptor. This idea is agreed with the previous report that oxprenolol, which is a typical β-antagonist, had an agonist-like action to β3-receptor[6].

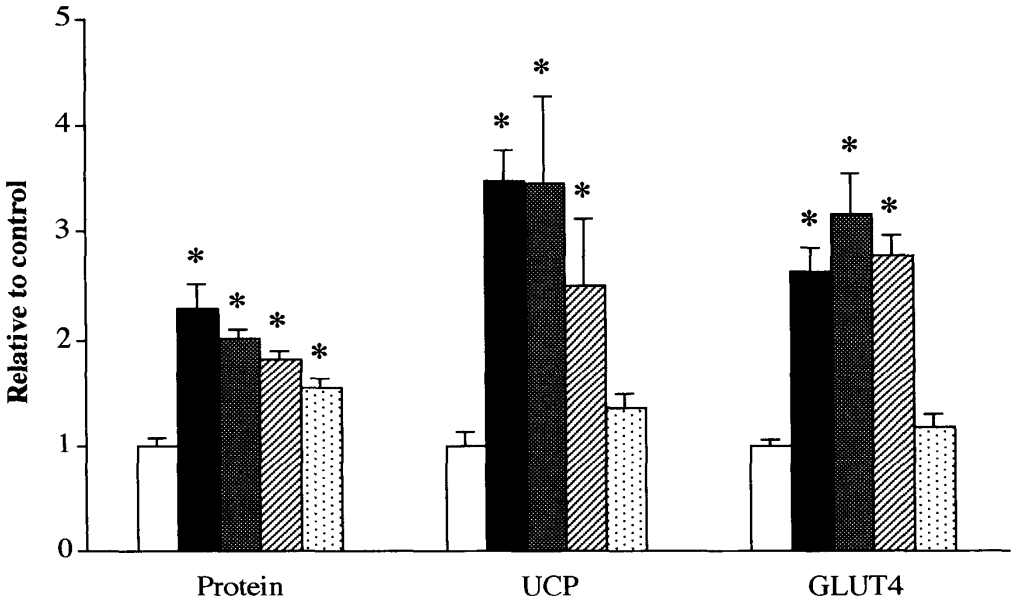

Fig. 3 Effects of chronic administration of various β3-agonists on BAT. Open columns, control; Solid columns, CL316,243 (1mg/day/kg); Shadow columns, BRL 26830A (1mg/day/kg); Hatched columns, ICI D7114 (1mg/day/kg); Dotted columns, arotinolol (10mg/day/kg). Values are means ± SEM for 5 rats. *Significantly different from the controls at $P<0.05$.

The trophic action of β3-agonists to BAT implies that chronic treatment with these agonists increases the capacity of BAT thermogenesis and energy expenditure, suggesting the usefulness of β3-agonists as an anti-obesity drug. In fact, previous reports demonstrated that these agonists were very effective to ameliorate the abnormalities in obese mice and rats[2,25]. It is particularly interesting that α/β-antagonist, arotinolol, may have some agonist-like effects on β3-receptor. Arotinolol is used as a drug of hypertention, and obesity is frequently complicated by hypertention. Thus, it may be possible that some α/β-antagonists, like arotinolol, could be effective not only to hypertention but also for obesity at once. Further studies are needed to explore this intriguing idea.

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