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Increased growth of brown adipose tissue but its reduced thermogenic activity in creatine-depleted rats fed β-guanidinopropionic acid

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Abstract

To study the responses of thermogenic activity in brown adipose tissue (BAT) to creatine depletion, male Wistar rats were fed creatine analogue β-guanidinopropionic acid (β-GPA) for about 10 weeks. Compared to control rats, a marked decrease in the levels of high-energy phosphates, such as phosphocreatine and ATP, was noted in BAT of β-GPA rats. Conversely, upward trends in other chemical components (DNA, glycogen, and total protein) in BAT as well as an increase in BAT mass were observed in β-GPA rats, suggesting a tendency to hyperplasia of the BAT. The thermogenic activity (which was assessed by guanosine 5'-diphosphate binding to BAT mitochondria) in the mitochondria recovered from BAT of β-GPA rats, however, was not increased in response to such changes but rather decreased. Moreover, uncoupling protein (UCP) content in the mitochondrial fraction of β-GPA rats was significantly lower than that in control rats (the relative amounts were 77 ± 6 and 100 ± 4%, respectively). Nevertheless, surprisingly, the level of UCP mRNA was remarkably greater in β-GPA rats than in control rats. These observations indicate that there is a discordance between BAT growth and activity in β-GPA rats, thereby suggesting that a failure on and after UCP translation may be involved in the impairment of BAT thermogenic activity with creatine depletion. The impairment of BAT thermogenic activity, that is, UCP activity may indicate that uncoupling or heat production was inhibited in order to increase the ATP synthesis in BAT of β-GPA rats in compensation for a reduction in the levels of high-energy phosphates (including ATP), with resultant hypothermia.

Keywords: Brown adipose tissue; Creatine depletion; β-Guanidinopropionic acid; Hyperplasia; Nonshivering thermogenesis; Uncoupling protein

1. Introduction

Creatine is well known to play an essential role in the biochemical energetics of skeletal muscle contraction. With the demonstration of functional compartmentation of creatine kinase (CK) on the mitochondrion, it became clear that the actual form of energy transport in the muscle fibre is phosphocreatine (PCr), indicating the traffic of energy to be a shuttle between two isoenzymes of CK, one bound to the mitochondrion and one to the M band of the myofibril [1]. In our previous study, intramitochondrial inclusions running parallel to the long axis of mitochondrion were observed in skeletal muscles following creatine depletion by creatine analogue β-guanidinopropionic acid (β-GPA) [2]. Similar intramitochondrial inclusions have also been found in patients with mitochondrial myopathies and identified as deposits of mitochondrial CK [3]. Moreover, quite recently we have revealed that an elevation of β-adrenoceptor density in rat soleus muscle is induced after chronic depletion of high-energy phosphates by feeding β-GPA [4], without a change in the muscle fatigue resistance [5].

In addition, creatine has been detected elsewhere in the body in such tissues as nervous tissue, testes, kidney, and liver, although in much lower concentrations [6]. Berlet et al. [7] have shown that creatine also occurs both in white adipose tissue and in brown adipose tissue (BAT) of rats, the values of the latter prevailing over those of the former by a factor of 100, indicating that creatine plays a role in energy metabolism of both adipose tissues, especially BAT. BAT contains many mitochondria which give it its brown colour. The thermogenic function of BAT mitochondria is related to loose coupling of respiration due to the activity

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of a unique 32 kDa uncoupling protein (UCP), thereby providing a capacity for nonshivering thermogenesis (NST) and diet-induced thermogenesis (for review see Himms-Hagen [8]).

These considerations led us to study the effect of administration of β-GPA on BAT activity in rats in order to know a role of creatine in NST.

2. Materials and methods

Ten newly weaned male Wistar rats were divided randomly into the control and β-GPA diet groups ($n = 5$ each). Rats were housed individually in a stainless steel cage and pair-fed. For approx. 10 weeks control rats were fed a commercial powdered food (Nihon CLEA, CE-2, Tokyo), and the same food containing 1% β-GPA was fed to other group to deplete high-energy phosphates. The β-GPA was synthesized as reported earlier [9]. The daily food supply was gradually increased following the growth and each rat was fed 20 g of diet from week 4 to the end of the experiment. Water was supplied ad libitum. The temperature and humidity in the animal room with a 12:12 h light–dark cycle were maintained at ≈ 23° C and 55%, respectively. The animals were cared for in accordance with the 'Guiding Principles for the Care and Use of Animals' approved by the Council of the Physiological Society of Japan, based upon the Helsinki Declaration, 1964. Each colonic temperature was measured by a thermistor thermometer inserted 5 cm into the rectum on the morning of the last day. On the same day, rats were anesthetized with injection of pentobarbital sodium (5 mg/100 g body wt. i.p.), and then the content and distribution of phosphorus compounds in BAT were evaluated by ^{31}P-nuclear magnetic resonance (NMR) spectroscopy (BEM 170/200, Otsuka Electronics, Osaka), as stated earlier [4]. Further, interscapular BATs were removed, dissected free from all recognizable white adipose tissue and other connective tissue, and weighed. The BATs were then frozen in liquid nitrogen and stored at −80° C until later analysis.

The contents of total protein and DNA in BAT were determined by the methods of Lowry et al. [10] and Schneider [11], respectively. The glycogen level was measured according to the method described by Dubois et al. [12].

BAT mitochondria to assay guanosine 5′-diphosphate (GDP) binding capacity (an index of UCP activity) [8] were isolated according to the method of Cannon and Lindberg [13]. Mitochondrial protein was measured in the presence of Triton X-100 by the procedure of Lowry et al. [10]. The final mitochondrial pellet was suspended in 100 mM sucrose/10 mM sodium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (pH 7.2) at a final concentration of 0.8–1.3 mg/ml. [^{3}H]GDP binding was measured according to the method of Scarpace et al. [14]. Specific binding was determined from the difference in binding with and without 1 mM unlabelled GDP.

BAT mitochondrial proteins (3.75 μg/lane) were solubilized in sodium dodecyl sulfate (SDS) and separated on a SDS-polyacrylamide gel (10%) [15]. Western blotting of the proteins separated was performed as described before [16] after electrotransfer onto a nitrocellulose filter [17]. Antibodies used were anti-mouse UCP serum raised in rabbit, which was offered by Dr. T. Kawada (Kyoto University, Kyoto). The relative amounts of UCP were estimated using the photograph of the Western blot and a LKB Ultroscan XL Enhanced Laser Densitometer.

Total RNAs of BAT were isolated by the acidic guanidinium isothiocyanate method of Chomczynski and Sacchi [18]. RNA electrophoresis and Northern blotting were performed as described before [16], originally demonstrated by Feinberg and Vogelstein [19]. The RNA samples separated were hybridized to cDNA probe (which was labelled with [^{32}P]dCTP by the random-priming method of Feinberg and Vogelstein [19] encoding rat UCP (positions 743 to 1103 in the sequence given by Bouillaud et al. [20]). To ascertain the integrity and amount of sample RNAs, the same RNA samples were rehybridized later to β-actin cDNA probe.

Student's *t*-test was applied to the data; differences were considered significant at $P < 0.05$.

3. Results and discussion

The patterns of ^{31}P-NMR spectra in response to β-GPA feeding are shown in Fig. 1. In response to β-GPA feeding, PCr and ATP heights were reduced, and a new peak for phosphorylated β-GPA (β-GPAP) was noted in the ^{31}P-NMR spectra. This result was in agreement with that in skeletal muscle of rats also fed β-GPA [4], thereby indicating depletion of high-energy phosphates in BAT. Chevli and Fitch [21] demonstrated that β-GPA either might be relatively inaccessible to CK or was a poor substrate for the enzyme. Therefore, ATP synthesis might not be fully replaced by β-GPAP, so that the results in the current study might suggest a change in mitochondrial energy metabolism.

The body mass in β-GPA rats was significantly less than in normal controls (Table 1). Since control and β-GPA rats were pair-fed except for β-GPA, the difference in body mass between both groups was considered to be due to β-GPA feeding. The DNA and glycogen contents in BAT of β-GPA rats showed an upward trend compared to those in the control rats ($P < 0.10$; Table 1). The BAT from β-GPA rats had a significantly higher level of total protein than BAT from control rats (Table 1). These results indicated a tendency to hyperplasia of BAT in β-GPA rats.

Conversely, the protein content in mitochondria recovered from BAT of β-GPA rats seemed to be lower than that in the control rats ($P < 0.10$), which was similar to the

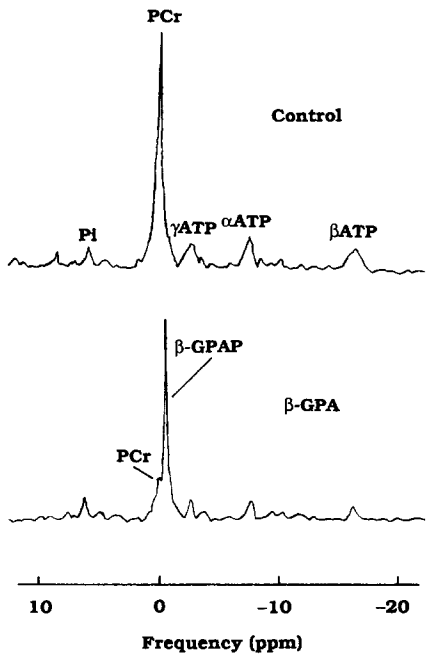

Fig. 1. ^{31}P-Nuclear magnetic resonance spectroscopy of phosphorus compounds in the brown adipose tissue of control rats and of rats fed β-GPA. PCr, phosphocreatine; β-GPA, β-guanidinopropionic acid; β-GPAP, phosphorylated β-GPA.

finding on GDP binding, albeit not statistically significant (Table 1). Scatchard analysis showed that the change in GDP binding with β-GPA feeding was caused by a decrease in the number of GDP binding sites, whereas affinity was similar (Fig. 2). These findings suggested that the BAT thermogenic activity decreased substantially with β-GPA feeding. Because BAT thermogenesis is primarily regulated by the sympathetic nervous system [8] and an increase in the number of GDP binding sites is also mediated by sympathetic activation [22], a β-GPA-related decline in β-adrenergic signal transduction may be a factor in the decline in BAT thermogenic activity.

Table 1
BAT mass, chemical components, mitochondrial GDP binding, and colonic temperature in control and β-GPA-fed rats

Variable	Control	β-GPA-fed
Body mass (g)	356 ± 14 (5)	298 ± 8 (5) [a]
BAT mass (mg)	443 ± 35 (5)	616 ± 28 (5) [a]
DNA (μg)	466 ± 31 (5)	775 ± 137 (3)
Glycogen (μg)	169 ± 21 (5)	339 ± 78 (4)
Total protein (mg)	8.90 ± 1.34 (5)	17.4 ± 1.4 (5) [a]
Mitochondrial protein (mg recovered)	1.09 ± 0.15 (5)	0.745 ± 0.096 (5)
GDP binding		
(pmol recovered)	160 ± 53 (3)	80.8 ± 6.9 (5)
(pmol/mg mitochondrial protein)	151 ± 45 (5)	114 ± 15 (5)
(pmol/kg body mass)	456 ± 129 (5)	251 ± 21 (5)
Colonic temperature (° C)	37.0 ± 0.4 (5)	36.0 ± 0.1 (5) [a]

Values are means ± S.E. Numbers in parentheses indicate numbers of animals. BAT, brown adipose tissue; GDP, guanosine 5′-diphosphate.
[a] Significantly different from the control rats: $P < 0.05$.

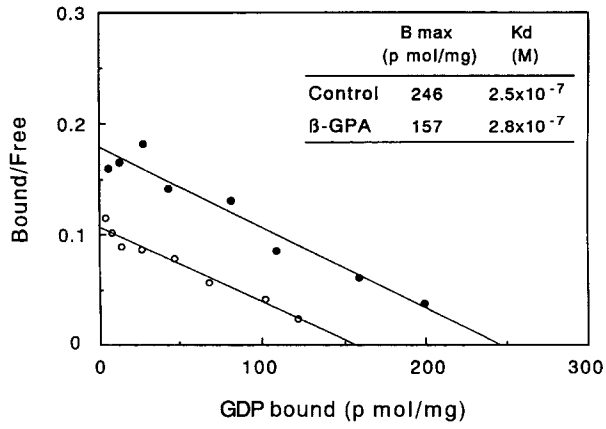

Fig. 2. Influence of β-GPA feeding on GDP binding specific to the mitochondria recovered from brown adipose tissue. Scatchard representations of specific binding are illustrated for control (●) and β-GPA (○) rats. GDP, guanosine 5′-diphosphate.

It was found, on the contrary, that the level of UCP mRNA was markedly greater in β-GPA rats than in control rats (Fig. 3), suggesting that the function of β-adrenergic signal transduction operated sufficiently on the expression of UCP in the BAT of β-GPA rats because UCP gene expression is basically controlled via β-adrenergic pathway [23]. As previously described, our recent study demonstrated that the β-adrenoceptor density in rat soleus muscle was significantly increased by feeding β-GPA (about +36%); however, whether or not the re-

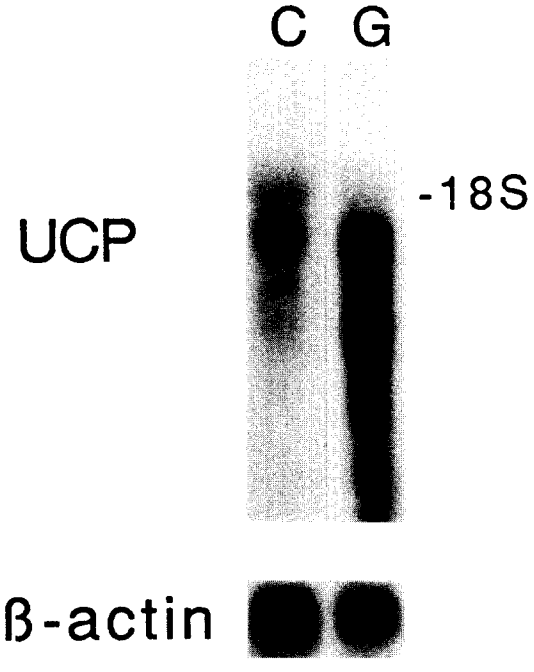

Fig. 3. Northern blot analysis of uncoupling protein (UCP) mRNA. Total RNAs (10 μg) isolated from the brown adipose tissue of control (C) and β-GPA-fed (G) rats were hybridized with oligonucleotide probes to UCP or β-actin.

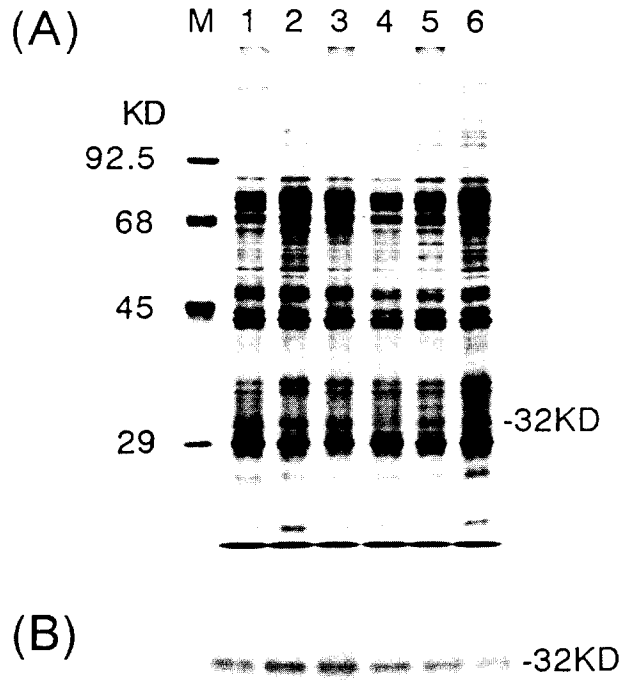

Fig. 4. SDS-polyacrylamide gel electrophoresis of mitochondrial proteins (A) and Western blot analysis of uncoupling protein (B) in brown adipose tissue. Lane M, markers; lanes 1–3, control rats; lanes 4–6, β-GPA rats.

sponses of β-adrenoceptors in BAT to β-GPA feeding in the current study may be extrapolated from such findings on rat skeletal muscle remains to be clarified. Likewise, we cannot, at present, explain the difference in β-adrenergic response between GDP binding and UCP mRNA expression in the BAT of β-GPA rats.

Nevertheless, surprisingly, UCP content in the BAT mitochondrial fraction of β-GPA rats was significantly lower than that in control rats (the relative amounts were 77 ± 6 and $100 \pm 4\%$, respectively) (Fig. 4B), although the difference between the levels of 32 kDa proteins (including 32 kDa UCP) was not great (Fig. 4A). These results may lead to the speculation that UCP synthesis in the BAT of β-GPA rats was affected by a translational process and/or a post-translational process.

Meanwhile, the possibility exists that the increased expression of UCP mRNA in the BAT of β-GPA rats accompanied by the tissue growth might be due in part to the decreased body temperature (Table 1). After 10 weeks of β-GPA feeding, however, the rats could not compensate a decrease in body temperature with the changes of BAT for hyperplasia, probably because of the unincreased level of GDP binding. In BAT mitochondria, substrate oxidation is poorly coupled to ATP synthesis owing to the presence of UCP, thereby leading to energy dissipation, i.e., heat production [8]. In the current study a notably depleted level of high-energy phosphates (including ATP) was observed in the BAT of β-GPA rats. Therefore the unincreased level of GDP binding in BAT of β-GPA rats may suggest that uncoupling or heat production was inhibited in order to increase the ATP synthesis.

Depletion of high-energy phosphates by feeding β-GPA stimulates mitochondrial protein synthesis in rat skeletal muscle accompanied by the elevated metabolic rate (resting oxygen consumption) [24]. In the current study, on the other hand, the content of mitochondrial proteins (including UCP) in BAT of β-GPA rats appeared to be lower than that in the control rats, suggesting that the mitochondrial protein synthesis in BAT is controlled by a mechanism specific to this tissue. Further studies, however, are needed to clarify the BAT-specific mechanism for mitochondrial protein synthesis. It appears thus that of special interest are the profiles of mitochondrial enzymes, such as CK and Krebs cycle enzymes, in BAT of rats fed β-GPA.

In conclusion, a discordance between BAT growth and activity was found in β-GPA rats in the current study, suggesting that a failure on and after UCP translation may be involved in the impairment of BAT NST with creatine depletion.

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