The Effect of Acute Cold Exposure and Norepinephrine on Uncoupling Protein Gene Expression in Brown Adipose Tissue of Monosodium Glutamate-Obese Mice

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ABSTRACT—Abnormal regulation of mitochondrial uncoupling protein (UCP) gene expression was studied in brown adipose tissue (BAT) of monosodium glutamate (MSG)-induced obese mice. UCP mRNA levels in control mice increased markedly after acute cold exposure; however, MSG-obese mice showed an impaired response. In contrast, an injection of norepinephrine (NE) induced a comparable increase in UCP mRNA levels in control and MSG-obese mice. These results suggest that the impairment in the cold-induced increase in UCP mRNA is due to a deficient sympathetic input to BAT and/or to a diminished response of BAT to endogenous NE, which constitutes the mechanism of impaired thermoregulation in obese mice in a cold environment.

Keywords: Monosodium glutamate, Brown adipose tissue, Uncoupling protein

The neonatal administration of monosodium glutamate (MSG) destroys arcuate and ventromedial nuclei of the hypothalamus and induces an obesity syndrome without hyperphagia (1). The regulation of body temperature in a cold environment is impaired in the MSG-induced obese mice (2). However, the pathogenesis of the defective thermoregulation in this obese animal has not been clarified. We have recently reported that in MSG-obese mice, there is an impairment in cold- and norepinephrine (NE)-induced increase in type II thyroxine 5'-deiodinase (T₄ 5'DII) activity in brown adipose tissue (BAT) (3). BAT is the most important site of cold-induced non-shivering thermogenesis, and the *in situ* generation of 3,5,3'-triiodothyronine (T₃) from T₄ by T₄ 5'DII is required for the optimal synthesis of mitochondrial uncoupling protein (UCP) (4), which is a rate-limiting factor for thermogenesis in BAT (5). UCP gene expression is regulated primarily by the sympathetic nervous system, and UCP mRNA is induced by both cold exposure and adrenergic agonists in normal conditions (6, 7). In the present study, we examined the effects of cold exposure and NE administration upon UCP gene regulation in MSG-obese mice.

To raise MSG-obese mice, neonatal random-bred ICR mice were subcutaneously injected with MSG dissolved in 0.9% saline (Junsei Chemical Co., Ltd., Tokyo) (2 mg/g

body weight) on days 1, 3, 5, 7 and 9 following birth as described previously (3). The control mice received an equal volume of vehicle (0.9% NaCl at 12.5 μ l/g body weight). The mice were housed in an environment temperature of 22–24°C for 8–12 weeks after birth, and then they were exposed to 4°C or remained at 22°C for 4 hr. Some mice received a subcutaneous NE (Sankyo Co., Ltd., Tokyo) injection (0.8 mg/kg) at 22°C. Animals were killed by exsanguination 4 hr after injection of NE or at the end of 4-hr cold exposure. BAT in the interscapular region was rapidly removed, frozen in liquid nitrogen and stored at –80°C for RNA extraction.

The abundance of UCP mRNA was measured by Northern or slot blot analysis as described previously (6). Briefly, total RNA was isolated by a guanidium thiocyanate/phenol/chloroform extraction procedure (8). For Northern analysis, a 10- μ g sample of total RNA was applied for each lane and electrophoresed in 1% agarose gels. For quantitative analysis, slot blots were performed, in which a 2- μ g sample of total RNA was applied to the slot blot apparatus. ³²P-labeled UCP cDNA (pUCP rat 15), generously provided by Dr. K. Freeman (9), was used as a probe. Hybridization was performed at 65°C for 16–24 hr under appropriate conditions. Membranes were washed with 0.1 $\times$ SSC, 0.1% SDS at 65°C and then exposed to X-ray film for 24–48 hr. The abundance of

RNA was measured by densitometry of the film. All data were expressed as means $\pm$ S.E.M. Statistical significance of differences was evaluated by the non-parametric Tukey's multiple range test.

There was no difference in UCP mRNA levels in BAT between MSG-obese and control mice kept at the ambient temperature of 22°C (Fig. 1). In the saline control mice, the cold exposure for 4 hr resulted in a 1.7-fold increase in UCP mRNA levels. However, this cold-induced up-regulation in UCP mRNA was markedly attenuated in MSG-treated obese mice. As shown in Fig. 2, subcutaneous administration of 0.8 mg/kg NE induced a comparable increase in UCP mRNA levels in BAT of control and MSG-obese mice. Cold exposure increases sympathetic nerve activity to BAT and thereby stimulates β -adrenoceptors on the BAT cells, which, in turn, increases

UCP gene expression (6, 7). Therefore, the impairment of a cold-induced increase in UCP mRNA levels in these mice may be due to the deficient sympathetic outflow from the central nervous system to BAT. However, the distribution pattern of β -adrenergic receptors on the BAT may change in MSG-obese mice, and thereby endogenous NE may hardly bind to these receptors in a cold environment. If such is the case, the sympathetic outflow to BAT may not be necessarily deficient.

As described earlier, our previous report showed that both cold- and NE-induced increase in T_4 5'DII activity was markedly reduced in BAT of MSG-obese mice (3). UCP gene expression is controlled by β -adrenergic stimulation (6, 7), while T_4 5'DII activity is controlled by α_1 -adrenergic stimulation (10, 11). T_3 in BAT, which is generated by T_4 5'DII in situ, exerts a permissive effect on

Fig. 1. Effect of cold exposure on UCP mRNA levels in BAT of control and MSG-obese mice. MSG-treated or control mice were either exposed to 4°C or maintained at 22°C for 4 hr followed by an extraction of total RNA of BAT. Representative Northern blot of mRNA (upper) and abundance of UCP mRNA determined by slot blot analysis (lower) are shown. Columns and bars represent the mean $\pm$ S.E.M. for results obtained from 11 to 14 mice. ** $P < 0.01$. □: 22°C, ▨: 4°C.

Fig. 2. Effect of NE on UCP mRNA levels in BAT of control and MSG-obese mice. MSG-treated or control mice were s.c. injected with 0.8 mg/kg of NE or 10 ml/kg saline (S) at 22°C. Four hours later, mice were sacrificed and total RNA of BAT was extracted. Representative Northern blot of mRNA (upper) and abundance of UCP mRNA determined by slot blot analysis (lower) are shown. Columns and bars represent the mean $\pm$ S.E.M. for results obtained from 9 to 11 mice. * $P < 0.05$, ** $P < 0.01$. □: S, ▨: NE.

optimal synthesis of UCP (4). It is interesting that NE exerts a concerted stimulation of UCP synthesis through the 2 different receptor subtypes.

UCP plays an important role in generating heat and burning calories by creating a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondrial membrane in BAT (7). Therefore, the defect in the induction of UCP mRNA by cold stimulation in MSG-obese mice may be one reason why MSG-obese mice are incapable of maintaining body temperature under a condition of cold exposure. Recently, two proteins related to UCP have been identified and designated as UCP2 (12, 13) and UCP3 (14, 15). Further characterization of these UCP isoforms may clarify abnormal heat-generating mechanisms in obesity.

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