Up-regulation of mitochondrial uncoupling proteins by stimulation of nuclear and β-adrenergic receptors in L6 myotubes

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Citation
Japanese Journal of Veterinary Research, 50(1), 31-32

Issue Date
2002-05-31

Doc URL
http://hdl.handle.net/2115/2935

Type
bulletin (article)

File Information
KJ00002400419.pdf
amino acid sequences were highly homologous to those of other species. The tissue distribution of each isoform mRNA was also similar to those of other species. Sympathetic stimulation increased UCP 1 mRNA expression in adipose tissue of beagles. These results suggest that UCP may be involved in the regulation of energy expenditure in the dog, as in rodents.

4. On the basis of the above results, serum leptin analysis and CT were performed in obese beagles before and after treatment with a low calorie diet containing either fish oil or tallow. Body weight decreased on both diets, slightly but significantly more on the fish oil diet. Serum leptin and total fat area in CT also decreased similarly on the two diets. The decrease of subcutaneous fat seemed more strikingly than that of visceral fat. Analysis of UCP mRNA revealed that the UCP 3 mRNA level in skeletal muscle increased on the fish oil diet. These results further support the usefulness of serum leptin assay and CT for diagnosis of obesity in the dog, and suggest that fish oil has an anti-obesity effect probably due to the increase of UCP expression and energy expenditure.


Up-regulation of mitochondrial uncoupling proteins by stimulation of nuclear and \( \beta \)-adrenergic receptors in L6 myotubes

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Obesity is the most common nutritional disorder in humans and also in companion animals. The principal causes of obesity have been thought to be increased energy intake and decreased exercise or both. Recent studies have shown a significant involvement of metabolic heat production in the regulation of energy balance and its impairment as a possible cause of obesity. The most likely mechanism of metabolic heat production is thought to be uncoupling of mitochondrial oxidative phosphorylation, which is accelerated by an uncoupling protein (UCP) family. UCP 1 is present only in brown adipose tissue, a specific site of cold-induced heat production in small rodents. Other isoforms of UCP are widely expressed, and particularly UCP 2 and UCP 3 in skeletal muscle are expected to be important in metabolic heat production in larger mammals. There have been many literatures that the gene expression of UCP 2 and UCP 3 in skeletal muscle is changed by physiological and pharmacological conditions affecting energy balance, but these in vivo studies are not suitable to clarify the cellular and molecular mechanisms regulating UCP gene expression in skeletal muscle. To this end, in the present study, I investigated the mRNA expression of UCP 2 and UCP 3 in vitro using a widely used muscle cell line, L6, particularly focusing on the possible involvement of nuclear and \( \beta \)-adrenergic receptors.
L6 cells expressed both UCP2 and UCP3 mRNA after differentiation to myotubes in vitro. The mRNA levels of UCP2 and UCP3 were increased when the cells were treated for 24 hr with increasing concentrations of triiodothyronine (T3), and also non-selective ligands of the peroxisome proliferator-activated receptor (PPAR) such as α-bromopalmitate and carbacyclin. However, selective ligands of PPARα (WY14643) and PPARγ (troglitazone) were not effective in the UCP mRNA induction. mRNA analysis of individual PPAR isoforms revealed that L6 myotubes expressed significantly PPARδ but undetectable levels of PPARα and PPARγ. A ligand of retinoid X receptor (RXR), 9-cis retinoic acid, was also effective by itself and in combination with carbacyclin. These results indicate that UCP2 and UCP3 in L6 myotubes were up-regulated by various nuclear receptors including T3 receptor (TR), RXR and PPARδ.

Since long-chain fatty acids are known to have ligand activity for PPAR, the effects of fatty acids on UCP mRNA expression were examined in L6 myotubes. All fatty acids so far examined including oleic acids, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and conjugated linoleic acid dose-dependently increased mRNA expression of UCP2 and UCP3. The arachidonic acid-induced UCP expression was attenuated by an inhibitor of lipooxygenase, but not by those of cyclooxygenase. These results suggest that long-chain fatty acids up-regulate UCP2 and UCP3 expression in L6 myotubes by itself and also by their lipooxygenase metabolites probably through the activation of PPARδ.

Possible involvement of β-adrenergic receptor (β-AR) in UCP expression in L6 myotubes also investigated. Stimulation of the cells with epinephrine increased the UCP3 mRNA level transiently at 6 hr, and also the UCP2 mRNA level at 6-24 hr. The stimulatory effects of epinephrine were also observed in the presence of carbacyclin and 9-cis retinoic acid, and were mimicked by isoproterenol and salbutamol (β2-AR agonist), but abolished by propranolol (non-selective β-AR antagonist) and ICI-118, 551 (β2-AR antagonist). mRNA analysis revealed the existence of β2-AR, but not β1- and β3-ARs, in L6 myotubes. Analysis of cellular cAMP responses also revealed the existence of functional β2-AR in the cells. It was thus shown that UCP2 and UCP3 were up-regulated by stimulation of β2-AR in L6 myotubes.

From these results, I concluded that UCP2 and UCP3 expression in L6 myotubes were up-regulated directly by β2-AR and also by nuclear receptors such as PPARδ, RXR and TR. This is compatible with the previously reported in vivo observations that sympatho-adrenomedullary activation and increased plasma levels of T3 and free fatty acids induced UCP2 and UCP3 in skeletal muscle. It is expected that specific activation of these receptors would lead to increased energy expenditure and thereby an anti-obesity effect.