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this study, the effect of chronic treatment with CL316,243 on mRNA expression of UCP1, UCP2 and UCP3 was examined.

1) The single injection of CL316,243 (0.1 mg/kg) to mice caused an acceleration of lipolysis. Same result was obtained even after daily injection of CL316,243 (0.1 mg/kg) for 9 days.

2) Compared to UCP1 mRNA, UCP2 mRNA was expressed in all tissues examined, and its expression in the WAT, BAT and skeletal muscle of obese yellow KK mice was higher than that of C57BL control mice. UCP3 mRNA expressed abundantly in skeletal muscle and BAT, and there

was no difference in its expression between yellow KK and C57BL mice.

3) Chronic treatment with CL316,243 resulted in an increased UCP1 expression in WAT and even in skeletal muscle. Moreover, CL316,243 increased both UCP2 and UCP3 mRNA expression in skeletal muscle, but not in WAT or BAT.

These results suggest that anti-obesity effect of β 3-AR agonist might be attributable to increased energy expenditure not only by UCP1 but also by UCP2 and UCP3.

Canine leptin: molecular cloning, characterization and development of enzyme-linked immunosorbent assay

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Obesity is defined as a state of excessive body fat accumulation, and is due to an imbalance between energy intake and energy expenditure. Since obesity is also known as a major risk factor of a lot of number of diseases such as non-insulin dependent diabetes, atherosclerosis and hypertension, control of obesity is needed as well in small animal practices as in humans. For this purpose, it is necessary to evaluate the extent of obesity objective and quantitatively. However, no reliable method for assesment of obesity in small animals has not yet been established.

Leptin is the product of the gene responsible for obesity in the *ob/ob* mice strain. Leptin is synthesized and secreted by adipose tissues, and has been shown to regulate food intake and energy expenditure. Interestingly, the serum leptin level positively correlates with body fat

content and body mass index, and is higher in obese human and rodents. Therefore, it is expected that serum leptin level in dogs could be also proportional to body fat content, and this trend may enable to assess obesity and adiposity in dogs. In the present study, in order to clarify the properties of canine leptin, cDNA molecular cloning, recombinant protein expression in *E. coli*, and its functional analysis were performed. Moreover, a new method to measure dog serum leptin levels was established.

1) Canine leptin cDNA was cloned by RACE method using mRNA from omental adipose tissue as a template; nucleotide sequence in the coding region and the deduced amino acid sequence were 80-90% homologous to other species.

2) Northern blot analysis revealed abundant leptin mRNA expression in adipose tissues, but not in other tissues.

3) His-tagged recombinant canine leptin was expressed in *E. coli* and subsequently purified. This purified recombinant canine leptin was shown to be a single band in SDS-polyacrylamide electrophoresis, with a molecular weight approximately 19kDa.

4) In CHO-OBRb cells expressing rat leptin long form receptor, when stimulated by recombinant canine leptin, STAT3 (signal transducer and activator of transcription 3) and MAPK (mitogen activated protein kinase) phosphorylation was induced.

5) Rabbits were immunized with recombinant canine leptin as antigen; then specific anti-canine leptin antibody was obtained. Western blot analysis revealed that this anti-canine

leptin antibody cross-reacted both mouse and human leptins.

6) Using anti-canine leptin antibody, sandwich ELISA (enzyme-linked immunosorbent assay) was established. This ELISA system can measure a range from 0.5 to 32.0 ng/ml of canine leptin. Among 14 normal dogs serum tested, leptin levels were from 1.4 to 5.6 ng/ml (3.0 ± 0.3 ng/ml, mean \pm S.E.), except one sample.

Conclusively, my results indicate that leptin in dog is produced mainly adipose tissues as well as in other species, and this recombinant canine leptin is physiologically active. Moreover, the ELISA system established in this study is useful for serum leptin concentration measurement in the dog.

Role of brain interleukin-1 in immobilization stress-induced increase of blood interleukin-6 level in rat

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Interleukin(IL)-6 is known to increase its blood level in response to inflammatory stress and exerts various body defenses. Recently, physical and/or psychological stress without any invasion have been shown to elevate serum IL-6 level. However, the mechanism is little understood how these stresses cause the peripheral responses.

IL-1 is a cytokine which transduces inflammatory signals not only to immune responding cells but also to the brain. Intracerebroventricular (icv) administration of IL-1 has been shown to cause fever and anorexia, and also to induce an increase of serum IL-6 level. Moreover, it has been reported that Non-invasive stress increases the expression of IL-1

β itself in the brain. Thus it is assumed that the increased IL-1 β in the brain after non-invasive stress might regulate the peripheral IL-6 response. In the present study, to clarify this assumption, I analyzed the responses of peripheral IL-6 and brain IL-1 in rats after application of short-term immobilization (IM) stress.

When rats were immobilized, serum IL-6 level was increased progressively to reach a peak at 3 hour. Similarly, IM stress increased significantly IL-1 β mRNA levels in the hypothalamic region 1 hour after the IM. An IL-1 receptor antagonist given icv 15 minutes before the IM stress partially prevented the increase of serum IL-6 level. Moreover, icv administration of