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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 ± 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 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
anti-IL-1 \( \beta \) and anti-IL-1 \( \alpha \) antibodies also suppressed the increase of serum IL-6 level. These results indicate that IL-1 in the brain mediates the signals of the IM stress and causes the increase in blood IL-6 levels.

Next, hepatic expression of IL-6 mRNA was determined during IM stress, since recent findings suggest the liver as one of the main organs for peripheral IL-6 production. IM stress enhanced the expression of IL-6 mRNA in the liver. However, icv administration of the IL-1 receptor antagonist did not affect the changes in the hepatic IL-6 mRNA expression by IM stress. These results suggest that the liver is a responsible for blood IL-6, but IL-6 production in this organ in not under the control of brain IL-1.

Conclusively, brain IL-1 is important in the control of peripheral IL-6 production induced by non-invasive stress, as well as inflammatory stress.

Effects of a new cardiotonic agent, pimobendan on contractile responses in single muscle fibers of the frog

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1. The effects of a new cardiotonic agent, UD-CG 115 (pimobendan) on contractile activity were investigated in single twitch skeletal muscle fibers of the frog.
2. Pimobendan dose-dependently potentiated twitch responses to electrical stimuli (0.1 \( \mu \)M–100 \( \mu \)M) regardless of the presence or absence of Ca\(^{2+}\) without any effects on tetanic tension and electrical membrane properties. The half decay time of twitch responses was significantly prolonged by pimobendan.
3. Pimobendan caused further increase in twitch tension potentiated by caffeine (1 mM).
4. Adenine, an inhibitor of Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR), did not inhibit twitch responses potentiated by pimobendan, suggesting the lack of involvement of CICR.
5. Contractures induced by caffeine (2–3 mM) or KC1 (15–90 mM) were also potentiated by pimobendan. Concentration-response curves for caffeine or KC1 were shifted to the left by pimobendan.
6. Pimobendan increased Ca\(^{2+}\) (1 \( \mu \)M) -induced contraction in the skinned fibers.
7. 8-Bromo cyclic AMP slightly potentiated twitch responses, and further increase in twitch tension was observed with pomobendan in the presence of 8-bromo cyclic AMP. Pimobendan did not directly affect ATP-dependent Ca\(^{2+}\) uptake into fragment SR.
8. These results suggest that the positive inotropic effect of pimobendan is due to increase Ca\(^{2+}\) affinity to contractile apparatus in frog skeletal muscles.