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# *Influence of salmon calcitonin on the analgesic effect of selective κ-opioid agonist in mice*

Calcitonin is a polypeptide hormone that can exert various pharmacological effects besides its well-known function as a regulator of calcium metabolism. Clinical observations have shown that salmon-calcitonin (S-CT) has an analgesic effect in a variety of painful pathologies in human (5, 8). This effect of S-CT has been demonstrated in laboratory animals too (11, 14). The mechanism underlying the antinociceptive effect of S-CT is unknown and opioid or non-opioid mechanisms have been postulated (1, 2, 4). The opioid mechanism may be suggested because of the enhancement of endogenous opioid release found after s-CT administration (3) and because of the modifications induced by S-CT *in vivo* and *in vitro* on the opioid effects (7, 9). The level of this interaction, that could be one of the basis of the analgesic effect of S-CT, remains to be elucidated. It has been suggested that opioid mechanisms linked to the effects of calcitonin may be mediated by a non-m-opioid system.

The aims of the present work are: 1) to study the possible analgesic activity of S-CT administered intraperitoneally (i.p.) using writhing test in mice, 2) to study the influence of S-CT on the analgesia induced by the selective κ-opioid agonist, U-50, 488H.

## MATERIAL AND METHODS

A. Animals. Male Albino Swiss mice weighing 22-27g were used. Each group consisted of 8 mice. Animals were given food and water *ad libitum* and were kept under controlled environmental conditions (20±4°C, 55±5% humidity) with a 12-h light-dark cycle for at least 5 days before experiments. The mice were housed for at least 1 day in the test-room before experimentation.

B. Substances. The following substances were used in the experiments: salmon calcitonin (Calcihexal, Hexal AG, Holzkirchen, Germany); U-50, 488H, (trans-3,4-dichloro-methyl-N-[2-(1-pyrrolidynyl)cyclohexyl] benzeneacetamide methane sulphonate) (Sigma); NorBNI; nor-binaltorphimine (Sigma).

C. Methods. The writhing test was used as analgesic test. The mice were injected i.p. with 2% acetic acid solution to produce the typical writhing reaction which is characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs. The mice were then placed in individual transparent containers and the number of stretches was counted during a 10-min period beginning 5 min after the acetic acid injection. Each mouse was used only once.

Four sets of experiments, consisting of different treatments, were carried out: 1) S-CT treatment: three groups of mice were injected i.p. with: saline solution (0.01ml/g b.w., control group) or with S-CT(2.5 UI/kg and 10UI/kg). The writhing test was carried out 60 min later; 2) Opioid treatment: three groups of mice were injected i.p. with: saline solution (0.01ml/g b.w., control group) or with κ-opioid receptor agonist, U-50, 488H (1mg/kg and 10mg/kg). The writhing test was carried out 30 min later; 3) S-CT + opioid treatment: four groups of mice were treated i.p. with 2.5 UI/kg of S-CT 30 min before the i.p. administration of the previously described doses of the κ-opioid agonist. The test was carried out 30 min after opioid administration. 4) Nor-BNI + S-CT + opioid treatment: four groups of mice. The procedure as in point 3 but the mice were pretreated additionally with 5mg/kg of Nor-BNI (κ-opioid receptor antagonist), given 5 min before s-CT.

D. Statistics. Results are expressed as mean ±SE. Statistical analysis was performed by Student's t-test for unpaired data. p values of $< 0.05$ were regarded as significant.

## RESULTS

Figure 1 and Figure 2 show the effect of the i.p. administration of S-CT and U-50, 488H respectively on the number of stretches. S-CT administration induced analgesia shown as a decrease in the number of stretches when the writhing test was carried out 60 min later if the dose of 10 UI/kg was used (2.5 UI/kg:12.25±2.0; 10 UI/kg:3.25±1.45).

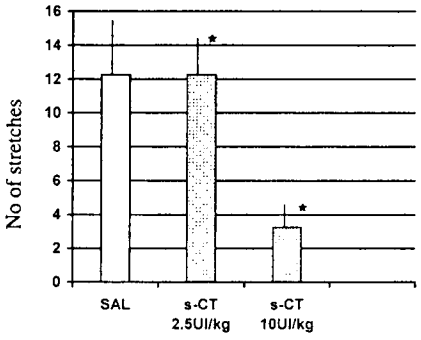


Fig.1. Effect of the i.p. administration of S-CT on the number of stretches in mice (writhing test). The data represent means ± S.E.M. *P<0.05 vs. saline control group

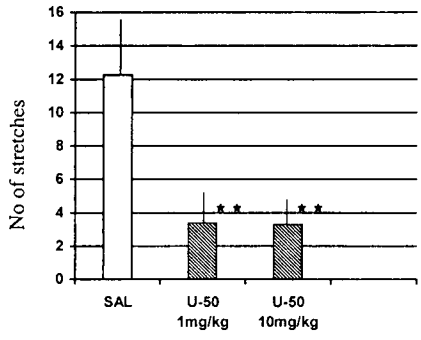


Fig.2. Effect of the i.p. administration of U-50,488H on the number of stretches in mice (writhing test). The data represent means ± S.E.M. **P<0.01 vs. saline control group

The i.p. administration of U-50, 488H produced analgesia shown as a decrease in the number of stretches induced by acetic acid 30 min later (1mg/kg:3.38±1.85; 10mg/kg 3.3±1.5). This last effect was significant and similar with both of the used doses. The difference vs. saline-treated mice (12.25±3.14) was statistically significant with both of substances. To study the effect of the combined administration of S-CT and U-50, 488H (Fig. 3) the lower dose of S-CT (subanalgesic dose, 2.5 UI/kg) was selected in order to avoid additive effects. When S-CT was administered 30 min before U-50, 488H the analgesic effect of this opioid was increased in comparison with the action of opioid alone. This effect was not dependent on the U-50, 488H dose (1mg/kg: 1.6±0.4; 10mg/kg: 1.5±0.7). The

differences between the effect of U-50, 488H in control animals and in S-CT-treated animals were statistically significant. Pretreatment with the selective κ-opioid receptor antagonist,Nor-BNI, given 5 min before S-CT, reversed the calcitonin-induced potentiation of the analgesic effect of U-50, 488H (Fig. 4).

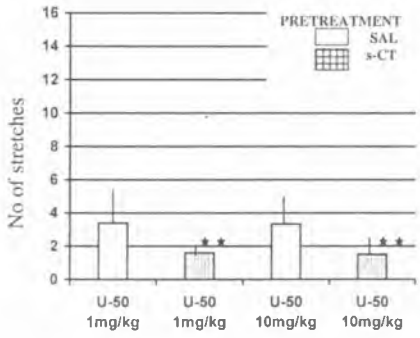


Fig.3. Effect of the i.p. administration of U-50,488H on the number of stretches in mice pretreated with saline S-CT at a dose 2.5 UI/kg. The data represent means ± S.E.M. *P<0.05 vs. its control group. **P<0.01 vs. its control group

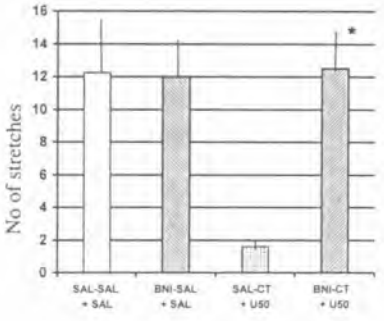


Fig.4. Effect of the i.p. injection of Nor-BNI (5 mg/kg) on the U-50,488H-induced decrease of the number of stretches in mice pretreated with S-CT (2.5 UI/kg). The data represent means ± S.E.M. *P<0.05 vs. its control group

## DISCUSSION

Although the existence of regulatory mechanisms between opioid and calcitonin systems in the control of pain have been suggested, the results of different studies are controversial. *In vitro* studies have shown that calcitonin induces significant increases in the inhibitory effect of the κ-opioid receptor agonist bremazocine, on the guinea-pig ileum and mouse vas deferens (9), whereas it produced no changes in the effects of the selective m-opioid receptor agonist, DAMGO (7). This effect has been reproduced using an analgesic test: the analgesia induced by [D-Pen$^2$,D-Pen$^5$]enkephalin (δ-agonist) and by U-50, 488H(κ-agonist) was significantly improved after pretreatment with a subanalgesic dose of S-CT (8,10). However, differences between *in vivo* and *in vitro* results may be justified since there is an involvement of more complicated systems and pathways in analgesia, which is absent in isolated organs. Naloxone has been demonstrated both to antagonize and to be ineffective to block calcitonin effects. The present data suggest that analgesia induced by S-CT may be mediated, at last partially, through modifications of the κ-opioid receptors. The antagonism was observed after the injection of selective κ-opioid antagonist, Nor-BNI corroborates this suggestion. These results agree with those previously described by many investigators (12). S-CT was administered i.p. because it is accepted that after peripheral administration it could be detected in the cerebrospinal fluid, and evidence demonstrates that i.p. injection of this peptide induces changes in effects mediated through the central nervous system (6, 8).

In contrast, other investigators have observed that systemic injection of calcitonin produces no analgesic effect (11). Calcitonin binding sites have been demonstrated in human or rodents brain and spinal cord homogenates (4). Laurian et al. (6) according to Collin et al. (3) suggested that S-CT liberates b-endorphins. Furthermore, subanalgesic doses of S-CT were able to increase the writhing tests (8,14). Studies on the hypothalamus-pituitary-adrenocortical axis have shown that

S-CT potentiates κ-, but not μ-opioid-induced release of cortison (10). The achieved results indicate the involvement of κ-opioid receptors in the analgesic effect of S-CT. Nevertheless, the underlying mechanism cannot be inferred from these data, and different possibilities could be suggested: an "up regulation" of the opioid receptors may take place, although the time that elapsed between the administration of S-CT and the increase of the effect of κ receptors was only 30 min. It is possible that activation of hypothalamic calcitonin receptors could positively modulate the κ-opioid system either directly (via binding of κ-opioid receptor agonists) or indirectly (via release of endogenous dynorphins). S-CT is widely used in clinical practice and generally well tolerated in human. The joint administration of S-CT and κ-opioid agonist may be useful in the treatment of painful diseases resistant to other treatments.

## CONCLUSIONS

1. Intraperitoneally administered S-CT at a dose of 10 UI/kg induced strong analgesic effect after 60 min, which was evaluated using writhing test in mice. S-CT at a dose 2.5 UI/kg was ineffective.

2. U-50, 488H, κ-opioid agonist, injected intraperitoneally caused significant analgesic effect, similar with both of the used doses, observed after 30 min.

3. The analgesic effect of U-50, 488H was enhanced in mice by pretreatment with subanalgesic dose of S-CT.

4. The potentiation by S-CT of the effect of U-50, 488H was blocked by prior injection of the selective κ-opioid antagonist, Nor-BNI, suggesting a role of κ-opioid receptors in mediating calcitonin-induced supersensitivity to U-50, 488H.

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## SUMMARY

In the present study the relationship between calcitonin and κ-opioid system was analysed. The possibility that salmon calcitonin (S-CT) exerts analgesic activity in mice as well as the influence of salmon calcitonin on the analgesic effect of κ-opioid agonist, U-50, 488H were examined. The writhing test in mice was used as analgesic test. S-CT given alone intraperitoneally at a high dose produced antinociceptive effect. S-CT given at a subanalgesic dose increased the antinociceptive effect of κ-opioid agonist, U-50, 488H. The obtained results suggest that the increase in the effectiveness of κ-opioid receptor agonist may be one of the mechanisms involved in the analgesia induced by salmon calcitonin.

## Wpływ kalcytoniny łososiowej na przeciwbólowy efekt selektywnego agonisty receptorów opioidowych kappa u myszy

W prezentowanej pracy analizowano zależność między kalcytoniną a układem opioidowym kappa. Badano, czy jest możliwe, że kalcytonina łososiowa (S-CT) wywiera działanie przeciwbólowe u myszy, a także czy wpływa ona na analgetyczny efekt selektywnego agonisty receptorów opioidowch kappa, U-50,488H. Jako testu analgetycznego użyto testu wicia u myszy. S-CT podana dootrzewnowo w wysokiej dawce wywoływała efekt przeciwbólowy. S-CT podana w dawce subanalgetycznej zwiększała przeciwbólowy efekt U-50,488H. Uzyskane wyniki sugerują, że zwiększenie efektywności agonisty receptorów opioidowych kappa może być jednym z mechanizmów analgezji indukowanej przez kalcytoninę łososiową.