CALCITONIN-INDUCED ANOREXIA IN RATS: A STRUCTURE-ACTIVITY STUDY BY INTRAVENTRICULAR INJECTIONS*

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Abstract—The anorectic potency of salmon, porcine and human calcitonins (sCT, pCT and hCT, respectively) and two sCT-fragments were compared in rats. Intraventricular injections of sCT (0.062 and 0.031 nmole/animal) significantly reduced the normal feeding and body weight. The effect appeared to be dose-dependent, reversible and lasted longer than 6 hr. No anorexia ensued, however, on injections of mammalian hormones though tested in relatively high doses (pCT: up to 3.7 nmole, hCT: 3.7 nmole). The C-terminal fragments of sCT, sCT (10–32) and sCT (22–32) were also found to be devoid of anorectic activity; but when administered with sCT, the longer fragment (1.2 nmole) significantly decreased the effect of sCT and even the shorter one (18 nmole) tended to act as an antagonist. This property was not recorded with pCT and hCT in the doses examined. On the one hand, these results indicate a novel specificity of the anorectic receptor in rat brain; and on the other hand, they seem to strongly argue against the hypothesis that in mammals thyroïdal calcitonin secreted postprandially might participate in the regulation of subsequent feeding, unless the presence of the sCT-like molecule can be detected in mammals. All the more because detection of such a molecule must await development of a specific assay, the antagonistic property of the sCT fragment found herein would have use for clarifying the physiological significance of the anorectic receptor which is possibly in the hypothalamus.

The anorectic effect of salmon calcitonin (sCT) was first reported by Freed et al. (1, 2). When injected subcutaneously, the fish hormone strongly reduced food intake in rats and rhesus monkeys and reduced the body weight in humans. A marked increase in the

potency by direct injection into rat brain has led them to suggest that the peptide would exert its effect by directly acting on the central nervous system. Later, using a synthetic derivative of eel calcitonin, Miyahara and Oomura (3) electrophysiologically identified a group of glucose-sensitive neurons in the rat hypothalamus as a possible locus of the anorectic effect. Since it has been demonstrated that the calcitonin-like immuno-activity increases postprandially in adult and

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infant rats (4, 5), these results seem to be in support of the hypothesis that calcitonin secreted after a meal or suckling might participate in the regulation of subsequent feeding in mammals.

As far as we know, there are no concrete evidences, however, to show that the mammalian calcitonins do actually share such anorectic activity or that a sCT-like molecule is endogenously secreted in mammals, no matter what the cell origin may be. Cooper and his coworkers were the only researchers who have briefly noted that human and porcine calcitonin exhibit if any weak anorectic effect in rats (6, 7). This has prompted us, first of all, to compare the anorectic potency of known mammalian calcitonins with that of sCT in detail. We now report an unusual specificity for the effect of sCT which would not have been predictable based on the other biological activities reported so far for calcitonins, with one exception (8).

MATERIALS AND METHODS

sCT, porcine calcitonin (pCT) and sCT fragments were synthesized and/or purified by the Armour Pharmaceutical Company (Kankakee, Ill., U.S.A.). Human calcitonin (hCT) was purchased from The Peptide Institute (Minoh, Japan).

Under pentobarbital Na anesthesia (50 mg/kg, i.p.), a stainless steel guide cannula with a matched stylet was implanted into the lateral ventricle of Wistar male rats (200–250 g). After a post-surgical recovery period of 7 to 10 days, the animal received 10 μ l of a peptide solution or saline alone between 17:00 and 18:00 with an injection speed of 10 μ l/2 min; and unless otherwise described, food and water in the cage were recorded at –24, 0, 6 and 24 hr after the injection and body weight recorded at –24, 0 and 24 hr. Throughout this experiment, animals were allowed free access to pelleted food and water.

RESULTS

Figure 1 compares food intake recorded in the first 6 hr period (18:00 to 24:00) after injection of saline and various doses of sCT. Rat feeding, which usually started around 18:00, was significantly reduced by sCT (31 or 62 pmole/animal) in a dose-dependent manner. The lower dose (12 pmole/animal) was not active in this experiment, though Freed et al. (1) reported the occurrence of anorexia with this dose. The anorexia induced by sCT lasted longer than 6 hr, and food intake during the second period (6 to 14 hr after injection) also remained low. Body weight appeared to decrease in parallel to the reduction in food intake (Table 1), but decreased drinking may also contribute to the loss of body weight (a high dose of sCT significantly decreased drinking in the first period).

Some members of the sCT-treated groups exhibited sporadic daytime eating on the next day; this behavior occurred less frequently in the control groups. Within 24 hr after injection, sCT-treated rats resumed the normal feeding behavior, and the feeding pattern returned to the normal one having four peaks (refer to reference 9, the data not shown here). It should be noted that rats did not develop detectable tolerance to the

Fig. 1. Dose-dependency of sCT-induced anorexia. Each point represents the mean $\pm$ S.E. of four to ten rats. ★ and ★★ indicate significant inhibition at $P < 0.05$ and $P < 0.01$, respectively.

second dose of sCT given at the interval of 7 days following the first.

As shown in Fig. 2, no anorexia ensued after pCT injection, even when it was given in a large dose, 3.7 nmole, which would be more potent than 62 pmole sCT in terms of the peripheral-hypocalcemic activity. In the doses tested, hCT and two sCT fragments were also found to be inactive (Table 1, Figs. 3 and 4). These results not only confirm the finding of Freed et al. (1), suggesting that the whole structure of sCT would be

essential for its anorectic activity, but also seems to disclose a novel hormone-specificity of the effect.

An interesting observation was made when the sCT fragments were injected with sCT. The longer fragment was able to, though partially, block the sCT effect (Fig. 3); and even the shorter one exhibited a tendency to apparently act as an antagonist (Fig. 4). In contrast, such a property has never been detected in the mammalian hormones (Fig. 2 and Table 1).

DISCUSSION

The structure-activity study reported herein clearly demonstrates that there exists an anorectic receptor in the rat brain which can specifically recognize the intact sCT molecule alone as a signal to stop eating. A part of our results seems to be generally in accord with the findings of Cooper and his colleagues who compared the anorectic potency of sCT, pCT and hCT (6, 7). This peculiar hormone-specificity apparently suggests that the anorectic activity would bear no direct relevancy with the other biological activities described so far for this family of hormones: hypocalcemia (10), decreased gastric acid secretion (11), analgesia (12, 13), etc. Even

Fig. 2. Effect on rat feeding of pCT injected alone or with sCT. Each column represents the mean $\pm$ S.E. of five to eight rats. ** significant inhibition as compared with the vehicle-treated group ($P < 0.01$).

Fig. 3. Effect on rat feeding of sCT(10-32) injected alone or with sCT. Each column represents the mean $\pm$ S.E. of five to seven rats. ** significant inhibition as compared with the vehicle-treated group ($P < 0.01$) and ☆☆ significant difference as compared with the sCT-treated group ($P < 0.01$).

Fig. 4. Effect on rat feeding of sCT(22-32) injected alone or with sCT. Each column represents the mean $\pm$ S.E. of five to seven rats. * and ** indicate significant inhibition as compared with the vehicle treated group at $P < 0.05$ and $P < 0.01$, respectively.

the binding assay using some specified regions of rat brain (14) has failed to predict such a specificity. In this sense, one exception would be the *in vitro* effect of sCT recently observed as the inhibition of Ca-uptake by rat hypothalamus slices (8). Since the effect appeared to be specific not only to sCT but also to the hypothalamus where the "appetate" is known to reside, it is expected that further study may disclose more relevancy of the effect to the anorectic effect.

No anorexia by two mammalian hormones in large doses seems to strongly argue against the hypothesis that the calcitonin serves as a feeding regulating factor in mammals, unless the animals are found to be endowed with the ability to synthesize and secrete a sCT-like molecule. Recently Galan et al. (15) have histochemically detected a sCT-like immunoreactive substance in the pigeon brain. Though immunoreactivity does not always mean a hormone-active molecule, this type of study with a refined binding assay as proposed (8) may help to clarify the physiological function of the anorectic receptor. In this context, the finding of an antagonist would be of practical value, and it is interesting to see how the feeding behavior of rats would be affected by the antagonist. Such a study is now underway.

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