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Central administration of cholecystokinin stimulates gastric pepsinogen secretion from anaesthetized rats

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Abstract

Intracerebroventricular administration of CCK-8S was associated with a stimulation of gastric pepsinogen secretion from anaesthetized rats; similar effects were induced by CCK-8S given intravenously. The excitatory effect of intracerebroventricular CCK-8S was not modified by central injection of L-364,718 or L-365,260, whereas both these antagonists, given by intravenous route, prevented the pepsigogue action of parenteral CCK-8S. Intracerebroventricular or intravenous CCK-8S also increased basal acid secretion, this latter effect being prevented by parenteral L-365,260 but not L-364,718. It is suggested that centrally applied CCK-8S evokes pepsinogen secretion through the activation of peripheral CCK-A and CCK-B receptors.

Keywords: Central nervous system; Stomach; Pepsinogen secretion; Cholecystokinin; CCK-A and CCK-B receptors

The cholecystokinin (CCK) family of peptides is widely distributed throughout the gastrointestinal tract as well as the central nervous system (CNS), where the predominant molecular form is represented by CCK octapeptide (CCK-8) [5,16]. At both central and peripheral levels, the receptors mediating the physiological actions of CCK can be distinguished into two subtypes, named CCK-A and CCK-B [11]. CCK-A receptors show high sensitivity for sulphated analogues of CCK and the antagonist L-364,718, whereas CCK-B receptors display high affinity either for sulphated and nonsulphated analogues or for the antagonist L-365,260 [11,17].

CCK is known to affect gastric motor and secretory functions through the activation of both CCK-A and CCK-B receptors [7,13,14]. In particular, CCK is able to stimulate both pepsinogen and acid secretions through CCK-A and CCK-B receptors located on gastric chief and parietal cells, respectively [13,14]. As far as the CNS is concerned, CCK-8-sulphate (CCK-8S), injected into the lateral cerebral ventricle of anaesthetized rats, caused a dose-dependent increase in gastric acid secretion [9]. However, data concerning the influence of central CCK-ergic pathways on pepsinogen secretion as well as the relevance of central CCK receptor subtypes in the regulation of gastric secretory functions are still lacking. Accordingly, the present study was designed to investigate the effects of centrally administered CCK-8S and selective antagonists for CCK receptor subtypes on gastric pepsinogen secretion in rats.

The experiments were carried out on male Wistar rats, weighing about 200 g and fasted for 24 h. Four days before the experiments, a chronic cannula was implanted into the lateral ventricle of the brain, during a short anaesthesia induced with pentobarbital sodium (30–40 mg/kg) given intraperitoneally (i.p.), as previously reported [2]. At the time of the experiment, the drugs under investigation were administered by intracerebroventricular (i.c.v.) or intravenous (i.v.) route. In the former case, the drug solutions (2.5 μl) were injected through the cannula using a 10-μl Hamilton microsyringe, followed by 2.5 μl of saline solution (154 mM NaCl). The solutions were injected within 20 s. In agonist–antagonist interaction experiments, the antagonist was administered 10 min before the agonist.

Perfusion of the rat stomach in situ was carried out following a procedure previously reported [4]. The ani-

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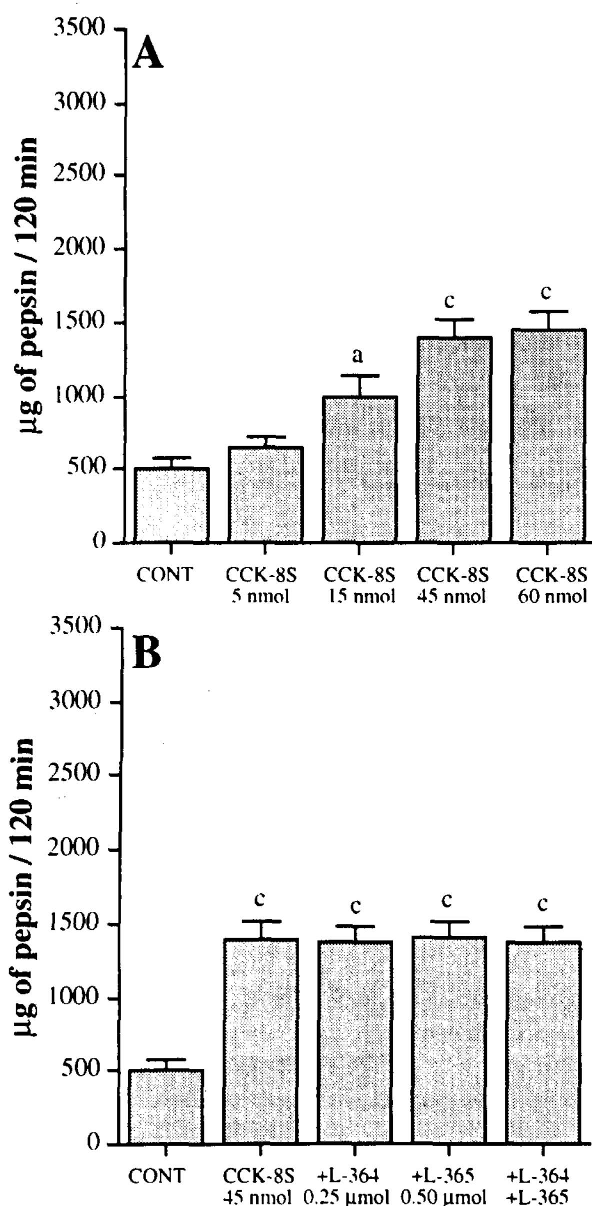

Fig. 1. Effects of CCK-8S (5, 15, 45 and 60 nmol/kg i.c.v.) (A), and CCK-8S (45 nmol/kg i.c.v.) given alone or following L-364,718 (0.25 μmol/kg i.c.v.), L-365,260 (0.5 μmol/kg i.c.v.) or L-364,718 (0.25 μmol/kg i.c.v.) plus L-365,260 (0.5 μmol/kg i.c.v.) (B) on basal pepsinogen secretion (μg of pepsin/120 min) in anaesthetized stomach-perfused rats. Each column indicates the mean value obtained from 8 experiments ± SEM (vertical lines). Statistical analysis for (A): ANOVA ($F = 14.35$; degrees of freedom, among groups = 4, within groups = 35, total = 39; $P < 0.001$); Student–Newman–Keuls test ($^{a}P < 0.05$ and $^{c}P < 0.001$ versus control value [CONT]). Statistical analysis for (B): ANOVA ($F = 14.88$; degrees of freedom, among groups = 4, within groups = 35, total = 39; $P < 0.001$); Student–Newman–Keuls test ($^{c}P < 0.001$ versus control value [CONT]).

mals were anaesthetized with urethane (1.2 g/kg i.p.), and the rectal temperature was monitored and maintained between 37 and 39°C with an infrared lamp. The gastric lumen was perfused continuously with saline solution at 37°C, at a rate of 1 ml/min, and 15-min effluent fractions were collected for 120 min after 30-min stabilization. Pepsin levels in the gastric effluent were determined by the method of Berstad [1] with minor modifications. Briefly, 0.5 ml of gastric effluent was added to tubes containing 2 ml of 2.5% bovine haemoglobin and 0.5 ml of 0.3 N HCl. Samples were then incubated for 10 min at 37°C and the reaction was stopped by addition of 5 ml of 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by an Uvikon 930 spectrophotometer (Kontron Instruments, Milan, Italy). The results were compared to a standard curve obtained by using known amounts of porcine pepsin (1 μg = 3 peptic units). Data were expressed as μg of pepsin/15 min. The acidity in the gastric perfusate was measured with an autotitrator pH meter (PHM 85, Radiometer, Copenhagen) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH, and expressed as μEqH^{+}/15 min. The total pepsinogen or acid output obtained in each experiment was also calculated and expressed as μg of pepsin/120 min and μEqH^{+}/120 min, respectively.

The following drugs and reagents were used: [Tyr-$(SO_3H)^{27}$]carboxyl terminal octapeptide (26–33) of cholecystokinin (CCK-8S), urethane ethyl carbamate, crystallized porcine pepsin, lyophilized bovine haemoglobin (Sigma, St. Louis, MO, USA); L-364,718 and L-365,260 (kindly provided by Merck Research Laboratories, Rahway, NJ, USA); pentobarbital sodium (Clin-Midy, Paris, France). Results are given as means ± SEM, each mean value being obtained from 8 experiments. The significance of differences was evaluated by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test, and P values lower than 0.05 were considered significant.

In control animals, receiving drug vehicle only, basal pepsinogen and acid secretions, assessed after 30-min stabilization, accounted for 74.7 ± 9.8 μg of pepsin/15 min and 3.6 ± 0.5 μEqH^{+}/15 min, respectively, and these values remained at a steady level up to the end of the experimental period (120 min).

Under these conditions, i.c.v. administration of CCK-8S (5, 15, 45 and 60 nmol/kg) caused a dose-dependent stimulation of pepsinogen secretion, the maximal effect occurring at the dose of 45 nmol/kg (Fig. 1A), with a peak increase of 292.5 ± 27.3 μg of pepsin/15 min at 45 min (ANOVA $F = 36.77$, $P < 0.001$; Student–Newman–Keuls test for CCK-8S 45 nmol/kg versus control value at 45 min; $P < 0.001$). The antagonists L-364,718 (0.25 μmol/kg i.c.v.) and L-365,260 (0.50 μmol/kg i.c.v.), given either alone or in combination, did not affect either basal pepsinogen secretion (not shown) or the pepsigogue stimulant action exerted by i.c.v. CCK-8S (45 nmol/kg) (Fig. 1B).

When injected by i.v. route, CCK-8S (1.5, 5, 15 and 45 nmol/kg) elicited a marked and dose-dependent increase in pepsinogen secretion (Fig. 2A). In this case, the

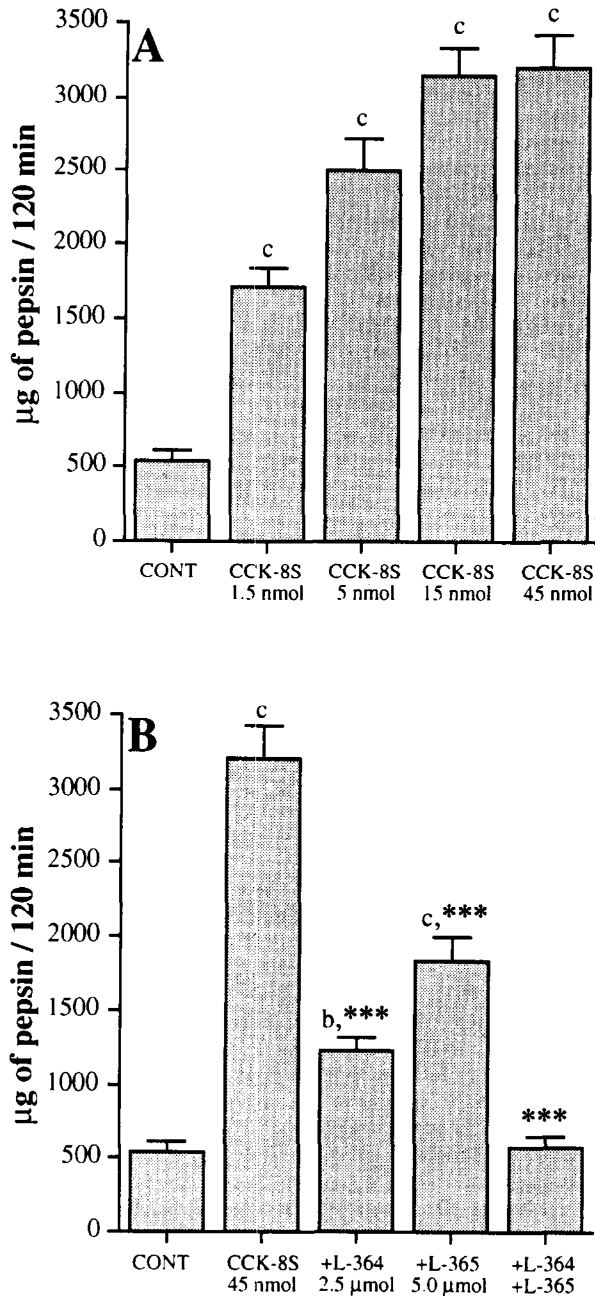

Fig. 2. Effects of CCK-8S (1.5, 5, 15 and 45 nmol/kg i.v.) (A), and CCK-8S (45 nmol/kg i.v.) given alone or following L-364,718 (2.5 μmol/kg i.v.), L-365,260 (5.0 μmol/kg i.v.) or L-364,718 (2.5 μmol/kg i.v.) plus L-365,260 (5.0 μmol/kg i.v.) (B) on basal pepsinogen secretion (μg of pepsin/120 min) in anaesthetized stomach-perfused rats. Each column indicates the mean value obtained from 8 experiments ± SEM (vertical lines). Statistical analysis for (A): ANOVA ($F = 44.18$; degrees of freedom, among groups = 4, within groups = 35, total = 39; $P < 0.001$); Student–Newman–Keuls test ($^{c}P < 0.001$ versus control value [CONT]). Statistical analysis for (B): ANOVA ($F = 74.51$; degrees of freedom, among groups = 4, within groups = 35, total = 39; $P < 0.001$); Student–Newman–Keuls test ($^{b}P < 0.01$ and $^{c}P < 0.001$ versus control value [CONT]; $^{***}P < 0.001$ versus CCK-8S 45 nmol/kg alone).

maximal increment of pepsinogen output was observed at the dose of 15 nmol/kg (Fig. 2A), with a peak of 656.3 ± 36.4 μg of pepsin/15 min at 30 min (ANOVA: $F = 63.47$, $P < 0.001$; Student–Newman–Keuls test for CCK-8S 15 nmol/kg versus control value at 30 min; $P < 0.001$). Following administration by i.v. route, L-364,718 (2.5 μmol/kg) and L-365,260 (5.0 μmol/kg), given either alone or in combination, did not modify basal pepsinogen secretion (not shown). However, L-364,718 or L-365,260 partly antagonized the excitatory effect exerted by i.v. CCK-8S (45 nmol/kg) on pepsinogen output (Fig. 2B). In addition, the combined parenteral administration of L-364,718 and L-365,260 completely prevented the pepsigogue action induced by i.v. CCK-8S (Fig. 2B).

Concomitant with the stimulation of pepsinogen output, both i.c.v. and i.v. CCK-8S caused a dose-dependent increase in basal acid secretion. The peak effects obtained with the dose of 45 nmol/kg were 11.8 ± 0.8 and 17.2 ± 1.3 μEqH^{+}/15 min for the i.c.v. or i.v. route, respectively. The acid hypersecretory action elicited by i.v. CCK-8S (45 nmol/kg) was not affected by i.v. L-364,718, whereas it was completely antagonized by i.v. L-365,260 (Fig. 3). In addition, after combined treatment with L-364,718 and L-365,260, the blockade of CCK-8S-induced acid hypersecretion was not significantly different from that obtained with L-365,260 alone (Fig. 3).

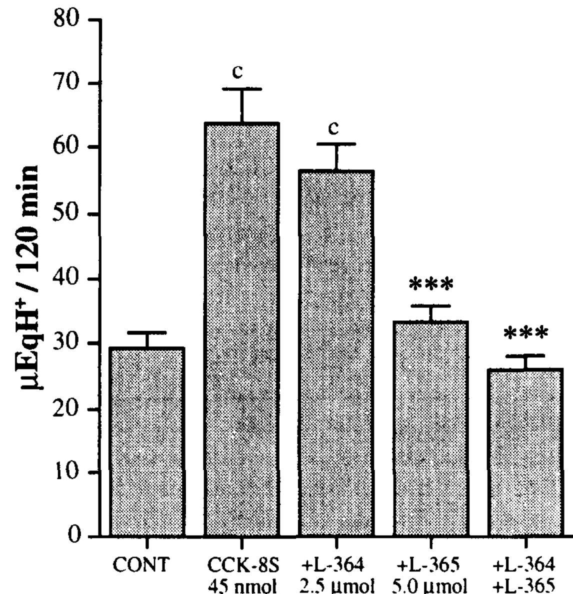

Fig. 3. Effect of CCK-8S (45 nmol/kg i.v.), given alone or following L-364,718 (2.5 μmol/kg i.v.), L-365,260 (5.0 μmol/kg i.v.) or L-364,718 (2.5 μmol/kg i.v.) plus L-365,260 (5.0 μmol/kg i.v.), on basal acid secretion (μEqH^{+}/120 min) in anaesthetized stomach-perfused rats. Each column indicates the mean value obtained from 8 experiments ± SEM (vertical lines). Statistical analysis: ANOVA ($F = 23.11$; degrees of freedom, among groups = 4, within groups = 35, total = 39; $P < 0.001$); Student–Newman–Keuls test ($^{c}P < 0.001$ versus control value [CONT]; $^{***}P < 0.001$ versus CCK-8S 45 nmol/kg alone).

The present findings suggest that centrally applied CCK-8S stimulates the pepsinogen output through activation of peripheral-type receptor mechanisms. In this regard, the following points are worth noting: (1) centrally injected CCK-8S was quite less effective and potent in eliciting peptic hypersecretion than did i.v. CCK-8S given at equimolar doses; (2) the time for reaching the peak increase in peptic output was longer after central (45 min) than parenteral (30 min) administration of CCK-8S; (3) both i.c.v. L-364,718 and L-365,260 failed to affect the stimulant action of i.c.v. CCK-8S, although these antagonists have been reported to effectively block central CCK-mediated effects at lower doses than those adopted in the present study [18]. On the other hand, the pepsigogue effect exerted by i.v. CCK-8S could be significantly antagonized by parenteral administration of L-364,718 and L-365,260, both administered at doses found to afford a significant blockade of peripheral CCK receptors in in vivo studies [6,8].

Thus, it is conceivable that centrally applied CCK-8S exits the cerebrospinal fluid and reaches CCK receptors located in the periphery through the systemic circulation at concentrations sufficient to stimulate gastric pepsinogen secretion. In support of this view, it has been previously shown that radiolabelled CCK-8S can be detected in plasma after central injection and that i.c.v. CCK-8S, given at a dose of about 20 nmol/kg, is able to inhibit food consumption by diffusing from the brain to sites located in peripheral organs [3,12]. Furthermore, analogous to our results, a maximal stimulation of pepsinogen secretion from conscious rats was obtained with parenteral CCK at the dose of 19 nmol/kg·h [15].

The partial sensitivity of peptic hypersecretion induced by i.v. CCK-8S to either i.v. L-364,718 or L-365,260 indicates that both peripheral CCK-A and CCK-B receptors are involved in this excitatory effect. In this respect, it is well known that CCK-A receptors located on gastric chief cells mediate a direct increase in pepsinogen secretion without affecting the acid output [13]. In addition, since gastric luminal acid is known to enhance the pepsinogen secretion via local nervous reflexes [10], CCK-B receptors located on gastric parietal cells [14] may be responsible for an indirect increase in peptic output through the activation of acid secretion. Our data, showing that parenteral L-365,260, but not L-364,718, antagonized CCK-8S-induced acid hypersecretion, are consistent with this hypothesis.

In conclusion, under the experimental conditions adopted in the present study, central CCKergic pathways do not appear to play an important role in the regulation of gastric pepsinogen secretion. It is suggested that, following central administration of CCK-8S, the stimulation of peptic output results directly from an activation of CCK-A receptors on chief cells and indirectly from an increase in acid secretion mediated by CCK-B receptors on parietal cells.

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