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Cholecystokinin-octapeptide antagonizes morphine analgesia in periaqueductal gray of the rat

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The analgesic effect of systemic morphine (5 mg/kg, s.c.) was dose-dependently antagonized by CCK-8 administered to the periaqueductal gray (PAG) of the rat. This effect could be reversed by proglumide, a CCK-receptor antagonist. The effect of morphine analgesia was potentiated by proglumide administered to PAG. These results are compatible with the notion that PAG is a strategic site where CCK-8 exerts an antiopioid activity.

INTRODUCTION

Vanderhaeghen[28] was the first to describe a gastrin-like peptide(s) in the mammalian brain. Subsequent studies have shown that the carboxyl-terminal octapeptide cholecystokinin carries all the known biologic activities of the larger 33 amino acid peptide and is thought to be the predominant species of cholecystokinins in the central nervous system (CNS)[5,20,24]. This peptide is located throughout the brain in a characteristic distribution[1,2,3,14], which is parallel to a large extent to the distribution of its specific binding sites[13,17]. These findings, together with the fact that CCK is localized in vesicles and released in a calcium-dependent fashion by depolarization[6,7,9,23,25], suggest that CCK-8 may be regarded as a neurotransmitter or neuromodulator[18,19,32].

The sulfated CCK-8 has a wide variety of physiological and behavioral effects. One of them is that CCK-8 acts as an opiate antagonist following its systemic or central administration (intrathecally or intracerebroventricularly)[4,8,11,12,16]. However little is known on the exact sites of action of CCK in the brain. Since PAG is known as one of the most important areas for pain modulation and contains both opiates and CCK and their receptors[13,15,17,21,26,27,29,33], we decided to study the effect of CCK-8 on morphine analgesia in the PAG of the rat.

MATERIALS AND METHODS

Surgical procedures

Male Wistar rats weighing 200–250 g were anaesthetized with chlorohydrate (0.4 g/kg, i.p.) and mounted on a stereotaxic instrument. Stainless steel guide cannulae of 0.7 mm outer diameter (o.d.) were implanted on the right lateral PAG at the coordinate of P6.2, R0.7 and H4.0 mm[22], and fixed in situ with dental acrylic. Seven days were allowed for surgical recovery. All rats received 5 daily habituation sessions (80 min each) in the restraining cylinders before they were used for the main experiment.

Measurements of nociceptive thresholds

Rats were restrained in a cylindrical plastic holder with tail extending. Room temperature was maintained at 20 ± 1 °C. Nociceptive threshold was measured by the latency of the tail flick response (TFL) induced by radiant heat applied on the lower ⅓ of the tail. The average of the first 3 TF trials recorded 5

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min apart was taken as the baseline latency, usually within the range of 4–6 s. Subsequent TFL measurements were performed at 10-min intervals following subcutaneous or intracerebral administration. The values of TFL were expressed as the percentage changes from basal TFL with a cutoff limit of +150% to avoid tissue damage.

Microinjection procedures

A constant infusion pump was used to deliver 0.5 or 1.0 μl of solution at the speed of 0.125 μl/min through a stainless steel injection tube (0.3 mm o.d.), extending 2.0 mm beyond the tip of the guide cannula to reach PAG.

Location of site of injection

At the end of the experiment a stainless steel tubing of the same size as the injection tube was inserted into the guide cannula. The rat was decapitated and its head removed to be fixed in 10% formalin for 15 days. The site of injection was identified in serial frozen sections of 50 μ thickness. Fig. 1 shows the sites of the PAG injection in 3 coronal brain sections.

Data analysis

The data were expressed as mean ± S.E.M. and the significance of difference between two groups was assessed by analysis of variance (ANOVA) and Student's t-test. A level of $P < 0.05$ was accepted as an indication of significance.

Drugs

CCK-8 and unsulfated CCK-8 (CCK-us) were gifts from Squibb and Sons Inc. They were dissolved in artificial cerebrospinal fluid (CSF) for PAG injection at a dose range of 0.25–1.0 ng. CCK-us was used as a control for CCK-8. Proglumide was a gift from Rotta Research Laboratorium, Milano, Italy. It was used as sodium salt and dissolved in CSF (PH = 7.2). Morphine hydrochloride is a product of Shenyang Drug Company (China). It was dissolved in normal saline (1 mg/ml) for s.c. injection.

RESULTS

Antagonistic effect of CCK-8 on morphine analgesia in the PAG

Twenty rats were divided into 3 groups and given s.c. injection of 5 mg/kg of morphine. Twenty min after morphine injection, the rats were given intra-PAG injection of (1) 1 ng CCK-8 in 1 μl ($n = 6$), (2) 1 ng CCK-us in 1 μl ($n = 7$) or (3) 1 μl of CSF ($n = 7$), respectively. The percentage increases of TFL 10 min after the beginning of PAG injection were 130 ± 9% and 130 ± 4% in groups 2 and 3 respectively, whereas in group 1 the corresponding value was only

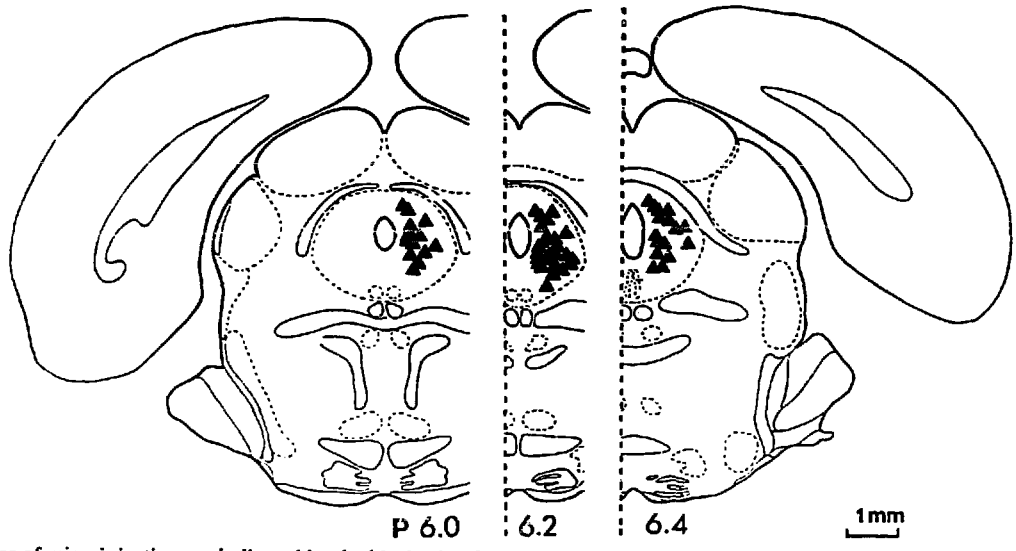

Fig. 1. Sites of microinjection are indicated by the black triangles plotted on diagrams of coronary sections of the medulla oblongata Data from rats with injection sites outside of PAG were not included in the present study.

8 ± 3%. The analgesic effect of morphine was almost completely abolished by CCK, but not by CCK-us. In another 2 groups of rats, the dose of CCK was reduced to 0.5 and 0.25 ng. Fig. 2 depicted the dose–response relationship on CCK-8 antagonism of morphine analgesia, with ID_{50} calculated to be 0.22 ng.

To assess whether CCK-8 itself affects TFL, 3 groups of 8 rats each were given intra-PAG injection of 1 ng of CCK-8, 1 ng of CCK-us, or 1 μl of CSF respectively 20 min after s.c. injection of 1 ml normal saline (NS). TFL was measured for a period of 70 min. The results are shown in Fig. 3. No significant difference in TFL was found between any two of the 3 groups.

Reversal of CCK-8 effect by proglumide

Three groups of rats were given one s.c. injection, followed by two intra-PAG injections (PAG1 and PAG2). PAG1 was an injection 20 min after s.c. injection. PAG2 was an injection 10 min after the beginning of PAG1 injection. In Group 1, half of the rats was given morphine (2 mg/kg, s.c.) + CCK-8 (0.5 ng in 0.5 μl, PAG1 inj) + PROG (0.2 μg in 0.5 μl, PAG2 inj), while the other half received 0.5 μl of CSF (PAG2 inj) instead of PROG. Five days later, similar experiments were performed in a counter-balanced manner. As shown in Fig. 4, at the time of the second PAG injection, the TFLs were similar for the proglumide group (3 ± 3%) and for the CSF control group (2 ± 3%). Ten min after proglumide administration,

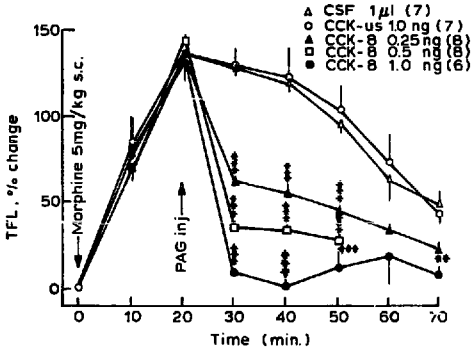

Fig. 2. Antagonism of morphine analgesia by CCK-8 administered to PAG of the rat. Mean percentage change ± S.E.M. (%) is plotted against time in min. Morphine was injected s.c. at time 0 and CCK-8 was injected at the time indicated by the black arrow. The drugs and doses are indicated in the upper right corner of each panel. Numerals in the parentheses are the numbers of animals used. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; as compared with the corresponding control groups.

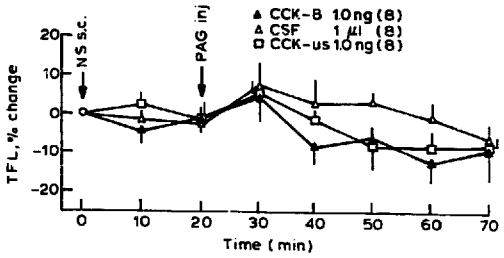

Fig. 3. Changes in pain threshold following s.c. injection of 1 ml NS and intra-PAG injection of CCK-8. The symbols are the same as in Fig. 2.

the TFL was increased to 48 ± 6%, while that of the control group was only 3 ± 3%. The difference was statistically significant ($P < 0.001$). This result indicates that proglumide, a CCK receptor blocker, was capable of reversing the antagonistic effect of CCK-8 on morphine analgesia (ANOVA, $P < 0.01$ for time-course recorded from 40–80 min). The rats in group 2 were given s.c. injection of morphine (2 mg/kg) and PAG1 injection of 0.5 μl CSF. Half of the rats received PAG2 injection of 0.2 μg proglumide in 0.5 μl, while the other half received 0.5 μl CSF as control. Five days later, the same experiment was repeated in a counter-balanced manner. The results are shown in Fig. 4. Morphine analgesia was significantly increased by proglumide administered to PAG (ANOVA, $P < 0.01$). Group 3 was given PAG2 injection of 0.2 μg proglumide 10 min after the PAG1 injection of 0.5 μl CSF, which followed the s.c. injection of 0.5 ml of NS. This group was set as a control to

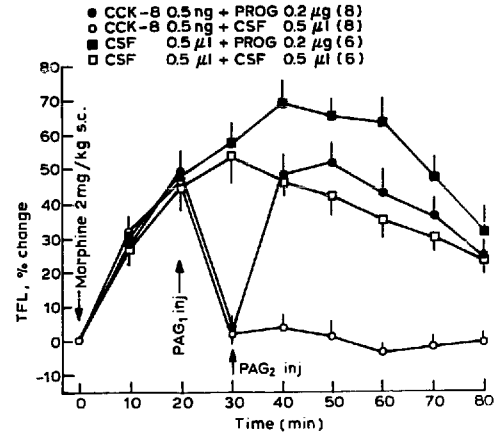

Fig. 4. The antagonistic effect of CCK-8 on morphine analgesia, and the reversal of the effect of CCK-8 by proglumide. The symbols are the same as in Fig. 2.

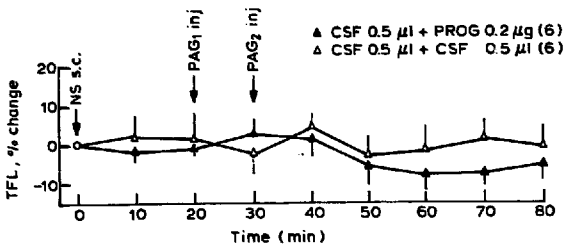

Fig. 5. Changes in pain threshold following intra-PAG injection of proglumide after s.c. injection of N.S. and PAG injection of CSF. The symbols are the same as in Fig. 2.

assess whether proglumide itself affects TFL after the multiple injections. As shown in Fig. 5, proglumide exerted neither analgesic nor hyperalgesic effect (ANOVA, $P > 0.05$).

Potentiation of morphine analgesia by proglumide administered to the PAG

Eight rats were given s.c. injection of 2 mg/kg of morphine, followed 20 min later by intra-PAG injection of 0.2 μg of proglumide in 1 μl ($n = 4$) or 1 μl of CSF ($n = 4$) as control. The same tests were repeated in a counter-balanced manner 5 days later. As shown in Fig. 6, the TFLs in the proglumide group were significantly higher than that in the CSF group, indicating an overall potentiation of morphine analgesia (ANOVA, $P < 0.01$).

Two groups of 8 rats were given intra-PAG injection of either 0.2 μg proglumide in 1 μl or 1 μl of CSF 20 min after s.c. injection of 0.5 ml of NS. As can be seen from Fig. 6, no significant difference was found between the two groups (ANOVA, $P > 0.05$).

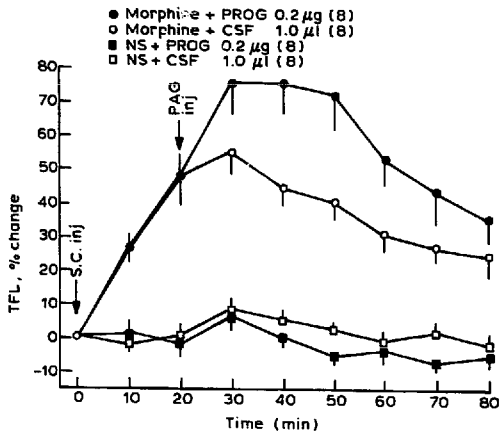

Fig. 6. Potentiation of morphine analgesia by proglumide administered to PAG. The symbols are the same as in Fig. 2.

DISCUSSION

We have reported that i.c.v. injection of CCK-8 in rats antagonizes the analgesia induced by s.c. injection of morphine (5 mg/kg), with an ID_{50} of 1.7ng[12]. In the present study CCK-8 was injected into PAG of the rat. A similar antagonistic effect on morphine analgesia was obtained, with an ID_{50} of only 0.22 ng (calculated from the data shown in Fig. 2). That the same extent of antagonism can be achieved with only 13% of the dose of CCK-8 needed for i.c.v. injection seems to emphasize PAG as a strategic site for CCK to antagonize morphine analgesia.

The aforementioned effect of CCK-8 is supposed to be mediated by CCK receptors, as was suggested by the fact that antagonism to morphine analgesia was totally reversed by proglumide, a CCK receptor blocking agent[10] injected into the same site of PAG. It should be mentioned that since proglumide per se was capable of potentiating morphine analgesia (Fig. 6), it seemed to be rational to suppose that the 'reversal' is simply a potentiation of morphine analgesia by proglumide. However, quantitative comparison revealed that intra-PAG injection of 0.2 μg of proglumide produced a more obvious increasing of TFL (45%) in rats injected with CCK-8 (Fig. 4) than in rats injected with CSF (30%, Fig. 6). In the former case (Fig. 4), proglumide antagonized not only the endogenously released CCK but also the CCK exogenously injected into PAG.

It is interesting to note that while injection of 0.2 μg of proglumide into PAG of the naive rat produced little influence in TFL (Fig. 5), injection of the same dose of proglumide produced a marked potentiation of the analgesia induced by morphine (Fig. 6). A plausible explanation would be that the effect of CCK-8 is not to increase the pain sensitivity by itself, but to antagonize the action of opioids, be it released endogenously (as in the case of stress) or injected exogenously. Since our rats had been habituated to the experimental condition for 5 days, the amount of opioid peptides released in the PAG during the main experiments might be very low. This would account for the fact that intra-PAG injection of CCK-8 produced no hyperalgesia (Fig. 3) and injection of proglumide produced no hypoalgesia (Fig. 5). Administration of morphine may accelerate the release of CCK-8 in the PAG area, forming a negative feed-

back control to the effect of morphine. In this case, proglumide would induce a potentiation of morphine analgesia by removing the feedback control exerted by CCK-8.

Results obtained in the present study are in line with those reported by Watkins' group[30,31] in that analgesia induced by intra-PAG injection of morphine was potentiated by proglumide injected into the same site. Our data provide an even stronger support to the viewpoint that PAG is one of the most important sites for CCK-8 to antagonize morphine analgesia.

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