Research report

Cholecystokinin octapeptide (CCK-8) antagonizes morphine analgesia in nucleus accumbens of the rat via the CCK-B receptor

Su-Fen Pu [a],*, Hui-Xin Zhuang [b], Ji-Sheng Han [a]

[a] Neuroscience Research Center and [b] Department of Cell Biology, Beijing Medical University, Beijing, 100083, People's Republic of China

Abstract

The analgesic effect of systemic morphine (4 mg/kg, s.c.) was antagonized in a dose-dependent manner by cholecystokinin octapeptide (CCK-8) (0.1–0.5 ng) administered bilaterally to the nucleus accumbens of the rat. This effect of CCK-8 could be reversed by devazepide, a CCK-A receptor antagonist, at 50 ng and 200 ng and by L-365,260, a CCK-B receptor antagonist, at 5 ng administered bilaterally to the nucleus accumbens. A marked potentiation of morphine analgesia was achieved by intra-nucleus accumbens injection of 200 ng devazepide or 5 ng L-365,260. Since the effect of L-365,260 in antagonizing the anti-opioid effect of CCK-8 in the nucleus accumbens is 40 times more potent than devazepide, it is suggested that the anti-opioid effect of CCK-8 is mediated by CCK-B receptors. In conclusion, nucleus accumbens is a strategic site where CCK-8 exerts an anti-opioid activity, most probably via the CCK-B receptors.

Key words: Morphine analgesia; CCK-8; Nucleus accumbens; CCK-A receptor; CCK-B receptor

1. Introduction

Previous reports from this [10,11,19,41,42] as well as several other laboratories [1,2,6,7,9] have shown that one important function of the cholecystokinin octapeptide (CCK-8) is to act selectively as an opiate antagonist in modulating pain and in regulating food intake. CCK-8 has been shown to be the most potent endogenous anti-opiate substance. In the studies of nociception, Faris et al. [9] first reported that CCK, as a specific opiate antagonist antagonizes morphine analgesia. Later, Han et al. [10,11] confirmed this finding and additionally found that CCK-8 could antagonize electroacupuncture (EA)-induced analgesia (mediated by EA-induced endogenous opiate release).

Additional studies have demonstrated that systemic administration of proglumid [26,32,34–36], a CCK antagonist, devazepide (formerly, L-364,718, MK-329) [5], a selective CCK-A receptor antagonist, or L-365,260 [8], a selective CCK-B receptor antagonist, enhances

morphine analgesia in rats, but does not affect the analgesia induced by serotonin or norepinephrine. This indicates that CCK-8 is not a universal antagonist for analgesia.

The development of potent, specific, non-peptide CCK receptor antagonists devazepide [4] and L-365,260 [21] greatly facilitated the research in this field. Dourish et al. have shown that morphine analgesia is significantly potentiated by L-365,260 [8], devazepide [5], or L-365,031 [8] (another selective CCK-A receptor antagonist). Since L-365,260 is 5–40 times more potent than devazepide or L-365,031 to produce the same effect, they concluded that this anti-opioid effect of CCK-8 is mediated by CCK-B receptors. Little information, however, is available concerning the site of action of CCK for its anti-opioid activity.

Using microinjections of opioid antibody, opiate receptor agonists, or antagonists, our previous studies [39,40] have shown that nucleus accumbens is an important site for induction of morphine analgesia. In the present study, specific CCK-A and CCK-B receptor antagonists, devazepide and L-365,260, are microinjected bilaterally to nucleus accumbens to test if nucleus accumbens is an important site where CCK-8 exerts an anti-opiate activity.

* Corresponding author. Present address: Department of Physiology, Colorado State University, Fort Collins, CO 80523, USA. Fax: (1) (303) 491-7569.

2. Materials and methods

2.1. Surgical procedures

Male Wistar rats weighing 300-350 g were anesthetized with chlorohydrate (0.4 g/kg, i.p.) and mounted on a stereotaxic instrument (Narishige Inc. Japan). Stainless steel guide cannulae of 0.7 mm outer diameter (o.d.) were implanted on both sides of nucleus accumbens at coordination of A 2.0 mm, L 1.5 mm, and H 5.0 mm according to the atlas of Paxinos and Watson [27], and fixed in situ with dental acrylic. Seven days were allowed for surgical recovery.

2.2. Measurements of nociceptive thresholds

Rats were restrained in a cylindrical plastic holder with tail extending. Room temperature was maintained at $20 \pm 1°C$. Nociceptive threshold was measured by the latency of the tail flick (TFL) response induced by radiant heat applied on the lower 1/3 of the tail [28]. The average of the first 3 TFL trials recorded 5 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 intra-nucleus accumbens administration. The values of TFL were expressed as percentage changes from basal TFL with a cutoff limit of + 150% to avoid tissue damage.

2.3. Microinjection procedures

A constant infusion pump (Palmer company, England) was used to deliver 0.5 (for double microinjections) or 1.0 μl (for single microinjection) 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 nucleus accumbens. All intra-nucleus accumbens injections were done bilaterally.

2.4. 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 cannulae. The rats were decapitated and the heads were removed to be fixed in 10% formalin for 15 days. The sites of injection were identified in serial 50 μm frozen sections. Fig. 1 shows the sites of the nucleus accumbens injection in 3 coronal brain sections.

2.5. Data analysis

The data were expressed as mean ± S.E.M. Statistic significance was determined by analysis of variance (ANOVA) and Newman-Keul's post comparison test. A level of $P < 0.05$ was considered statistically significant.

2.6. Drugs

CCK-8 was a gift from Squibb and Sons Inc. (UK). Devazepide (3S(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1-H-1,4-benzodiazepin-3-yl)-H-indole-2-carboxamide) and L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)N'-(3-methyl-phenyl)urea) were donated by Dr. R.F. Freidinger of the Merck, Sharp and Dohme Research Laboratories (USA). Devazepide and L-365,260 were first dissolved in dimethylsulfoxide (DMSO) and then diluted with propylene glycol to a final ratio of 20% DMSO:80% propylene glycol (v/v) and then diluted with normal saline to the final concentration. Morphine hydrochloride was purchased from Qinghai Drug Company (China).

3. Results

3.1. Antagonistic effect of CCK-8 on morphine analgesia in the nucleus accumbens

Thirty rats were divided into 4 groups and given s.c. injection of 4 mg/kg of morphine. Twenty minutes later, the rats were given bilaterally an intra-nucleus accumbens injection of 0.1 ng ($n = 8$), 0.5 ng ($n = 8$), and 1.0 ng ($n = 7$) of CCK-8 or 1 μl of normal saline ($n = 7$), respectively. The results show that CCK-8 (0.1-1.0 ng) dose-dependently antagonized the analgesic effect of morphine (Fig. 2).

To assess whether CCK-8 itself affects TFL, two groups of 17 rats were given an intra-nucleus accumbens injection of 0.5 ng of CCK-8 ($n = 9$), and 1 μl of normal saline ($n = 8$), respectively after 20 min s.c. injection of normal saline. TFL was measured for a period of 70 min. No significant difference in TFL was found between the two groups (Fig. 3).

3.2. Reversal of CCK-8 anti-analgesic effect by devazepide and L-365,260

Four groups of rats were given one s.c. injection of morphine (3 mg/kg) followed by two intra-nucleus accumbens injections (acb1 and acb2). acb1 was an intra-nucleus accumbens injection of CCK-8 (0.5 ng in 0.5 μl) 20 min after s.c. injection. acb2 was an intra-
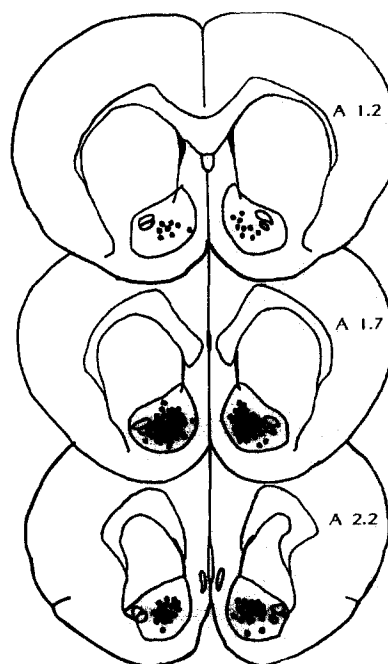

Fig. 1. Distribution of injections in nucleus accumbens. The sites of microinjection are indicated by the black dots on diagrams of coronary sections of nucleus accumbens (A: 1.2-2.2). Data from rats with injection sites outside of nucleus accumbens were not included in the present study.
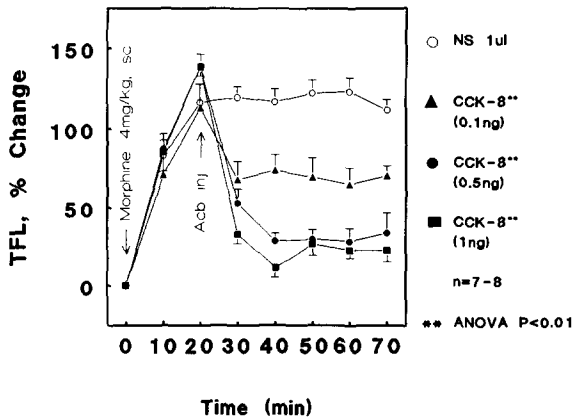
Fig. 2. Antagonism of morphine analgesia by CCK-8 administered to nucleus accumbens of the rat. The data shown (percentage of the means ± S.E.M.) were recorded during 70 min. Morphine (4 mg/kg) was injected s.c. at time 0 and CCK-8 was injected into nucleus accumbens at time 20 (Acb Inj). The drugs and doses are indicated on the right side of the figure. ** $P < 0.01$ as compared with the corresponding control group.
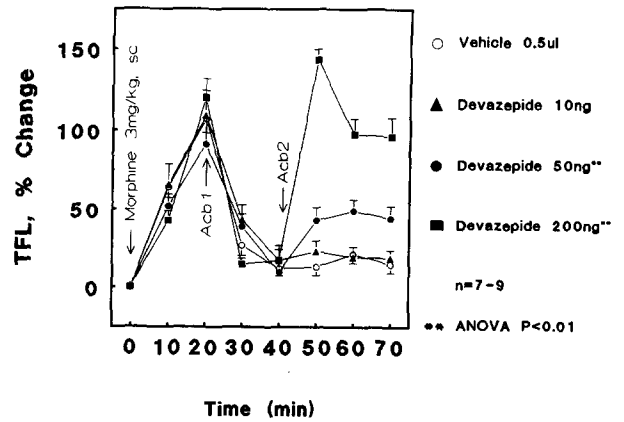

Fig. 4. The reversal of the anti-analgesic effect of CCK-8 by devazepide. The data shown (percentage of the means ± S.E.M.) were recorded during 70 min period. Morphine (3 mg/kg) was injected s.c. at time 0, 0.5 ng of CCK-8 was injected intra-nucleus accumbens at time 20 (Acb1), and 0.5 μl of vehicle ($n = 7$), 10 ng ($n = 9$), 50 ng ($n = 8$), or 200 ng ($n = 8$) of devazepide (Acb2) was injected intra-nucleus accumbens at time 40. The drugs and doses are indicated on the right side of the figure. ** $P < 0.01$ as compared with the corresponding control group.

nucleus accumbens injection of 10 ng ($n = 9$), 50 ng ($n = 8$), 200 ng ($n = 8$) of devazepide or 0.5 μl of vehicle ($n = 7$) 20 min after the beginning of acb1 injection. As shown in Fig. 4, although 10 ng devazepide did not affect the anti-analgesic effect of CCK ($P > 0.05$), 50 ng (45 + 8%) and 200 ng (112 + 6%) of devazepide dose-dependently reversed the anti-analgesic effect of CCK-8 ($P < 0.01$).

To compare the effect of L-365,260 in reversing the effect of CCK-8 to devazepide, the same experimental procedure was carried out where devazepide was replaced by L-365,260. As in Fig. 5, only 5 ng of L-365,260 could produce the same effect of blocking anti-analgesic effect of CCK-8 as it does by 200 ng of devazepide. That means the effect of L-365,260 in antagonizing the anti-opioid effect of CCK-8 in the nucleus accumbens is 40 times more potent than devazepide.
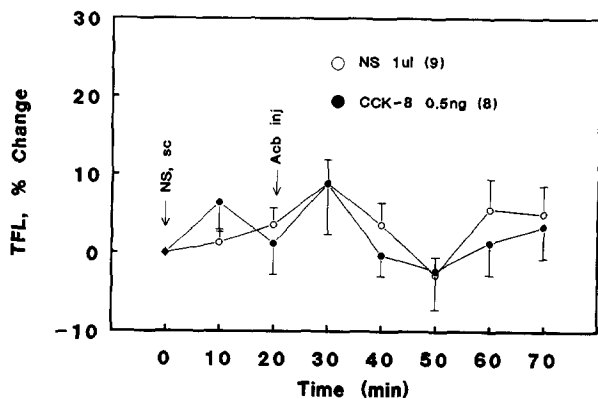
3.3. Potentiation of morphine analgesia by devazepide or L-365,260 administered to the nucleus accumbens

Eight rats were given s.c. injection of 3 mg/kg of morphine. Ten minutes later, the TFL increased 70%. Four rats were then injected bilaterally to nucleus accumbens with vehicle (1 μl), another 4 rats were injected with 200 ng of devazepide. The same tests were repeated in a counter-balanced manner 5 days later. As shown in Fig. 6, the TFLs in the devazepide treatment group were significantly higher than that in the vehicle treatment group, indicating an overall potentiation of morphine analgesia ($P < 0.01$).
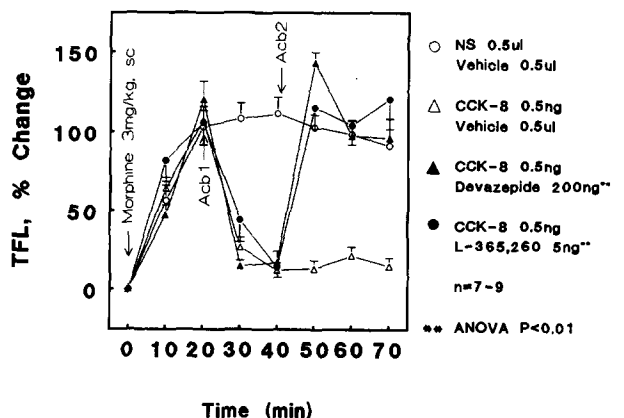

Fig. 3. Changes in pain threshold following s.c. injection of 0.5 ml of normal saline and intra-nucleus accumbens injection of 1 μl of normal saline or 0.5 ng of CCK-8 (Acb Inj). The data shown (percentage ± S.E.M.) were recorded during 70 min period.

Fig. 5. The reversal of the anti-analgesic effect of CCK-8 by L-365,260. The data shown (percentage of the means ± S.E.M.) were recorded during 70 min period. Morphine (3 mg/kg) was injected s.c. at time 0, 0.5 ng of CCK-8 was injected intra-nucleus accumbens at time 20 (Acb1), and 0.5 μl of vehicle or 5 ng of L-365,260 was injected intra-nucleus accumbens at time 40 (Acb2). The symbols are the same as in Fig. 4.
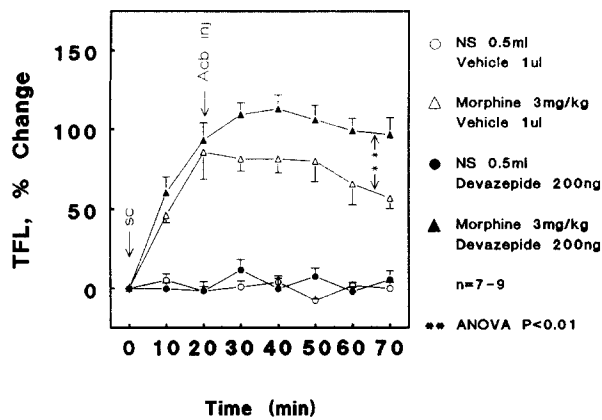
Fig. 6. Potentiation of morphine analgesia by devazepide administered to nucleus accumbens and the effect of devazepide on the pain threshold. The data shown (percentage of the mean ± S.E.M.) were recorded during 70 min period. Morphine (3 mg/kg) or normal saline (0.5 ml) was injected s.c. at time 0, 200 ng of devazepide or 1 μl of vehicle was injected intra-nucleus accumbens at time 20 (Acb Inj). The symbols are the same as in Fig. 4.

In a control experiment, two groups of 8 rats were given intra-nucleus accumbens injection of either 200 ng of devazepide or 1 μl of vehicle after s.c. injection of normal saline. As can be seen from Fig. 6, no significant difference was found between the two groups ($P > 0.05$).

The above experiments were repeated with L-365,260 (5 ng) instead of devazepide (200 ng). 5 ng of L-365,260 significantly potentiated the analgesic effect of morphine, but L-365,260 alone could not change the TFL threshold (Fig. 7).

These experiment indicated that 5 ng of L-365,260 and 200 ng of devazepide significantly potentiated the analgesic effect of morphine and that L-365,260 or
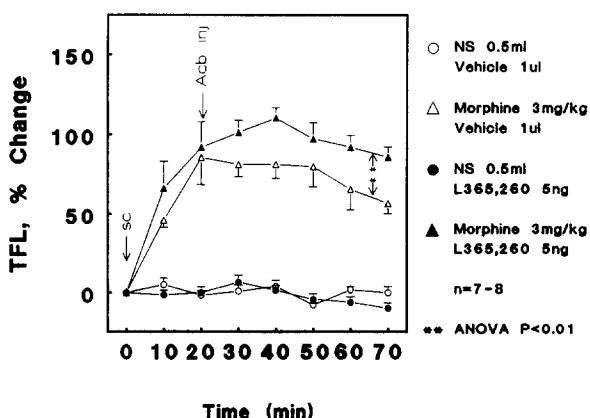

Fig. 7. Potentiation of morphine analgesia by L-365,260 administered to nucleus accumbens and the effect of L-365,260 on the pain threshold. The data shown (percentage of the means ± S.E.M.) were recorded during 70 min period. Morphine (3 mg/kg) or 0.5 ml of normal saline was injected s.c. at time 0 and 5 ng of L-365,260 or 1 μl of vehicle was injected intra-nucleus accumbens at time 20 (Acb Inj). The symbols are the same as in Fig. 4.

devazepide administered alone did not affect the basic TFL threshold.

4. Discussion

The present study demonstrated that (a) administration of CCK-8 into nucleus accumbens antagonized the analgesic effect of systemic morphine; (b) administration of CCK-A receptor antagonist, devazepide, or CCK-B receptor antagonist, L-365,260, reversed the anti-analgesic effect of CCK-8, and potentiated morphine analgesia. Together, these results strongly suggested that the nucleus accumbens is a strategic site where CCK-8 exerts an anti-opioid activity. Since the effect of L-365,260 in antagonizing the anti-opioid effect of CCK-8 in nucleus accumbens is 40 times more potent than devazepide, it is suggested that the anti-opioid effect of CCK-8 in nucleus accumbens is probably mediated by CCK-B receptors.

Previous studies have shown that the release of CCK from spinal cord was increased after intrathecal injection of morphine [32]. Recently, Zhou et al. [42] reported that systemic morphine produced a marked (89%) increase of CCK immunoreactivity in the perfusate of the rat spinal cord. A marked increase in preproCCK mRNA was found in the brain of rats receiving chronic morphine treatment by using dot blot hybridization [41]. Several other studies have demonstrated that systemic administration of CCK antagonist, proglumide [26,32,35,36], devazepide [5], or L-365,260 [6,8], potentiate morphine analgesia and attenuate the development of tolerance to morphine analgesia. In the present study, we found that there was no effect on the pain threshold by administration of CCK alone. However, there was a marked anti-opioid effect when CCK-8 was administered bilaterally into nucleus accumbens after systemic administration of morphine. The administration of devazepide or L-365,260 bilaterally into nucleus accumbens had a marked potentiation effect on morphine analgesia. The results of these pharmacological studies in pain modulation indicate that CCK-8 functions physiologically as an opiate antagonist, but not an opiate agonist.

Considerable anatomical experiments have shown that the distribution of CCK protein and CCK binding sites parallels that of endogenous opiate and opiate receptors in the nucleus accumbens [3,17,18,24,25,30, 31,33,38]. Chronic morphine administration leads to an increase in CCK immunoreactivity in rat nucleus accumbens (Pu, Zhou and Han, to be published). A 75.7% increase in acute morphine tolerance, 86.3% increase on the third day and 128.9% on the sixth day of chronic morphine tolerance were observed. These findings support our hypothesis that nucleus accumbens is a strategic site for opiate/CCK interaction.

Several lines of evidence raised the possibility that nucleus accumbens, amygdala, and periaqueductal gray (PAG) may form a complex network with 'all or none' like characteristics to modulate the analgesic action of morphine. This may explain why (1) microinjection of an opioid into one of these nuclei could produce an increase in TFL almost equal to the effect of systemic morphine [29,37,39,40], (2) microinjection of the opioid antagonist naloxone into one of these nuclei could block the analgesic effect of systemic morphine [39,40], (3) no analgesic effect was observed if the microinjections of morphine were made outside of these nuclei [23], and (4) intracerebral microinjection of morphine to one of these nuclei could cause the release of opioid peptides in one nucleus after another. For example, microinjection of morphine into nucleus accumbens increases the content of immunoreactive enkephalin and β-endorphin in the perfusate of PAG and amygdala [22,23]. Additionally, fluorescent retrograde labeling studies have shown that some ascending fibers from PAG/dorsal raphe nucleus project to the forebrain regions, including nucleus accumbens and amygdala. It has also been suggested that morphine administration to the PAG activates ascending serotoninergic pathway from PAG and releases 5-HT in the nucleus accumbens, which in turn activates an enkephalinergic mechanism within the nucleus accumbens [20]. Although the exact centrifugal pain modulating circuitry hasn't been elucidated, it is obvious that nucleus accumbens, amygdala, and PAG are greatly involved in pain modulation within the central nervous system. It is also possible that the anti-analgesic effect of CCK-8 induced by its microinjection into these nuclei may occur by activating the same centrifugal pain modulating circuitry.

Two CCK receptors have been elucidated, CCK-A and CCK-B. Although both CCK receptors were found in the rodent central nerve system (CNS), the distribution of CCK-A receptors was limited to the regions of the mid- and hindbrain and CCK-B receptors predominated in the CNS [12–16]. Recently, the development of the potent, specific, and non-peptide CCK receptor antagonists greatly facilitated the studies of opiate/CCK interaction. Receptor binding studies [12] have shown that devazepide has high affinity to CCK-A receptor ($IC_{50} = 0.92$ nmol/l), but can bind to CCK-B receptor at high dose ($IC_{50} = 120$ nmol/l); on the other hand, L-365,260 has high affinity to CCK-B receptors ($IC_{50} = 15$ nmol/l), but it can bind to CCK-A receptor at high concentration ($IC_{50} = 2800$ nmol/l). Therefore L-365,260 has at least 8 times higher affinity to CCK-B receptor than devazepide. Dourish et al. [8] have shown that L-365,260 was 40 times more potent than L-365,031 (a selective CCK-A receptor antagonist) and 5 times more potent than devazepide in enhancing morphine analgesia in the rat tail flick test. L-365,260 was 20 times more potent than devazepide

in enhancing morphine analgesia in the rat paw pressure test (another method to test pain threshold). The result from the present study that L-365,260 was 40 times more potent in reversal of anti-opioid effect of CCK-8 and enhancing morphine analgesia in the nucleus accumbens, was in good agreement with the above studies. Using ^{125}I-Bolton-Hunter-CCK-8, devazepide, and L-365,260 and competitive receptor autoradiographic study, we found that CCK-B receptor is enriched in nucleus accumbens.

In conclusion, although the precise mechanisms by which CCK-8 and CCK antagonists influence opioid analgesia or opiate/CCK interaction, are unknown, the present study suggest that nucleus accumbens is a strategic site where CCK-8 exerts an anti-opioid activity, and this activity is mediated, most probably, via the CCK-B receptors.

Acknowledgements

References

[1] Baber, N.S., Dourish, C.T. and Hill, D.R., The role of CCK, Caerulein and CCK antagonist in nociception, Pain, 39 (1989) 307–328.

[2] Barbaz, B.S., Autry, W.L., Ambrose, F.G., Hall, N.R. and Liebman, J.M., Antinociceptive profile of sulfate CCK: comparison with CCK-4, unsulfate CCK and other neuropeptides, Neuropharmacology, 25 (1986) 823–829.

[3] Beinfeld, M.C., Cholecystokinin in the central nervous system: a minireview, Neuropeptides, 3 (1983) 411–427.

[4] Chang, R.S.L. and Lotti, V.J., Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist, Proc. Natl. Acad. Sci. USA, 83 (1986) 4923–4926.

[5] Dourish, C.T., Hawley, D. and Iversen, S.D., Enhancement of morphine analgesia and prevention of morphine tolerance in the rat by the cholecystokinin antagonist L-364,718, Eur. J. Pharmacol., 147 (1988) 469–472.

[6] Dourish, C.T., Hawley, D., O'Neill, M.F. and Iversen, S.D., Enhancement of morphine analgesia and prevention of morphine tolerance by the selective CCK-B antagonist L-365,260, Br. J. Pharmacol., Suppl. 98 (1989) 631.

[7] Dourish, C.T., Rycroft, W. and Iversen, S.D., Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors, Science, 245 (1989) 1509–1511.

[8] Dourish, C.T., O'Neill, M.F., Coughlan, J., Kitchener, S.J., Hawley, D. and Iversen, S.D., The selective CCK-B receptor antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat, Eur. J. Pharmacol., 176 (1990) 35-44.

[9] Faris, P.L., Komisaruk, B.R., Watkins, L.R. and Mayer, D.J., Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia, Science, 219 (1983) 310-312.

[10] Han, J.S., Ding, X.Z. and Fan, S.G., Is cholecystokinin octapeptide (CCK-8) a candidate for endogenous anti-opioid substrates?, Neuropeptides, 5 (1985) 399-402.

[11] Han, J.S., Ding, X.Z. and Fan, S.G., Cholecystokinin octapeptide (CCK-8): antagonism to electroacupuncture analgesia and a possible role in electroacupuncture tolerance, Pain, 27 (1986) 101-115.

[12] Hill, D.R. and Woodruff, G.N., Differentiation of central CCK receptor binding sites using the nonpeptide antagonists MK-329 and L-365,260, Br. J. Pharmacol., Suppl. 98 (1989) 629.

[13] Hill, D.R., Campbell, N.J., Shaw, T.M. and Woodruff, G.N., Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonist, J. Neurosci., 7 (1987) 2967-2976.

[14] Hill, D.R., Shaw, T.M. and Woodruff, G.N., Species differences in the localization of peripheral type cholecystokinin receptors in rodent brain, Neurosci. Lett., 79 (1987) 286-289.

[15] Hill, D.R., Shaw, T.M., Dourish, C.T. and Woodruff, G.N., CCK-A receptors in the rat interpeduncular nucleus: evidence for a presynaptic localization, Brain Res., 454 (1988) 101-105.

[16] Hill, D.R., Shaw, T.M. and Woodruff, G.N., Binding sites for ^{125}I-cholecystokinin in primate spinal cord are of the CCK-A subclass, Neurosci. Lett., 89 (1988) 133-139.

[17] Innis, R.B., Correa, F.M.A., Uhl, G.R., Schreider, B. and Snyder, S.H., Cholecystokinin octapeptide immunoreactivity: histochemical localization in rat brain, Proc. Natl. Acad. Sci. USA, 76 (1979) 521-535.

[18] Innis, R.B. and Snyder, S.H., Distinct cholecystokinin receptors in brain and pancreas, Proc. Natl. Acad. Sci. USA, 77 (1980) 6917-6921.

[19] Li, Y. and Han, J.S., Cholecystokinin-octapeptide antagonizes morphine analgesia in periaqueductal grey (PAG) of the rat, Brain Res., 480 (1989) 105-110.

[20] Li, Y.Q., Takada, M., Matsuzaki, S., Shinonaga, Y. and Mizuno, N., Identification of periaqueductal gray and dorsal raphe nucleus neurons projecting to both the trigeminal sensory complex and forebrain structures: a fluorescent retrograde double-labeling study in the rat, Brain Res., 623 (1993) 267-277.

[21] Lotti, V.J. and Chang, R.S.L., A new potent and selective nonpeptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260, Eur. J. Pharmacol., 162 (1989) 273-280.

[22] Ma, Q.P. and Han, J.S., Noloxone blocks opioid peptide release in periaqueductal gray and amygdala elicited by morphine injected into N. accumbens, Peptides, 13 (1992) 261-265.

[23] Ma, Q.P., Shi, Y.S. and Han, J.S., Further studies on interactions between periaqueductal gray, nucleus accumbens and habenula in antinociception, Brain Res., 583 (1992) 292-295.

[24] Mansour, A., Khachaturian, H., Lewis, M.E., Aki, H. and Watson, S.J., Anatomy of CNS opioid receptors, Trends Neurosci., 11 (1988) 308-314.

[25] Niehoff, D.L., Quantitative autoradiographic localization of cholecystokinin receptors in rat and guinea pig brain using ^{125}I-Bolton-Hunter-CCK-8, Peptides, 10 (1989) 265-274.

[26] Panerai, A.E., Rovati, L.C., Cocco, E., Sacerdote, P. and Mantegazza, P., Dissociation of tolerance and dependence to morphine: a possible role for cholecystokinin, Brain Res., 410 (1987) 52-60.

[27] Paxinos, G. and Watson, C., The Rat Brain in Stereotaxic Coordinates, 2nd edn., Acadmic Press, New York, 1982.

[28] Ren, M.F. and Han, J.S., An improved tail-flick test and its application in the study of acupuncture analgesia, Acta Physiol. Sinica, 30 (1978) 204-208.

[29] Rogers, R.T., Influence of intra-amygdaloid opiate injection on shock thresholds, tail-flick latencies and open field behavior in rats, Brain Res., 153 (1978) 211-216.

[30] Satio, A., Sankaran, H., Goldfine, I.D. and Williams, J.A., Cholecystokinin receptors in the brain: characterization and distribution, Science, 208 (1980) 1155-1156.

[31] Sharif, N.A. and Hughes, J., Discrete mapping of brain mu and delta opioid receptors using selective peptides: quantitative autoradiography, species differences and comparison with kappa receptors, Peptides, 10 (1989) 499-522.

[32] Tang, J., Chou, J., Iadarola, M., Yang, H.Y.T. and Costa, E., Proglumide prevents and curtail acute tolerance to morphine in rats, Neuropharmacology, 23 (1984) 715-718.

[33] Vanderhaeghen, J.J., Neuronal cholecystokinin. In A. Bjorklund and T. Hokfelt (Eds.), Handbook of Chemical Neuroanatomy, Vol. 4, GABA and Neuropeptides in the CNS, Elsevier, Amsterdam, 1985, pp. 406-435.

[34] Watkins, L.R., Kinscheck, I.B. and Mayer, D.J., Potentiation of morphine analgesia by the CCK antagonist proglumide, Brain Res., 327 (1985) 169-180.

[35] Watkins, L.R., Kinscheck, I.B. and Mayer, D.J., Potentiation of opiate analgesia and apparent reversal of morphine tolerance by proglumide, a cholecystokinin antagonist, Science, 224 (1984) 395-396.

[36] Watkins, L.R., Kinscheck, I.B., Rosenquist, G., Miller, J., Wimberg, S., Frenk, H., Kaufman, E., Coghill, R., Suberg, S.N. and Mayer, D.J., The enhancement of opiate analgesia and the possible reversal of morphine tolerance by proglumide, Ann. N.Y. Acad. Sci., 448 (1985) 676-677.

[37] Yaksh, T.L. and Rudy, T.A., Narcotic analgesia: CNS sites and mechanisms of action as revealed by intracerebral injection techniques, Pain, 4 (1978) 299-360.

[38] Zabin, M.A., Innis, R.B., Wamsley, J.K., Snyder, S.H. and Kuhar, M.J., Autoradiographic localization of cholecystokinin receptor in rodent brain, J. Neurosci., 3 (1983) 877-906.

[39] Zhou, Z.F., Ding, X.Z., Jiang, Y. and Han, J.S., Effect of intracerebral microinjection of naloxone on acupuncture and morphine analgesia in the rabbit, Scientia Sinica, 24 (1981) 1166-1178.

[40] Zhou, Z.F., Xuan, Y.T. and Han, J.S., Analgesic effect of morphine injected into habenula, nucleus accumbens and amygdala of rabbits, Acta Pharmacol. Sin., 5 (1984) 150-153.

[41] Zhou, Y., Sun, Y.H., Zhang, Z.W. and Han, J.S., Accelerated expression of cholecystokinin gene in the brain of rats rendered tolerant to morphine, NeuroReport, 3 (1992) 1121-1123.

[42] Zhou, Y., Sun, Y.H., Zhang, Z.W. and Han, J.S., Increased release of immunoreactive cholecystokinin octapeptide by morphine and potentiation of μ-opioid analgesia by CCK$_B$ receptor antagonist L-365,260 in rat spinal cord, Eur. J. Pharmacol., 234 (1993) 147-154.