Brain Research, 523 (1990) 5–10
Elsevier

BRES 15696

Cholecystokinin octapeptide antagonized opioid analgesia mediated by μ- and κ- but not δ-receptors in the spinal cord of the rat

Xiao-Jing Wang, Xiao-Hong Wang and Ji-Sheng Han

Department of Physiology, Beijing Medical University, Beijing (People's Republic of China)

(Accepted 23 January 1990)

Key words: Cholecystokinin octapeptide; (*N*-MePhe^3,D-Pro^4) Morphiceptin; (*N*-MeTyr^1,*N*-MeArg^7,D-Leu^8) Dynorphin(1–8) ethylamide; (D-Pen^{2,5}) Enkephalin; Proglumide; Intrathecal injection; Opioid analgesia; Antiopioid effect

Intrathecal (ith) injection of cholecystokinin octapeptide (CCK-8) to the rat with single dose of 4 or 40 ng, or successive doses from 0.1 to 1 μg at 10 min intervals produced neither analgesia nor hyperalgesia. However, the analgesia produced by ith injection of PL017, a specific μ-receptor agonist or 66A-078, a specific κ-receptor agonist could be markedly antagonized by CCK-8 at a dose as small as 4 ng. In contrast, analgesia produced by ith injection of δ-agonist DPDPE could not be blocked by CCK-8 even at a dose as high as 40 ng. Since the effect of CCK-8 could be totally reversed by the CCK receptor antagonist proglumide, this effect is most probably mediated by CCK receptors.

INTRODUCTION

Cholecystokinin octapeptide (CCK-8) has been known as a neuropeptide of abundant and wide distribution in CNS[1] with some important physiological functions including the anti-opioid effect[4,9]. Previous studies performed in our laboratory revealed that centrally administered CCK-8 antagonized the analgesic effect produced by parenterally administered morphine or the endogenously released opioids during the period of electroacupuncture (EA)[2,5] and that a profound release of CCK-8 might play an important role in the development of tolerance toward morphine or EA analgesia[5,7]. In contrast to these reports, some authors showed that CCK-8 was capable of producing naloxone-reversible analgesia when higher doses were used[8,24]. The aim of this study was to clarify (1) whether ith injection of a higher dose of CCK-8 would produce analgesia, (2) which of the 3 types of opioid receptors was most susceptible to CCK-8 antagonism. For the latter purpose we have adopted (*N*-MePhe^3,D-Pro^4)morphiceptin or PL017 as specific μ-agonist[3], (*N*-MeTyr^1,*N*-MeArg^7,D-Leu^8)dynorphin(1–8) ethylamide or 66A-078 as specific κ-agonist[16] and (D-Pen^{2,5})enkephalin or DPDPE as specific δ-agonist[14], to look into their interaction with CCK-8 at spinal level for pain control.

MATERIALS AND METHODS

Surgical procedures and intrathecal injection of drugs

Male Wistar rats weighing 200–250 g were anesthetized with chlorohydrate (0.4 g/kg, i.p.). PE-10 polyethlene catheter of 7.5 cm long was implanted through the atlanto-occipital membrane down to the lumbar enlargement of the spinal cord following Yaksh and Rudy[23]. The outer part of the catheter was plugged and fixed onto the skin. Experiment with ith injection started about 20–26 h after operation. Drugs were dissolved in normal saline (NS) and injected via the ith catheter at a volume of 10 μl, followed by 10 μl of NS for flushing. Injection was complete within 30 s.

Nociceptive test[13]

The rat was restrained in a special plastic holder with tail and hind legs protruding. Nociceptive threshold was measured by the latency of the tail flick response (TFL) elicited by radiant heat applied on lower 1/3 of the tail. The mean TFL of the three measurements assessed at the beginning of experiment with 5 min apart was taken as the basal threshold. The TFL values taken after drug administration at 10 min intervals were expressed as the percentage changes from basal TFL, with a cutoff limit of +150% to avoid unnecessary skin damage.

Data analysis

Data were expressed as mean ± S.E.M. Average effect of analgesia in each rat shown in dose-response curves was calculated from the mean % change in TFLs of the 5 time points after CCK-8 administration. The significance of difference between two groups was assessed by analysis of variance (ANOVA) followed by *Q*-test.

Drugs

CCK-8 was a gift of Squibb and Sons Inc., PL017 was purchased from Peninsula, and DPDPE from Sigma Co., U.S.A. 66A-078 was donated by Dr. Tachibana, Tsukuba Res. Lab. of the Eisai Co., Japan. Proglumide was a gift of Rotta Res. Lab., Italy.

Correspondence: J.S. Han, Department of Physiology, Beijing Medical University, Beijing 100083, People's Republic of China.

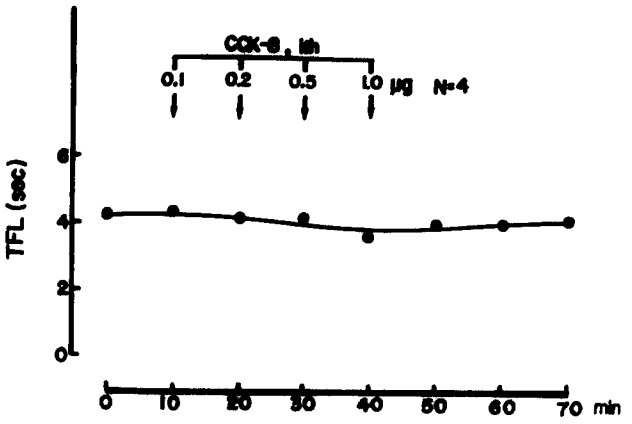

Fig. 1. Changes in pain threshold following successive intrathecal injection of CCK-8. Mean TFL (s) ± S.E.M. is plotted against time in min. S.E.M. are within the dot. CCK-8 was injected in 10 min intervals, the doses were shown above the arrows.

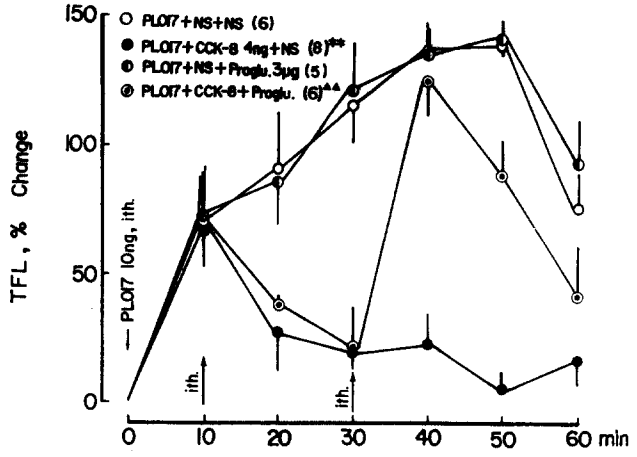

Fig. 2. The antagonistic effect of CCK-8 on PL017 (10 ng)-induced analgesia, and the reversal of the effect of CCK-8 by proglumide. Mean percentage change of TFL ± S.E.M. (%) is plotted against time in minutes. PL017 was injected intrathecally at time 0 and CCK-8 or normal saline (NS) was injected at 10 min as indicated by the arrow directed upward. Numerals in the parentheses indicate the number of animals in each group. Proglumide (proglu.) was injected intrathecally at 30 min as indicated by the second upward arrow. $^{**}P < 0.01$, as compared with the control (PL017 + NS + NS). $^{▲▲}P < 0.01$, as compared with the group of PL017 + CCK-8 + NS.

RESULTS

The effect of CCK-8 and proglumide on nociceptive threshold

Rats were divided into 4 groups of 4–6 each and given

TABLE I

The effect of intrathecal injection of CCK-8 and proglumide on the pain threshold

Figures in the table are mean ± S.E.M. Drugs were injected at time 0. n = number of animals in each group. No significant differences were found ($P > 0.05$, ANOVA) among the 4 groups.

Groups	*n*	*Basal threshold (s)*	*TFL (% change)*					
			10′	*20′*	*30′*	*40′*	*50′*	*60′*
NaCl	4	4.2 ± 0.2	−1.5 ± 2.0	2.9 ± 2.7	−2.5 ± 1.0	−6.8 ± 5.0	−1.3 ± 0.8	−0.2 ± 1.6
CCK-8 (4 ng)	6	4.7 ± 0.2	5.9 ± 3.0	−2.7 ± 5.5	−3.6 ± 6.5	5.1 ± 6.8	11 ± 5.6	16 ± 8.3
CCK-8 (40 ng)	6	4.4 ± 0.3	12 ± 5.1	6.8 ± 7.0	3.3 ± 4.4	8.1 ± 9.9	8.8 ± 5.2	8.7 ± 11
Proglumide (3 μg)	5	3.9 ± 0.2	−1.6 ± 15	−11 ± 10	−12 ± 6.4	−10 ± 3.0	−6.4 ± 5.7	0.5 ± 9.4

TABLE II

The antagonistic effect of CCK-8 on PL017-induced analgesia

Figures in the table are mean ± S.E.M. Intrathecal injection of P1017 was given at time 0 followed 10 min later by ith injection of CCK-8 4 ng or normal saline (NS) 10 μl. n = number of animals in each subgroup.

PL017 (ng)	*Subgroup*	*n*	*Basal TFL (s)*	*TFL (% change)*					
				10′	*20′*	*30′*	*40′*	*50′*	*60′*
2.5	NS	6	4.3 ± 0.5	44 ± 7.3	42 ± 21	42 ± 17	47 ± 8.2	40 ± 20	43 ± 17
	CCK-8	7	4.3 ± 0.5	42 ± 13	11 ± 5.9**	13 ± 6.0**	−2.4 ± 8.0**	7 ± 9.4**	−1.5 ± 8.9*
5	NS	7	4.4 ± 0.2	63 ± 17	66 ± 20	81 ± 19	31 ± 8.6	15 ± 7.0	18 ± 4.7
	CCK-8	7	4.6 ± 0.5	58 ± 17	13 ± 7.2**	0.76 ± 8.3**	3.4 ± 8.5**	8.0 ± 9.4	0.3 ± 7.8**
10	NS	6	4.3 ± 0.2	70 ± 19	91 ± 24	116 ± 16	136 ± 13	141 ± 5.4	76 ± 16
	CCK-8	8	4.5 ± 0.5	66 ± 19	26 ± 15**	20 ± 11**	23 ± 13**	5.3 ± 7.1**	17 ± 8.6**

$^{*}P < 0.05$, $^{**}P < 0.01$, compared with the corresponding data in NS control group using ANOVA followed by Q-test.

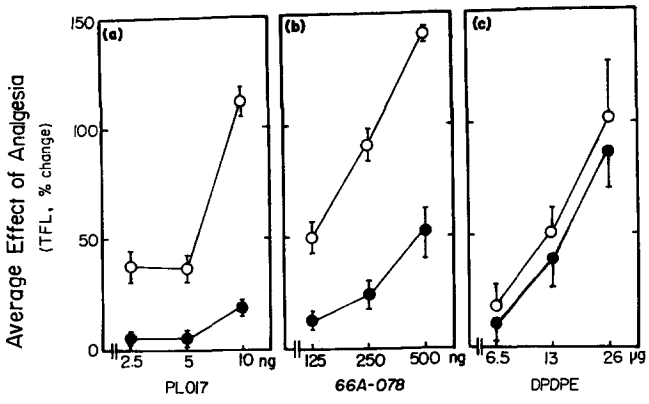

Fig. 3. Dose–response curve of opioid agonists with (●) or without (○) the injection of CCK-8. Average effect of analgesia in each rat was calculated from the mean % change in TFLs of 5 time points after CCK-8 administration. CCK-8 was injected intrathecally 10 min following the agonist administration.

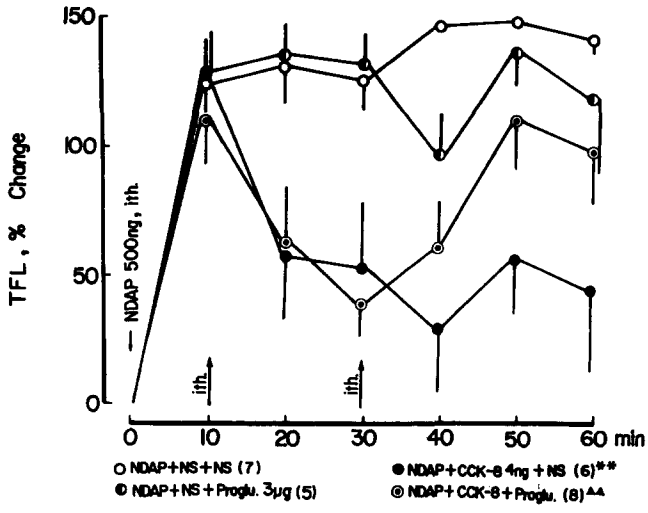

Fig. 4. The antagonistic effect of CCK-8 on 66A-078 (500 ng)-induced analgesia, and the reversal of the effect of CCK-8 by proglumide. The symbols are the same as in Fig. 2 except that 66A-078 was used instead of PL017.

an ith injection of NS 10 μl, CCK-8 4 ng, 40 ng and proglumide 3 μg, respectively. The TFLs determined every 10 min revealed that neither CCK-8 nor proglumide has significant effect on the basal pain threshold within 60 min (Table I, $P > 0.05$). Successive ith injections of CCK-8 with doses of 0.1, 0.2, 0.5 and 1 μg at 10 min intervals produced no significant changes in the pain threshold in a period of 70 min, either (Fig. 1).

Antagonistic effect of CCK-8 on PL017 analgesia

Intrathecal injection of PL017 2.5 ng ($n = 13$), 5 ng ($n = 14$) and 10 ng ($n = 14$) to 3 groups of rats produced dose-dependent analgesia (Table II and Fig. 2). Ten min following PL017 administration when TFLs increased dramatically, each group of rats was divided into two subgroups receiving an ith injection of 4 ng CCK-8 or 10 μl NS. After 10 min of CCK-8 administration, the TFL in rats receiving PL017 administration was dramatically decreased. The antagonistic effect of CCK-8 remained 50 min following its administration ($P < 0.01$). Fig. 3a shows that the average effect of analgesia as depicted by the dose–response curve of PL017 was almost abolished by ith injection of 4 ng CCK-8 ($P < 0.01$). It is also shown in Fig. 2 that while proglumide per se had no significant influence on PL017 induced analgesia, it did reverse the anti-opioid effect of CCK-8, suggesting that the effect of CCK-8 was mediated by the activation of CCK receptors.

Antagonistic effect of CCK-8 on 66A-078 induced analgesia

Three groups of rats were given ith injections of the κ-agonist 66A-078 125 ng ($n = 17$), 250 ng ($n = 16$) and 500 ng ($n = 13$), respectively. Ten min after the 66A-078

TABLE III

The antagonistic effect of CCK-8 on 66A-078-induced analgesia

Figures in the table are mean ± S.E.M. Intrathecal injection of 66A-078 was given at time 0 followed 10 min later by ith injection of CCK-8 4 ng or normal saline (NS) 10 μl. n = number of animals in each subgroup.

66A-078 (ng)	*Subgroup*	*n*	*Basal TFL (s)*	*TFL (% change)*					
				10′	*20′*	*30′*	*40′*	*50′*	*60′*
125	NS	9	4.5 ± 0.1	42 ± 14	62 ± 16	69 ± 16	48 ± 19	27 ± 8.1	40 ± 9.6
	CCK-8	8	4.8 ± 0.3	43 ± 16	13 ± 14**	11 ± 3.0**	37 ± 18	−4.9 ± 11**	4.8 ± 7.2**
250	NS	9	4.1 ± 0.2	64 ± 17	112 ± 15	120 ± 15	105 ± 17	72 ± 19	42 ± 15
	CCK-8	7	4.3 ± 0.3	60 ± 8.3	29 ± 21**	29 ± 18**	15 ± 15**	17 ± 21**	15 ± 18**
500	NS	7	5.1 ± 0.1	125 ± 13	132 ± 13	127 ± 12	148 ± 1.5	150 ± 0	144 ± 5.1
	CCK-8	6	5.1 ± 0.4	130 ± 12	58 ± 24**	55 ± 32**	33 ± 27**	59 ± 20**	47 ± 32**

**$P < 0.01$, compared with the corresponding values in NS control group using ANOVA followed by Q-test.

TABLE IV

The analgesia produced by DPDPE with or without the presence of CCK-8

Figures in the table are mean ± S.E.M. Intrathecal injection of DPDPE was given at time 0 followed 10 min later by ith injection of CCK-8 4 ng or normal saline (NS) 10 μl. n = number of animals in each subgroup. $P > 0.05$ (ANOVA), compared between the two subgroups.

DPDPE (μg)	*Subgroup*	*n*	*Basal TFL (s)*	*TFL (% change)*					
				10′	*20′*	*30′*	*40′*	*50′*	*60′*
6.5	NS	8	3.8 ± 0.2	59 ± 14	35 ± 12	13 ± 5.0	16 ± 4.4	13 ± 6.4	8.8 ± 6.7
	CCK-8	7	3.7 ± 0.2	55 ± 22	23 ± 13	9.7 ± 6.4	8.3 ± 5.8	1.4 ± 2.9	15 ± 7.7
13	NS	7	4.1 ± 0.3	105 ± 22	90 ± 15	60 ± 11	43 ± 17	29 ± 8.6	17 ± 12
	CCK-8	8	3.9 ± 0.3	106 ± 18	69 ± 16	56 ± 17	25 ± 7.2	20 ± 14	18 ± 8.1
26	NS	4	4.6 ± 0.2	118 ± 28	131 ± 13	124 ± 26	80 ± 35	83 ± 33	56 ± 28
	CCK-8	5	3.9 ± 0.3	124 ± 23	150 ± 0	97 ± 23	59 ± 22	45 ± 25	42 ± 20

injection when TFLs were increased, each group of rats was divided into two subgroups receiving ith injection of CCK-8 4 ng or NS 10 μl. The rats receiving NS showed a maximal analgesia of 69 ± 16%, 120 ± 15% and 150 ± 0% at the three doses mentioned above (Table III and Fig. 4), while the corresponding values in rats receiving CCK-8 administration were only 13 ± 14%, 29 ± 18% and 59 ± 20%, respectively. This antagonistic effect of CCK-8 remained 50 min after its injection ($P < 0.01$). Fig. 3b shows a right shift of the dose–response curve for 66A-078 analgesia after the addition of CCK-8 (4 ng, ith) on the top of 66A-078 ($P < 0.01$). That the anti-opioid effect of 66A-078 was a receptor-mediated event was evidenced by the experimental results shown in Fig. 4: (1) The antagonistic effect of CCK-8 on 66A-078-induced analgesia could be markedly reversed by ith injection of proglumide, a CCK receptor blocker, (2) proglumide per se had no significant influence on 66A-078 induced analgesia.

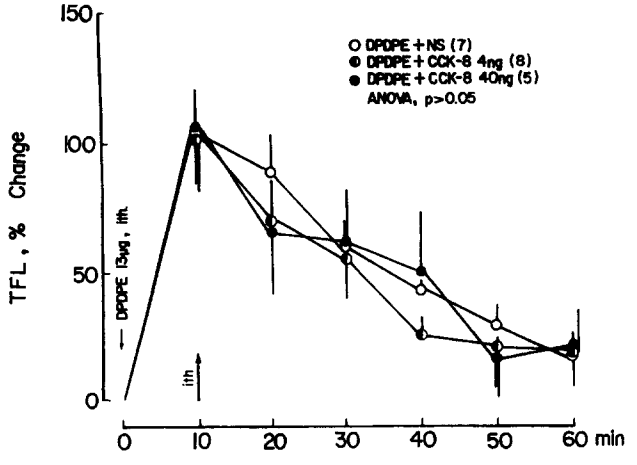

Fig. 5. The effect of CCK-8 on analgesia elicited by DPDPE. Mean percentage change of TFL ± S.E.M. (%) is plotted against time in min. DPDPE 13 μg was injected intrathecally at time 0. CCK-8 4 ng or 40 ng was injected at 10 min as indicated by an upward arrow. Numerals in the parentheses indicate the number of animals in each group. $P > 0.05$, as compared with the control group.

The effect of CCK-8 on DPDPE induced analgesia

Three groups of rats were injected ith with DPDPE 6.5 μg (n = 15), 13 μg (n = 15) and 26 μg (n = 9), respectively, followed 10 min later with ith injection of 4 ng CCK-8 or 10 μl NS. The results are summarized in Table IV. The dose–response curve of DPDPE-induced analgesia was not significantly affected by CCK-8 at a dose of 4 ng ith (Fig. 3c, $P > 0.05$), although it seems to be slightly inhibited by CCK-8. The same was true when the dose of CCK-8 was increased to 40 ng (Fig. 5).

DISCUSSION

Two different views exist concerning the relationship between CCK-8 and opioids, being antagonistic on one hand[2,4,7,9] and synergistic on the other hand[8,12]. Aside from the differences in the experimental setup such as animal species and route of injection, a major factor which may account for the differences in results from different laboratories is the dose of CCK-8. CCK-8 was found antagonistic to opioid analgesia when a pg or ng dose was used[2,4,9,11]; the same peptide produced a naloxone-reversible analgesia when the dose was increased to a μg or mg range[8,12]. Since our interest was to elucidate the function of CCK-8 in physiologically accessible level rather than using CCK-8 as a drug, we have been applying the smallest effective dose of CCK-8, and the results were compared with those obtained after the administration of CCK antiserum or CCK antagonist which inactivates the synaptically released CCK-8 in various conditions[5,7,11]. While the centrally effective dose of CCK-8 in antagonizing opioid analgesia has been in the range of 200 pg to 4 ng[2,11], we in the present study

intentionly increased the ith dose to 40 ng or to a cumulative dose of 1.8 μg (Fig. 1). Neither analgesia nor hyperalgesia was observed, which was consistent with the data reported by Pittaway et al.[12] in similar conditions.

The mechanisms underlying the antagonistic interaction between CCK-8 and opioids are obscure. At least two possibilities exist. One is that two separate pathways operated by opioids and CCK-8 converge on a common neuronal endpoint, as proposed by Faris et al.[4]. The other is that opioid receptor and CCK receptor reside at the same neuron and that binding of one receptor affects the other. Although the early work of Stengaard-Pedersen and Larsson[15] described that CCK-8 did not inhibit opioid binding, recent reports indicated that CCK-8 did affect the number or affinity of opioid receptors in neonatal[10] or adult rat brain[18]. The heterogeneity of opioid receptors[17] and the differential role played by μ-, δ- and κ-opioid receptors in producing analgesia at different levels of the CNS[3,14,22] raised the question as to whether CCK-8 has a special preference to antagonize analgesia mediated by a special type of opioid receptor. The results of the present study clearly indicate that CCK-8 antagonizes analgesia induced by PL017 (μ-agonist) and 66A-078 (κ-agonist) but not DPDPE (δ-agonist). This result fits very well with the data obtained in our recent study of receptor binding assay where CCK-8 was very effective in suppressing the binding of [^{3}H]DAGO to μ-receptor and [^{3}H]U69,593 to κ-receptor but not [^{3}H]DPDPE to δ-receptor[19]. Thus both in vivo and in vitro study pointed to the same conclusion that CCK-8 antagonism prefers μ- and κ- to δ-opioid receptor.

Proglumide, an antagonist for CCK receptor[6], has been reported to potentiate[20,21] or attenuate[12,20,21] opioid analgesia. The former is probably due to the blockade of the antiopioid effect of endogenously released CCK-8, resulting in a disinhibition of opioid analgesia. The latter effect appeared when a large (100–1000 fold) dose of proglumide was used[20,21], and the mechanism is so far unknown. In the present study we chose a dose for proglumide of 3 μg (ith) which is near the minimal dose used by Watkins et al. in attenuating morphine analgesia[20,21]. In our hands, this dose of proglumide did not affect the basal pain threshold or the analgesia induced by PL017 or 66A-078 in the present study, yet it reversed the anti-opioid effect of CCK-8 on μ- or κ-receptor-mediated analgesia almost completely. This result can be regarded as a strong evidence to suggest that the anti-opioid effect of CCK-8 was operative via the activation of CCK receptors.

In conclusion, CCK-8 in the spinal cord of the rat functions to antagonize opioid analgesia induced by μ- and κ-agonists but not δ-agonist. This anti-opioid effect of CCK-8 seems to be a receptor-mediated event, the biochemical mechanisms of which remain to be elucidated.

Acknowledgements. This work was supported by NIDA Grant DA 03983, and a grant from the National Natural Science Foundation of China. We thank Squibb and Sons Inc. for the generous donation of CCK-8, Rotta Res. Lab. for proglumide and Dr. Tachibana of Eisai Co. Japan for 66A-078.

REFERENCES

1 Beinfeld, M.C., Cholecystokinin in central nervous system: a minireview, *Neuropeptides*, 3 (1983) 411–427.
2 Ding, X.Z., Fan, S.G. and Han, J.S., Blockade of morphine analgesia by intracerebroventricular or subarachnoid injections of cholecystokinin in rats, *Acta Pharmacol. Sinica*, 6 (1985) 241–244.
3 Chang, K.J., Wei, E.T., Killian, A. and Chang, J.K., Potent morphiceptin analogs: structure activity relationships and morphine like activities, *J. Pharmacol. Exp. Ther.*, 227 (1983) 403–408.
4 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.
5 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) 101–105.
6 Hahne, W.F., Jensen, R.T., Lamp, G.F. and Gardner, J.D., Proglumide and Benzotript: members of different class of cholecystokinin receptor antagonists, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 6304–6308.
7 Han, J.S., Ding, X.Z. and Fan, S.G., Is cholecystokinin octapeptide (CCK-8) a candidate for endogenous antiopioid substrates?, *Neuropeptides*, 5 (1985) 399–402.
8 Hill, R.G., Hughes, J. and Pittaway, K.M., Antinociceptive action of cholecystokinin octapeptide (CCK-8) and related peptides in rats and mice: effect of naloxone and peptidase inhibitors, *Neuropharmacology*, 26 (1987) 289–300.
9 Itoh, S., Katsuura, G. and Maeda, Y., Caerulein and cholecystokinin suppress beta-endorphin-induced analgesia in the rat, *Eur. J. Pharmacol.*, 80 (1982) 421–425.
10 Johnson, F.E., Hudd, C., LaRegina, M.C., Beinfeld, M.C., Tolbert, D.L., Spain, J.W., Szucs, M. and Coscia, C.J., Exogenous cholecystokinin (CCK) reduces neonatal rat brain opioid receptor density and CCK levels, *Dev. Brain Res.*, 32 (1987) 139–146.
11 Li, Y. and Han, J.S., Cholecystokinin octapeptide antagonizes morphine analgesia in periaqueductal gray of the rat, *Brain Research*, 480 (1989) 105–110.
12 Pittaway, K.M. and Hill, R.G., Cholecystokinin and pain, *Pain Headache*, 9 (1987) 213–246.
13 Ren, M.F. and Han, J.S., Rat tail flick acupuncture model, *Chinese Med. J.*, 92 (1979) 576–582.
14 Rodriguez, R.E., Leighton, G., Hill, R.G. and Hughes, J., In vivo evidence for spinal delta-opiate receptor operated antinociception, *Neuropeptides*, 8 (1986) 221–241.
15 Stengaard-Pedersen, K. and Larsson, L.I., Localization and opiate receptor binding of enkephalin, CCK and ACTH/beta-endorphin in the rat central nervous system, *Peptides*, 2 Suppl. 1 (1981) 3–19.
16 Tachibana, S., Yoshino, H., Arakawa, Y., Nakazawa, T., Kaneko, T., Yamatsu, K. and Miyagawa, H., Design and

synthesis of metabolically stable analogues of dynorphin-A and their analgesia characteristics. In H. Takagi, Y. Oomura, M. Ito and M. Otsuka (Eds.), *Biowarning System in the Brain,* Tokyo University Press, 1988, pp. 101–109.

17 Traynor, J.R. and Wood, M.S., Distribution of opioid binding sites in spinal cord, *Neuropeptides,* 10 (1980) 313–320.

18 Wang, X.J., Fan, S.G., Ren, M.F. and Han, J.S., Cholecystokinin-8 suppressed [^{3}H]etorphine binding to rat brain opiate receptors, *Life Sci.,* 45 (1989) 117–123.

19 Wang, X.J. and Han, J.S., Modification by cholecystokinin-8 of binding of the mu, delta and kappa opioid receptors, *J. Neurochem.,* in press.

20 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.

21 Watkins, L.R., Kinscheck, I.B. and Mayer, D.J., Potentiation of morphine analgesia by CCK antagonist proglumide, *Brain Research,* 327 (1985) 169–180.

22 Xie, G.X. and Han, J.S., The analgesic effect of dynorphin A in spinal cord of the rat potentiated by dynorphin B, *Kexue Tongbao,* 30 (1985) 1688–1692.

23 Yaksh, T.L. and Rudy, T.A., Chronic catheterization of the spinal subarachnoid space, *Physiol. Behav.,* 17 (1976) 1031–1036.

24 Zetler, G., Apomorphine separates antinociceptive effects of cholecystokinin octapeptide and caeruletide from those of morphine, *Eur. J. Pharmacol.,* 92 (1983) 151–154.