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EFFECTS OF CCK RECEPTOR AGONISTS AND ANTAGONISTS ON MORPHINE-INDUCED ANTINOCICEPTION IN MICE

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ABSTRACT

In the present study the effects of both CCK receptor agonists and antagonists on antinociception induced by morphine in the tail-flick test have been evaluated. Morphine induced dose-dependent antinociception in mice. The response of morphine was potentiated by sulfated cholecystokinin-8 (CCK-8S) but not by unsulfated cholecystokinin-8 (CCK-8U). The CCK receptor antagonists MK-329 and L-365, 260 decreased the potentiation of morphine antinociception induced by CCK-8S. The antagonists even decreased the response induced by morphine in the presence of CCK-8U. High doses of MK-329 and L-365, 260 also potentiated morphine's antinociception. Single administration of the CCK receptor agonists CCK-8 and CCK-8U or CCK receptor antagonists did not elicit any response in the tail-flick test. It is concluded that CCK receptor mechanisms are involved in the modulation of pain response and/or morphine antinociception.

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Keywords: CCK agents, morphine, antinociception, mice.

INTRODUCTION

Cholecystokinin (CCK) is an important neuropeptide which is found within the mammalian nervous system.[1,8] Sulfated cholecystokinin-8 (CCK-8S) is the predominant form in the central nervous system although small amounts of cholecystokinin-4 (CCK-4) are clamied to the present.[8,13] CCK-8S is present in many regions of the midbrain, including the periaqueductal grey (PAG) and substantia nigra, that are important in pain modulation.[1] Caerulein is closely related to the naturally occurring octapeptide CCK.[1] CCK-8S and the related peptide caerulein produce several neural-mediated effects after both peripheral and central administration.[1]

Exogenously applied CCK-8S and related peptides have given different results on pain modulation in rodents.[20]

CCK-8S has been found to reduce the analgesic effect of morphine and β-endorphin.[4,6] Our previous results also show that pretreatment of mice with the CCK receptor agonist caerulein decreased or increased the morphine-induced antinociception depending on the pretreatment time.[22] There is evidence indicating that the CCK system may play an important role in pain transmission by modulating CNS opiate mechanisms.[4,5,7,9,15,17,23] Evidence has suggested that there may be multiple receptors for CCK. CCK receptor sites have been designated as CCK-A and CCK-B receptors.[10] Highly selective and potent CCK-A receptor antagonist MK-329 and CCK-B receptor antagonist L-365, 260 have become available, opening up possibilities to evaluate the role of CCK receptor subtypes in mechanisms of the pain system.[20]

The present study was undertaken to find out the receptor subtypes involved in the influence of CCK-8S and

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unsulfated CCK-8 on morphine antinociception.

MATERIALS AND METHODS

Groups of (n=9) male albino mice weighing 20-25 g were used in the experiments. The animals were housed in an animal room, with lights on 12h per day, and kept at 22 + 2°C. Food and water were continuously available. Antinociception was assessed using a tail-flick test (baseline 2-3 sec, cut-off 10 sec) with a tail-flick apparatus (type 812, Hugo Sachs Elektronic, Germany).

Comparison among animals was made by means of the change in tail-flick latency at 15, 30, 45 and 60 min after drug administration. Each animal was used only once and was euthanized immediately after the experiments.

Drugs

The following drugs were used: morphine HCl (Mac-Farlan Smith Ltd., Edinburgh), sulfated octapeptide cholecystokinin (CCK-8S), unsulfated octapeptide cholecystokinin-8 (CCK-8U) (Peninsula Lab. Inc., Belmont, U.S.A.). 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] and MK-329 [1-methyl-3-(2-indolyl) amino-5-phenyl-3H-1, 4-benzodiazepin-2-one], (Merck Sharp and Dohme, England) were dissolved in dimethylsulphoxide (DMSO) 40% and distilled water 60%.

The other drugs were prepared immediately before use and were injected in a volume of 10 mL/kg.

The stock solution of the peptides was prepared with bicarbonate solution (0.5M) and kept frozen. The doses of the drug and pretreatment times for the drug used were based on a survey of published studies in which these drugs were used,[3,23] [14,22]

Statistical analysis

Analysis of variance (ANOVA) followed by Newman Keul's test was used to evaluate the significance of the results obtained. Differences with a p-value <0.05 were considered significant.

RESULTS

Effect of morphine on tail-flick latency

Subcutaneous (s.c.) administration of different doses of morphine (1.5-9 mg/kg) to mice induced a dose-dependent antinociception [$F(5,48)= 594.1, p<0.0001$]. The maximum response was obtained with 9 mg/kg of the drug and at 45 min after drug injection (Fig. 1).

Effect of CCK receptor agonists on morphine-induced antinociception

When different doses of cholecystokinin-8 (CCK-8S; 0.025, 0.05 and 0.1 mg/kg, s.c.) were administered 30 min

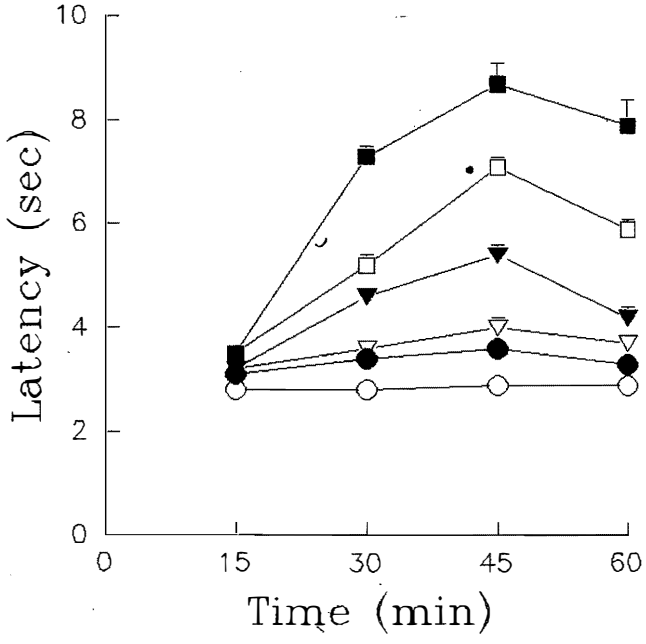

Fig. 1. Dose-response and time course of the antinociceptive effect of morphine in mice. Animals were administered subcutaneously (s.c.) either saline (O: 10 mL/kg) or morphine 1.5 (●), 3(▽), 4.5 (▼), 6 (□) and 9 (■) mg/kg. Antinociception was recorded 15, 30, 45 and 60 min after morphine injection. Each point is the mean±SEM of 9 animals.

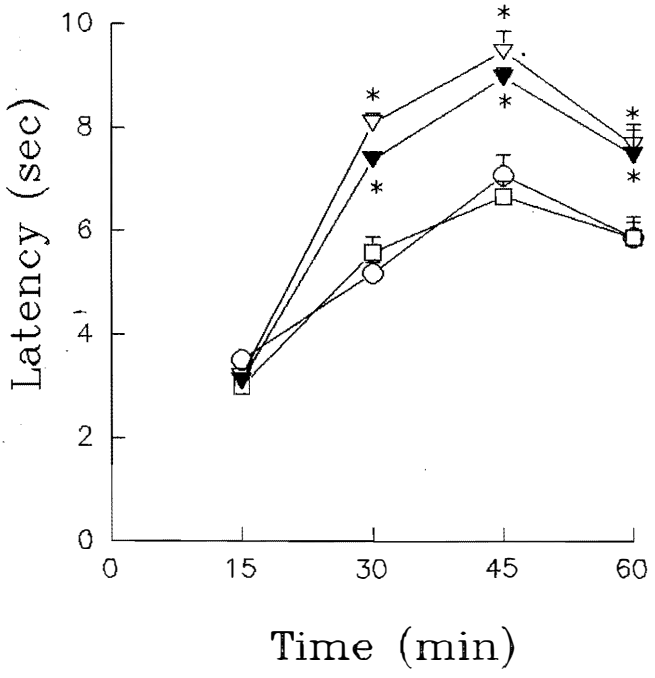

Fig. 2. Effects of sulfated cholecystokinin octapeptide (CCK-8) on morphine (6 mg/kg)-induced antinociception. Mice were pretreated (s.c.) with either saline (O; 10 mL/kg) or with CCK-8 (□) 0.025, (▼) 0.05, (▽) 0.1 mg/kg, 30 min prior to morphine (6 mg/kg, SC) administration. Antinociception was recorded 15, 30, 45 and 60 min after morphine injection. Each point is the mean±SEM of 9 animals. *$p<0.01$ different from morphine control group.

Table I. Effect of CCK-8 on antinociception induced by different doses of morphine.

Treatment	mg/kg	Latency after morphine injection (min)			
		15	30	45	60
Morphine + Saline	1.5 10 mL/kg	2.9±0.1	3.2±0.1	3.4±0.1	3.1±0.1
Morphine + CCK-8S	1.5 0.05	3.1±0.0	3.4±0.1	3.6±0.1	3.3±0.1
Morphine + CCK-8U	1.5 0.05	2.7±0.0	3.0±0.2	3.3±0.1	3.1±0.2
Morphine + Saline	3 10 mL/kg	3.2±0.1	3.6±0.1	4.0±0.2	3.7±0.1
Morphine + CCK-8S	3 0.05	3.4±0.1	4.1±0.1**	4.9±0.2**	4.1±0.2*
Morphine + CCK-8U	3 0.05	3.2±0.1	3.5±0.2	3.7±0.2	3.4±0.2
Morphine + Saline	6 10 mL/kg	3.5±0.1	5.2±0.2	7.1±0.4	5.9±0.4
Morphine + CCK-8U	6 0.1	3.3±0.1	5.5±0.3	6.8±0.2	5.7±0.2
Morphine + CCK-8U	6 0.05	3.3±0.1	5.5±0.2	6.9±0.3	5.8±0.3
Morphine + CCK-8U	6 0.01	3.5±0.1	5.4±0.2	6.7±0.2	5.8±0.2

Animals were treated (s.c.) with saline, sulfated cholecystokinin (CCK-8S) or unsulfated cholecystokinin (CCK-8U) 30 min prior to morphine administration and antinociception was recorded 15, 30, 45 and 60 min after the opioid injection. Each point is the mean±SEM of 9 experiments. *p<0.05, **p<0.01, different from control groups.

before morphine (6 mg/kg, s.c.), the antinociceptive response of morphine was increased [$F(15,128)= 54.1, p<0.0001$] (Fig. 2).

The peptide also potentiated the response of a low dose (3 mg/kg, s.c.) of morphine [$F(23,192)= 12.37, p<0.0001$] (Table I). ANOVA indicated significant differences between the animals which were treated with unsulfated CCK-8 (CCK-8U; 0.025, 0.05 and 0.1 mg/kg, s.c.) 30 min before high dose (6

Table II. Effect of CCK antagonists on antinociception induced by morphine (6 mg/kg).

Pretreatment	mg/kg	Latency after morphine injection (min)			
		15	30	45	60
Vehicle	10mL/kg	3.7±0.1	5.8±0.4	7.2±0.5	6.0±0.4
MK-329	0.125	3.1±0.1	5.4±0.4	7.5±0.4	6.5±0.5
MK-329	0.25	3.6±0.1	5.2±0.2	7.1±0.4	5.9±0.4
MK-329	0.5	3.5±0.1	7.0±0.4**	9.2±0.3**	8.1±0.5**
L-365, 260	0.125	3.2±0.1	5.8±0.5	7.1±0.4	6.4±0.2
L-365, 260	0.25	3.3±0.1	5.4±0.4	6.9±0.4	6.3±0.3
L-365, 260	0.5	3.1±0.1	8.5±0.2**	9.9±0.0**	8.9±0.3**

Animals were treated (s.c.) with either vehicle or CCK antagonists 30 min before morphine administration. Antinociception was recorded 15, 30, 45, and 60 min after morphine (6mg/kg) injection. Vehicle was 40% DMSO plus 60% distilled water. Each point is the mean± SEM of 9 experiments.
**p<0.01, different from control group.

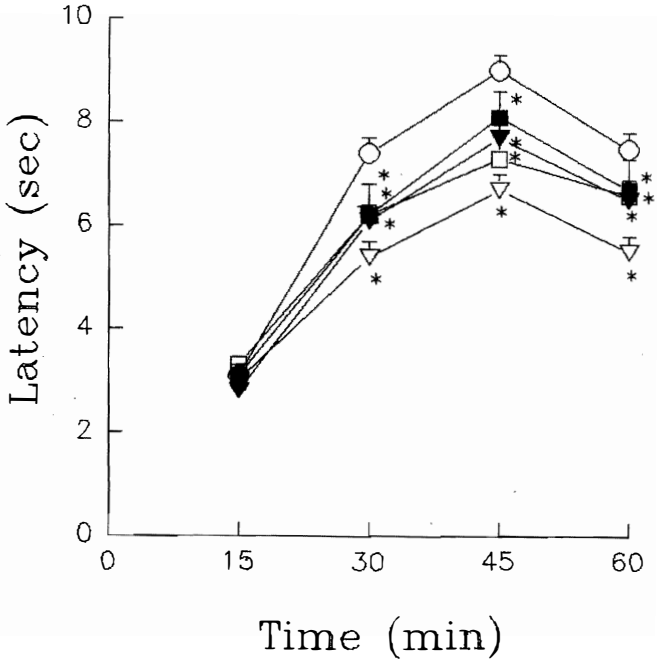

Fig. 3. Effects of cholecystokinin antagonists on response induced by morphine (6 mg/kg) plus CCK-8S. Animals were injected with CCK-8S (0.05 mg/kg) followed by saline (O; 10 mL/kg) MK-329 (▽) 0.25 and 0.5 (▼) mg/kg or with L-365, 260 (□) 0.25 and (■) 0.5 mg/kg, 35 min prior to morphine injection. CCK-8 was injected 30 min before morphine administration. Antinociception was recorded 15, 30, 45 and 60 min after morphine injection. Each point is the mean±SEM of 9 animals. *p<0.01 different from morphine control group.

Table III. Effect of CCK-antagonists on antinociception induced by morphine (6 mg/kg) in the presence of CCK-8U.

Pretreatment	mg/kg	Latency after morphine (min)			
		15	30	45	60
Saline + CCK-8U	10 mL/kg 0.05	3.5±0.1	5.8±0.2	7.1±0.6	5.5±0.4
MK-329 + CCK-8U	1.25 0.05	3.5±0.1	5.3±0.5	7.0±0.4	6.5±0.4
MK-329 + CCK-8U	0.5 0.05	3.1±0.1*	3.2±0.1**	3.5±0.2**	3.3±0.1**
L-365, 260 + CCK-8U	0.25 0.5	3.0±0.1*	3.7±0.2**	4.2±0.3**	3.7±0.2**
L-365, 260 + CCK-8U	0.5 0.05	2.7±0.1**	3.7±0.4**	4.5±0.5**	3.8±0.5**

Animals were treated (s.c.) with morphine (6 mg/kg) plus CCK-8U (0.05 mg/kg) in the presence or absence of CCK antagonists. MK-329 or L-365, 260 was injected 35 min prior to morphine (6 mg/kg) administration. Antinociception was recorded 15, 30, 45 and 60 min after morphine injection. Each point is the mean±SEM of 9 experiments. *$p<0.05$, **$p<0.01$, different from control group.

mg/kg, s.c.) [F(15, 128)= 29.9, $p<0.00001$] and low doses of morphine (1.5 and 3 mg/kg, s.c.) [F(23, 192)= 12.4, $p<0.0001$]. Further analysis showed that CCK-8U was not able to alter the response to morphine (Table I).

Effect of CCK receptor antagonists on analgesic effect of morphine

Pretreatment of animals with the CCK-A receptor antagonist MK-329 (0.125 and 0.25 mg/kg, s.c.) or the CCK-B receptor antagonist L-365, 260 (0.125 and 0.25 mg/kg, s.c.) 35 min before morphine (6 mg/kg) did not alter morphine-induced antinociception. However, a higher dose of both antagonists (0.5 mg/kg) increased the response to morphine [F(27,224)=29.2, $p<0.0001$] (Table II).

Effect of CCK antagonists on response induced by CCK peptides plus morphine

When the animals were treated with different doses of MK-329 and L-365, 260 (0.25 and 0.5 mg/kg) 5 min before CCK-8S (0.05 mg/kg), the antagonists decreased the response induced by CCK-8S (0.05 mg/kg, s.c.) plus morphine (6 mg/kg) [F(19, 160)= 30.1, $p<0.0001$]. (Fig. 3).

Pretreatment of animals with a high dose of MK-329 (0.5 mg/kg), but not a low dose (0.25 mg/kg), 5 min before unsulfated CCK-8S reduced the response of morphine (6 mg/kg) compared with the vehicle control group. Administration of CCK-8S receptor antagonist L-365, 260 (0.25 and 0.5 mg/kg) 5 min prior to CCK-8U (0.05 mg/kg, s.c.) also reduced the antinociception induced by CCK-8U plus morphine (6 mg/kg) [F(19, 160)= 18.2, $p<0.0001$] (Table III).

Effect of CCK agonists and CCK antagonists on tail-flick test

ANOVA indicated significant differences between animals which were administered CCK agonists CCK-8S (0.05 and 0.1 mg/kg, s.c.) and CCK-8U (0.05 and 0.1 mg/kg, s.c.) [F(19, 160)= 2.5, $p<0.01$] or MK-329 (0.125, 0.25 and 0.5 mg/kg, s.c.) and L-365, 260 (0.125, 0.5 and 0.1, s.c.) [27, 224)= 2.7, $p<0.0001$]. Further analysis showed that none of the agonists or antagonists were able to induce any reponse on the tail-flick test (Table IV).

DISCUSSION

In the present study, sulfated cholecystokinin octapeptide (CCK-8S) in different doses increased morphine-induced antinociception. Antinociception induced by both low and high doses of morphine were potentiated by CCK-8S. The results obtained are in agreement with our previous study in which pretreatment of animals with caerulein 30 min before morphine administration was shown to potentiate the antinociception of the opioid, although the analgesic effect was decreased when caerulein was given 5 min before morphine.[22]

CCK-8S binds with nanomolar affinities to both CCK-A and CCK-B receptors.[10] It may be suggested that potentiation of morphine's antinociception by CCK-8S is mediated through CCK receptors. Other reports have suggested that cholecystokinin attenuates opioid-mediated forms of analgesia.[4,6] The discrepancy with the data in different labs may be due to difference in dosage, animal species, route of administration, and time lapse between treatment and testing. The possibility may exist that cholecystokinin-related peptides have different roles in modulation of opioid-induced antinociception. After systemic adminstration, peptides pass the blood-brain barrier in sufficient concentration to exert central effects,[7] therefore there is the possibility that the response induced by CCK-8S is mediated through central mechanisms.

There is evidence that unsulfated CCK-8 (CCK-8U) has low affinity for CCK-A receptors, but shows selectivity for brain CCK receptors (CCK-B).[1,21] CCK-8U did not alter morphine's antinociception. However, the CCK-B receptor antagonist L-365, 260 decreased the antinociception induced by the CCK receptor agonist plus the opioid drug. This may indicate that CCK-B receptor subtypes are partly involved in the potentiation induced by the drug.

Table IV. Effect of CCK and CCK antagonists on tail-flick latency.

Treatment	mg/kg	Latency after drug injection (min)			
		15	30	45	60
Saline	10 mL/kg	2.7±0.1	2.8±0.1	2.8±0.1	2.8±0.1
CCK-8S	0.05	2.6±0.0	2.5±0.1	2.7±0.0	2.6±0.1
CCK-8S	0.1	2.6±0.1	2.7±0.1	2.7±0.0	2.6±0.0
CCK-8U	0.5	3.1±0.2	2.9±0.1	2.9±0.1	3.0±0.1
CCK-8U	0.1	3.0±0.1	2.8±0.1	2.9±0.2	2.6±0.1
Vehicle	10 mL/kg	3.1±0.1	3.1±0.1	3.3±0.1	3.2±0.1
MK-329	0.125	2.8±0.1	3.0±0.2	3.2±0.1	2.9±0.1
MK-329	0.25	2.5±0.0	2.9±0.1	3.0±0.2	3.1±0.2
MK-329	0.5	3.1±0.1	3.2±0.1	3.4±0.2	3.2±0.1
L-365,260	0.125	2.8±0.0	2.9±0.2	3.2±0.2	3.0±0.1
L-365,260	0.25	2.6±0.1	2.8±0.1	3.0±0.1	2.9±0.2
L-365,260	0.5	2.7±0.1	3.0±0.1	3.5±0.3	2.8±0.1

Animals were treated (s.c.) with saline, vehicle, CCK or CCK antagonists and antinociception was recorded 15, 30, 45 and 60 min after drug administration. Each point is the mean±SEM of 9 experiments.

It has been proposed that the effect of the peptides is produced indirectly through the induced release of endogenous opioid peptides or may result from direct interaction with opioid receptors.[15,19] Thus we examined the direct role of CCK receptors in CCK-8S-induced potentiation of morphine analgesia by using the selective non-peptide CCK receptor antagonists MK-329 and L-365, 260.[21] Pretreatment of animals with the CCK-A and CCK-B receptor antagonists attenuated the potentiated morphine antinociception induced by CCK-8S. On the basis of our results, it would seem that both CCK-A and CCK-B receptors appear to facilitate morphine-induced antinociception. The interpretation of these findings are difficult but by considering the presence of CCK receptors at pre- and post-synaptic sites and their interference with intracellular ion stores,[16] one can postulate different and even opposite roles for different CCK receptor subtypes.

Present results indicate that the selective CCK-A receptor antagonist MK-329 and selective CCK-B receptor antagonist L-365, 260[21] enhanced morphine antinociception in high dose, but not in low doses. The results are in agreement with other studies that have shown the enhancement of the analgesic effect of morphine by CCK receptor antagonists and decrease in tolerance to the opioid drugs.[11,20]

It is suggested that CCK-opioid interaction may be specific to thermal pain stimuli and play a role in pain transmission.[11,12] However, direct effects of the drugs on opiate receptors can not also be excluded.

It is proposed that the opioid and CCK receptors are present on the neurons at the spinal and supraspinal levels.[16] CCK/opiate interaction has been suggested for description of the effect of CCK on morphine analgesia.[1] It is possible that the blockade of CCK receptors by MK-329 or L-365, 260, and stimulation of a subtype of the CCK receptors by exogenous CCK-8U shifts the balance in the opposite direction and opioid analgesia is decreased. None of the peptides and antagonists were effective in the tail-flick test alone, consistent with results of other investigators,[2] which further indicates that the peptides may only have a role in modulation of antinociception. Clearly, further studies are needed in order to find the exact mechanism(s).

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