Cholecystokinin as a factor in the enhanced potency of spinal morphine following carrageenin inflammation

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1 Cholecystokinin (CCK) has been shown to diminish opioid analgesia. Here we investigate whether changes in the physiological levels of spinal CCK are responsible for the enhanced potency of spinal morphine in animals following carrageenin inflammation, as compared with normal animals.

2 Single dorsal horn nociceptive neurones were recorded in intact halothane-anaesthetized rats in the presence and absence of carrageenin-induced inflammation and comparisons were made between the two groups of animals. Inflammation was induced by the injection of 100 μ l of 2% λ -carrageenin into the hind paw.

3 The inhibitory effect of intrathecal morphine on the C-fibre-evoked responses of the neurones was enhanced in the carrageenin-treated animals such that the effects of 0.25 μ g and 10 μ g of morphine in normal animals were comparable to those of 0.01 μ g and 2.5 μ g in the carrageenin animals. The effect of 0.2 mg kg⁻¹ of the CCK_B antagonist, L-365,260, on the antinociceptive potency of intrathecal morphine was examined in both groups of animals. In normal animals, L-365,260 produced a significant enhancement in the effect of morphine indicating a tonic CCK modulation in these animals, but it had no effect on the inhibitions produced by either dose of morphine in the carrageenin animals.

4 The inhibition of the C-fibre-evoked response produced by intrathecal morphine in the presence of 1 μ g of CCK was examined in both groups of animals. CCK attenuated the effects of morphine only in animals with carrageenin inflammation, having no effect on the action of morphine in normal animals.

5 The effects of both CCK and L-365,260 were therefore dependent on the inflammatory state of the animal, with each drug being active in opposite situations.

6 We propose that in normal animals, morphine may produce a maximal stimulation of the release of CCK such that exogenous CCK is unable to reduce further the analgesic effects under these conditions. However, the differential effects of the agonist and antagonist in the normal and inflamed rats points to a role of CCK in the enhanced opiate actions. This enhancement of the potency of spinal morphine in inflammation is best explained by a reduction in spinal CCK release by morphine in this state.

Keywords: Morphine; cholecystokinin; L-365,260; inflammation; analgesia; spinal cord

Introduction

Over the years it has been demonstrated that there is great capacity for change in not only the transmission but also the modulation of nociceptive information (see recent reviews by Dickenson, 1991; Dubner & Ruda, 1992). Little is known about the transmitter systems responsible for alterations in the ability to produce analgesia. Whereas opiate sensitivity can be diminished in neuropathic pains (Arner & Meyerson, 1988; Portenoy *et al.*, 1990) the opposite can occur in inflammatory conditions. It is well established that there is an enhancement in the potency of systemically administered opiate agonists in a variety of chronic inflammatory states, gauged by behavioural approaches (Kayser & Guilbaud, 1983; Neil *et al.*, 1986; Joris *et al.*, 1990). Recently it has been shown that one site contributing to this increased potency of opiates following carrageenin-induced inflammation is the spinal cord (Hylden *et al.*, 1991; Stanfa *et al.*, 1992). We have demonstrated, in electrophysiological studies, that this enhancement in spinal potency is especially marked for the μ -opiate agonist, morphine compared with that seen with the δ -agonist DSTBULET and the κ -agonist, U69593. In addition, the facilitation of the C-fibre evoked response normally seen with low doses of intrathecal μ -agonists in this model was absent in animals with carrageenin-induced inflammation (Stanfa *et al.*, 1992).

Here we investigate one of the pharmacological systems within the spinal cord which may be responsible for the

relatively selective enhancement in the spinal potency of μ -opioids following peripheral inflammation. One candidate for this role is the peptide cholecystokinin (CCK) which acts predominantly at CCK_B receptors in the rat spinal cord (Hill & Woodruff, 1990). Although there are some anomalies in the results from studies using CCK and CCK receptor antagonists, there is good evidence that the peptide CCK selectively interferes with the action of μ - but not δ - or κ -agonists at the level of the spinal cord (Faris *et al.*, 1983; Barbaz *et al.*, 1989; Magnuson *et al.*, 1990). As yet, there has been no attempt to assign a physiological role to CCK in inflammatory states.

In this study we have used sulphated CCK-8 and the CCK_B receptor antagonist, L-365,260, to investigate whether physiological levels of CCK exert a tonic inhibition of the antinociceptive effect of intrathecal morphine in normal (control) animals and whether the same is true in animals with carrageenin-induced inflammation.

In addition, we used the CCK_B receptor antagonist to investigate whether the release of CCK is responsible for the facilitations seen with low doses of morphine in normal animals but absent in animals with carrageenin-induced inflammation (Stanfa *et al.*, 1992).

Methods

The method is essentially that previously described (Stanfa *et al.*, 1992). Briefly, male Sprague Dawley rats (200–250 g) were anaesthetized with 2% halothane in a mixture of N₂O

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and O₂ and after cannulation of the trachea a laminectomy was performed to expose the L1–L3 region of the spinal cord. A tungsten electrode was lowered into the dorsal horn and single unit extracellular recordings were made from convergent neurones responding to both innocuous and noxious stimuli with receptive fields on the ipsilateral hind paw. During the recording the level of halothane was reduced to 1–1.5% which was sufficient to maintain complete areflexia. Electrical stimulation (2 ms wide pulse, 0.5 Hz, 16 stimuli per trial) was used as the test stimulus for the experiment, applied at 3 times the threshold current for C-fibre activation. This type of stimulation provides a constant reproducible test stimulus for the experiment and avoids the problem of altered baseline responses which could result from hyperalgesia to natural stimuli as a consequence of the inflammation. Trials were performed every 10 min and we have previously shown that no change occurs in the thresholds for C-fibre activation during the period of the experiment (Stanfa *et al.*, 1992). C-fibre-evoked responses, which could be separated from the A β -fibre evoked responses on the basis of threshold and latency, were quantified from the post-stimulus histogram. The degree of wind-up of the cells (Mendell, 1966; Davies & Lodge, 1987; Dickenson & Sullivan, 1987), *W*, was also quantified and calculated as the total number of spikes to the 16 stimuli divided by *I*, where *I* is the cell's response to the initial C-fibre stimulus $\times 16$. Drugs were administered following 3 stable control trials.

In the animals with peripheral inflammation, 100 μ l of 2% λ -carrageenin was injected into the plantar region of the paw 3 h before drug administration. The evoked responses of the cells and the degree of wind-up of the cells was monitored during this 3 h period, with the 3 readings prior to the drug administration used as controls for the subsequent drug effect.

Morphine and CCK were applied directly to the surface of the cord (equivalent to an intrathecal injection) in a volume of 50 μ l. CCK was administered 20 min before the administration of morphine. L-365,260 0.2 mg kg⁻¹ (in 10% Tween/alcohol) in a volume of 0.25 ml was given subcutaneously 10 min before the application of morphine to the surface of the cord. The evoked response of the cell was then followed for a further 40 min. Intrathecal naloxone at a dose of 1 or 5 μ g was used to reverse the effects of morphine.

The effects of L-365,260 alone and the effects of the vehicle alone were examined in separate animals.

Materials

Morphine sulphate (Thornton and Ross), naloxone hydrochloride (Sigma), sulphated cholecystokinin-8 (CCK) (Sigma), λ -carrageenin (Sigma) and L-365, 260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N¹-(3-methyl-phenyl)urea) (a gift from Merck Sharp and Dohme) were used in this study.

Statistical analysis

The results are expressed as a percentage of control or as percentage inhibition. The data were analysed either by Student's *t* test or two factor analysis of variance (ANOVA) followed by Newman-Keuls Student's test. *P* values less than 0.05 were regarded as significant.

Results

The convergent neurones used in this study were located in the deeper laminae of the dorsal horn (500–1000 μ m, mean depth = 738 $\pm$ 31 μ m (*n* = 40)), with these neurones forming a similar population to those used in the previous study (Stanfa *et al.*, 1992).

Responses of the dorsal horn neurones following carrageenin injection

The C-fibre evoked response of 14 of the neurones was followed for 3 h after the injection of carrageenin. It was found that the C-fibre-evoked response of 6 (43%) of the neurones increased to a mean 132.5 $\pm$ 10% of control, while that of the other 8 (57%) decreased to 88.5 $\pm$ 4% of control. The degree and magnitude of this change was significantly correlated with the initial degree of wind-up of the neurones ($r = -0.51$, $P = 0.032$). Wind-up is the frequency-dependent increase in evoked neuronal responses of the cells to repeated constant intensity stimulation (Mendell, 1966; Davies & Lodge, 1987; Dickenson & Sullivan, 1987). Figure 1 shows that neurones that had little wind-up during the control period (low value of *W*) tended to have an increased response after carrageenin, whilst those with a large degree of wind-up initially (high *W*) had a decreased response. This pattern of changes is identical to that previously found in a more comprehensive study in this model (Stanfa *et al.*, 1992). In this earlier study we showed that the direction and magnitude of the change in the C-fibre evoked response following carrageenin was not correlated in any way with the subsequent responses to intrathecal opiates, thus allowing us to discard this as a factor in the altered potency of morphine in these animals. In addition neither the injection of saline into the paw nor the repeated stimulation had any effect on the magnitude of the C-fibre-evoked response (Stanfa *et al.*, 1992).

None of the neurones developed any spontaneous activity during the period of inflammation.

Effect of the CCK_B antagonist L-365, 260 on the potency of morphine in normal animals.

Two doses of intrathecal morphine (0.25 and 10 μ g) were tested on the C-fibre-evoked responses of convergent dorsal horn neurones in normal (untreated) rats and caused dose-dependent inhibitions of 7.9 $\pm$ 9% (*n* = 7) and 81.3 $\pm$ 6% (*n* = 6) respectively (see Figure 2).

In these normal animals, the subcutaneous administration of 0.2 mg kg⁻¹ of L-365,260 (the maximum effective dose

Figure 1 The relationship between the magnitude of the C-fibre evoked response in dorsal horn cells (3 h after the injection of carrageenin into the paw) and the initial degree of wind-up of the cell, *W*. Cells with little wind-up (low value of *W*) in the control period tended to have an enhanced C-fibre-evoked response after carrageenin, whilst those cells that exhibited a large degree of wind-up (high *W*) tended to have a decreased C-fibre evoked response after carrageenin. Correlation coefficient, $r = -0.51$ ($P = 0.032$).

Figure 2 The effect of L-365,260, 0.2 mg kg⁻¹, on the morphine-induced inhibitions of the C-fibre-evoked response in dorsal horn cells in normal animals. Note the significant enhancement of the potency of intrathecal morphine in the presence of L-365, 260 (solid columns) compared with the controls (open columns). $n = 5-7$ for each point. * $P < 0.05$.

Figure 3 The effect of L-365,260, 0.2 mg kg⁻¹, on the morphine-induced inhibitions of the C-fibre evoked response in dorsal horn cells in animals with carrageenin-induced inflammation. In the animals with peripheral inflammation, L-365,260 no longer potentiates the antinociceptive effects of morphine (solid columns) compared to morphine alone (open columns). $n = 5-9$ for each point.

(Dourish *et al.*, 1990)) 10 min before 0.25 µg and 10 µg of intrathecal morphine led to a significant enhancement in the potency of both doses. This produced a leftward shift in the dose-response curve with the degree of inhibition produced by 0.25 µg being enhanced to $34.7 \pm 9\%$ ($n = 6$, $P = 0.033$) and that produced by 10 µg to $99.4 \pm 1\%$ ($n = 5$, $P = 0.014$) (see Figure 2). Intrathecal naloxone at a dose of 5 µg reversed the morphine-induced inhibitions of the C-fibre evoked response to $84 \pm 12\%$ of control values within 20 min. These results indicate that in normal (control) animals the endogenous levels of CCK released in the dorsal horn are sufficient to attenuate partly the inhibitory effects of intrathecal morphine.

In some animals the effects of the vehicle alone ($n = 4$) or L-365,260 alone ($n = 4$) were examined. Neither substance had any effect on the C-fibre evoked response when followed over a 90 min time course.

Effect of the CCK_B antagonist L-365,260 on the potency of morphine in animals with carrageenin inflammation

In order to investigate whether endogenous levels of CCK attenuate the analgesic effects of intrathecal morphine in animals with carrageenin inflammation, two doses of morphine were chosen which produced a similar degree of inhibition of the C-fibre evoked response to those used in normal animals. We have previously shown that the dose-response curve for intrathecal morphine is shifted to the left in animals with carrageenin-induced inflammation (Stanfa *et al.*, 1992), hence these doses of morphine are lower than those used in the normal animals.

Intrathecal administration of 0.01 and 2.5 µg of morphine 3 h after the injection of carrageenin into the paw produced inhibitions of the C-fibre-evoked response of $11.5 \pm 9\%$ ($n = 5$) and $75.4 \pm 8\%$ ($n = 9$) respectively (Figure 3).

When the same two doses were applied 10 min after the subcutaneous administration of 0.2 mg kg⁻¹ of L-365,260 no significant enhancement was found in the antinociceptive potency of morphine (inhibitions of $12.0 \pm 4\%$ ($n = 6$) and $85.2 \pm 11\%$ ($n = 5$) respectively) which is in contrast to the situation in normal animals (see Figure 2). The intrathecal administration of 1 µg of naloxone reversed the inhibitions produced by 2.5 µg of morphine to $78 \pm 10\%$ of control

values within 20 min. These findings suggest that there is insufficient CCK released in the spinal cord of animals with carrageenin induced inflammation to attenuate significantly the antinociceptive actions of morphine under these conditions.

Effect of CCK on morphine analgesia in normal animals

CCK (1 µg) was given intrathecally 20 min before the application of 10 µg of morphine to the surface of the cord and the inhibition of the C-fibre evoked response of the dorsal horn neurones was followed for 40 min. There was found to be no difference between the inhibition produced by morphine alone and that produced in the presence of 1 µg of CCK (see Figure 4) with morphine alone causing an inhibi-

Figure 4 The time course for the inhibition of the C-fibre-evoked response by 10 µg of intrathecal morphine alone (O) and 20 min after the intrathecal administration of 1 µg of cholecystikinin (CCK, ●) in normal animals. In normal animals the presence of CCK had no effect on the potency of morphine. $n = 7-8$.

tion of $87.3 \pm 6\%$ ($n = 8$) after 40 min and $86.3 \pm 5\%$ ($n = 7$) when $1 \mu\text{g}$ of CCK was present. The intrathecal administration of $5 \mu\text{g}$ of naloxone reversed the morphine-induced inhibition of the C-fibre evoked response to $90 \pm 12\%$ of control within 20 min.

Effect of CCK on morphine analgesia in animals with carrageenin inflammation

The effect of $1 \mu\text{g}$ of intrathecal CCK (given 20 min before the intrathecal administration of morphine) was studied on the morphine-induced inhibition of the C-fibre evoked response of 7 dorsal horn neurones in carrageenin animals. Five of these cells showed substantially smaller inhibitions with $2.5 \mu\text{g}$ of morphine than the control neurones whilst two appeared to be unaffected by the presence of CCK. No other differences were found between the CCK-sensitive and insensitive neurones so all cells were pooled. Thus in the presence of $1 \mu\text{g}$ of CCK, $2.5 \mu\text{g}$ of morphine produced a significantly smaller inhibition of the C-fibre evoked response of the neurones ($F_{1,52} = 18.5$; $P < 0.0001$; $n = 7$ cells per group) (see Figure 5). The intrathecal administration of $1 \mu\text{g}$ of naloxone reversed the morphine-induced inhibition of the C-fibre evoked response to $93 \pm 3\%$ of control within 20 min. This demonstrates that exogenous CCK is still capable of reducing the antinociceptive actions of morphine in animals with carrageenin inflammation. Hence it is most likely that a reduced release of the peptide is responsible for the lack of effect of the CCK_B antagonist in these animals.

No significant facilitation of the C-fibre evoked response was seen with CCK as has been reported previously in this model and in other studies (Jeftinija *et al.*, 1981; Willetts *et al.*, 1985; Magnuson *et al.*, 1990), although we previously observed this effect to be maximal 5 min post CCK administration and this time point was not tested in this study.

Effect of L-365,260 on facilitations due to low dose morphine in normal animals

It has been reported that (1) morphine is able to cause the release of CCK in the dorsal horn of the spinal cord (Tang *et*

al., 1984; Benoliel *et al.*, 1991) (and see discussion), (2) CCK itself is capable of causing a facilitation of the C-fibre evoked response of neurones (Jeftinija *et al.*, 1981; Willetts *et al.*, 1985; Magnuson *et al.*, 1990), although this was not seen in the present study and (3) the facilitation of the C-fibre evoked response produced by low doses of intrathecal morphine in normal animals no longer occurs in animals with carrageenin inflammation (Stanfa *et al.*, 1992). The results of the present study suggest that morphine is no longer causing the release of CCK in animals with carrageenin-induced inflammation. This led us to investigate whether a morphine-induced release of CCK may be responsible for the facilitations seen with the low doses of morphine in normal animals, with μ -receptor inhibitions becoming predominant as the dose is increased.

The two doses of morphine chosen for this study are at the lower end of the morphine dose-response curve. The first dose ($0.01 \mu\text{g}$) is a subthreshold dose and had no significant effect on the C-fibre-evoked response ($93.5 \pm 9\%$ of control, $n = 6$) in normal animals. The second dose ($0.1 \mu\text{g}$) produced a facilitation of the C-fibre-evoked response to $126.2 \pm 8\%$ of control ($n = 5$) when given intrathecally. As the dose of morphine was increased to $0.25 \mu\text{g}$ and above, the facilitation turned to dose-dependent inhibitions (see Figure 6). When these low doses of morphine (0.01 and $0.1 \mu\text{g}$) were repeated 10 min after the subcutaneous administration of 0.2 mg kg^{-1} L-365,260, $0.01 \mu\text{g}$ of morphine, previously a subthreshold dose, facilitated the C-fibre evoked response to $118.8 \pm 8\%$ of control ($n = 5$) whilst $0.1 \mu\text{g}$ of morphine failed to have a significant effect on the C-fibre evoked response ($102.8 \pm 9\%$ of control, $n = 5$) (see Figure 6).

Thus it appears that the CCK_B receptor antagonist, L-365,260, does not block the facilitations produced by low doses of morphine suggesting that this phenomenon is not due to the release of CCK. There has however been a shift in the dose-response curve in the presence of the antagonist in the same way as occurs with the higher doses. As a result of this, the threshold dose for a significant effect (with these low doses, a facilitation) on the C-fibre evoked response has been lowered.

Figure 5 The time course for the inhibition of the C-fibre evoked response by $2.5 \mu\text{g}$ of intrathecal morphine alone (○) and 20 min after the intrathecal administration of $1 \mu\text{g}$ of cholecystokinin (CCK, ●) in animals with carrageenin-induced inflammation. In animals with carrageenin inflammation the presence of CCK leads to a significant decrease in the potency of intrathecal morphine ($P < 0.0001$, ANOVA). $n = 7$ cells per group.

Figure 6 The effects of low doses of intrathecal morphine alone (open columns) and in combination with CCK_B receptor antagonist, L-365,260 (solid columns) on the C-fibre-evoked responses. The vertical axis represents the % change from the controls so that a negative change signifies inhibitory effects and a positive change signifies facilitation. Note that in the presence of the antagonist the dose-response curve is shifted to the left such that the facilitation occurs with a lower dose of morphine but the magnitude remains the same. * $P < 0.05$ compared with morphine alone.

Discussion

The peptide CCK has been widely heralded as an endogenous modulator of the μ -opiate receptor whilst appearing to have little effect on effects mediated by the other subtypes of opiate receptor (Faris *et al.*, 1983; Barbaz *et al.*, 1989; Magnuson *et al.*, 1990). In this study we have used the CCK_B receptor antagonist, L-365,260, to demonstrate that in normal animals, under these experimental conditions, endogenous levels of CCK in the cord are sufficient to attenuate the antinociceptive actions of intrathecal morphine. This finding is in agreement with behavioural studies in normal animals (Baber *et al.*, 1989; Dourish *et al.*, 1990; Wiesenfeld-Hallin *et al.*, 1990) but this interaction has never been studied in models of pain states more relevant to the clinical situation. Here, in animals with carrageenin-induced inflammation, the CCK_B receptor antagonist produced no enhancement of the potency of morphine in direct contrast to the situation in normal animals.

The CCK receptors are mainly of the CCK_B type (Hill & Woodruff, 1990) in the rat spinal cord and although their exact location is not known, they may be located presynaptically since it has been reported that CCK_B receptors undergo axonal transport to the periphery in the vagus nerve (Mercer & Lawrence, 1992). Following early immunocytochemical studies, CCK was thought to be located within C-fibres although recent studies disagree with these findings (Hökfelt *et al.*, 1988; Zouaoui *et al.*, 1990). In addition cDNA probes have failed to localize CCK precursor mRNA within dorsal root ganglia in the rat (Seroogy *et al.*, 1990) suggesting that it is very unlikely that genuine CCK is found in primary afferents. It now seems most likely that the sources of endogenous CCK within the dorsal horn are the rich network of interneurons found in superficial laminae and possibly also some of descending fibres terminating there (Hökfelt *et al.*, 1988).

CCK does not appear to play a major role in nociceptive transmission since the peptide itself produces only mild increases in C-fibre responses (Jeftinija *et al.*, 1981; Willetts *et al.*, 1985; Magnuson *et al.*, 1990). Behavioural nociceptive thresholds are not altered by antagonists in all studies (Baber *et al.*, 1989; Dourish *et al.*, 1990; O'Neill *et al.*, 1990; Wiesenfeld-Hallin *et al.*, 1990) and where analgesic effects have occurred, they have been attributed to indirect opioid mechanisms (Wiesenfeld-Hallin *et al.*, 1990). In the present study both CCK and L-365,260 had no effect on the neuronal responses when given alone. The primary role of CCK in nociception would therefore appear to be as a modulator of opioid analgesia.

The exact sequence of events which triggers the release of CCK is not known although there is evidence that morphine stimulates the release of CCK within the dorsal horn (Tang *et al.*, 1984; Benoliel *et al.*, 1991). One possible physiological role of the morphine induced release of CCK is a mechanism whereby an endogenous 'brake' is applied to the antinociceptive actions of morphine or the endogenous ligand of the μ -receptor in times of acute pain. However, in animals with carrageenin-induced inflammation the antagonist no longer produced an enhancement of the antinociceptive effect of morphine, suggesting that CCK is no longer available. This lack of CCK is probably due to a reduced release of CCK and not an uncoupling of the interaction between morphine and CCK, (the exact nature of which is not known), since exogenous CCK applied to the cord caused a substantial reduction in the antinociceptive potency of morphine in these animals. It is possible that under these conditions of sub-chronic inflammation, morphine no longer evokes the release of CCK in the spinal cord and it is the absence of this autolimiting control that leads to the leftward shift in the dose-response curve seen in these animals compared with the normal state.

One explanation for the lack of effect of the CCK_B receptor antagonist in the carrageenin animals could be a reduced

opiate release of CCK as a result of the use of lower doses of morphine (0.25 and 2.5 μ g). This is unlikely since the CCK_B antagonist still produced a leftward shift in the responses to the even lower doses (0.01 and 0.1 μ g) of morphine used in normal animals. Different doses of morphine cannot therefore explain these findings leaving the presence of inflammation as the causal factor.

A feature of the morphine dose-response curve in normal animals is the facilitation of the C-fibre evoked response by low doses of morphine (Stanfa *et al.*, 1992). We speculated that this might be due to morphine releasing CCK which in turn causes facilitations of the neurones. These facilitations are absent in animals with carrageenin-induced inflammation where we now believe CCK release to be reduced. However since these facilitations still occur in the presence of L-365,260 it is unlikely that CCK is involved. This facilitation may then be due to an opiate-induced release of other excitatory neuropeptides such as substance P (Wiesenfeld-Hallin *et al.*, 1991). In addition, there are reports of direct opioid receptor-mediated increases in the duration of action potentials in dorsal root ganglion cells (Crain & Shen, 1990). Both mechanisms could be altered in inflammation.

The ability of CCK to modulate the actions of morphine is variable and depends on particular circumstances. CCK clearly attenuated the effects of morphine in the animals with carrageenin inflammation. The negative results obtained with the combination of CCK and morphine in normal animals in our model at first appears to conflict with reports by others showing that exogenous CCK blocks the antinociceptive actions of morphine (Faris *et al.*, 1983; Wiesenfeld-Hallin & Duranti, 1987; Baber *et al.*, 1989; Kellstein *et al.*, 1991). However Barbaz *et al.* (1989) found CCK to be effective in blocking morphine antinociception in the tail flick test but not in the hot plate or abdominal stretch test in the mouse.

We previously reported that 1 μ g of exogenous CCK caused a marked inhibition of the antinociceptive effect produced by the potent μ -agonist [D-Ala²,MePhe⁴,Gly-ol⁵]enkephalin (DAMGO) (Magnuson *et al.*, 1990). The difference between DAMGO and morphine may be due to morphine stimulating the release of CCK such that maximal CCK_B receptor activation results and so exogenous CCK can have no additional effect. Unlike morphine, DAMGO does not stimulate the release of CCK but in contrast has been shown to reduce the release of CCK at the level of the spinal cord (Rodriguez & Sacristan, 1989; Benoliel *et al.*, 1991). Thus when exogenous CCK is applied with DAMGO, attenuation of the analgesic effect of this opioid can occur. It is not clear how morphine produces the release of CCK, although it has been suggested that it may be through an action on the δ -receptor (Benoliel *et al.*, 1991). We suggest alternative explanations since in our model, in cross-tolerance studies, morphine does not appear to have any actions at the δ -receptor (unpublished observations).

One is that morphine and DAMGO may have different efficacies at the putative subtypes of the μ -receptor such as the μ_1 and μ_2 receptors (Pasternak & Wood, 1986). Indeed CCK blocks the antinociceptive action of meptazinol (a μ_1 -agonist) more successfully than that of morphine which binds to both μ_1 and μ_2 -receptors (Barbaz *et al.*, 1989). However the idea of μ_1 and μ_2 -receptors is a matter of controversy and there is evidence suggesting another receptor subclassification involving receptor complexes (Rothman *et al.*, 1989). Finally, there is a suggestion that DAMGO and morphine can be distinguished on the basis that different agonist-receptor complexes may couple with different G proteins (Sanchez-Blazquez & Garzon, 1991). These possibilities need further study before conclusions can be drawn.

Behavioural studies have suggested that the release of endogenous CCK is governed by the environment to which the rat is exposed. At present the results are conflicting with one group suggesting that CCK is released in 'safe' situations to prevent the analgesic actions of morphine (Wiertelak *et al.*, 1992) whilst another reports that CCK receptor

antagonists do not enhance morphine antinociception in rats in 'familiar' situations but do so in animals exposed to a novel environment (Lavigne *et al.*, 1992) suggesting that CCK release is associated with 'stress'. Our animals, being under general anaesthesia, could arguably be considered as being in a 'safe' situation. Equally the presence of inflammation could activate 'stress' systems although the anaesthetic would prevent any manifestation of these signs. Whatever the case, these results serve to indicate that the release of CCK is not fixed but is modified according to circumstances.

To summarize, we present evidence to show that morphine-mediated CCK release may be abolished in inflammatory states so providing a mechanism for the enhancement in the potency of spinal morphine seen following inflammation. In support of this hypothesis, not only does CCK have no effect on δ -opioid antinociception (Mag-

nuson *et al.*, 1990) but the effectiveness of δ -agonists in carrageenin inflammation is only moderately enhanced (Stanfa *et al.*, 1992). In more general terms, on the basis of studies with CCK antagonists, CCK has been implicated in morphine tolerance (see Baber *et al.*, 1989) where there is a reduced effect of morphine. These findings, together with the present study indicate that the enhanced and reduced effects of μ -opiates may be mediated through opposite extremes of the same mechanisms, in this case involving CCK. Hence further studies on the control of CCK may provide interesting insights into the role of CCK and possibly other neuropeptides in the modulation of nociceptive processing in the dorsal horn under different conditions.

This work was supported by the Medical Research Council and The Wellcome Trust. L.C.S. holds an MRC postgraduate studentship.

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(Received September 22, 1992

Revised November 26, 1992

Accepted November 27, 1992)