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Effect of CCK receptor antagonists on the antinociceptive, reinforcing and gut motility properties of morphine

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1 The ability of a selective CCK_A receptor antagonist PD 140548 and a selective CCK_B receptor antagonist CI-988 (formerly PD 134308) to modulate the various *in vivo* properties of morphine was investigated in the rat.

2 PD 140548 dose-dependently (0.001 – 1.0 mg kg^{-1}, i.p.) antagonised the development of conditioned place preference to morphine (2.0 mg kg^{-1}, s.c.). In contrast, CI-988 (0.01 – 1.0 mg kg^{-1}, i.p.) did not affect this morphine-induced behaviour. Neither of the CCK receptor antagonists blocked or generalised to the morphine (3.0 mg kg^{-1}, i.p.) discriminative stimulus.

3 CI-988 (0.001 – 10.0 mg kg^{-1}, s.c.) at doses of 0.05 and 0.1 mg kg^{-1} (s.c.), potentiated the antinociceptive action of a threshold dose of morphine (5.0 mg kg^{-1}, i.p.) in a radiant heat model of acute nociception, the rat tail flick test. Furthermore, at 0.01 mg kg^{-1} it potentiated the antinociceptive action of morphine (3.0 mg kg^{-1}) during the acute phase of the rat paw formalin test. And at doses of 0.01 and 0.1 mg kg^{-1} it also potentiated the antinociceptive action of morphine (1.0 mg kg^{-1}) during the tonic phase of the formalin test. However, in both models, higher doses of CI-988 were ineffective. In contrast, PD 140548 (0.001 – 10 mg kg^{-1}, s.c.) was only active at a dose of 1.0 mg kg^{-1} (s.c.) and only in the tonic phase of the formalin test. Neither CI-988 nor PD 140548 possessed any intrinsic antinociceptive action in either of the tests. Chronic treatment with CI-988 (0.01 mg kg^{-1}, s.c.) prevented the development of tolerance to morphine antinociception (4 mg kg^{-1}, s.c.) following a 6 day period of twice daily injections of morphine escalating from 1 to 16 mg kg^{-1} (i.p.).

4 Morphine dose-dependently (1 – 10 mg kg^{-1}, s.c.) reduced the distance travelled by a charcoal meal in the rat intestine. Neither PD 140548 (0.01 – 1.0 mg kg^{-1}, i.p.) nor CI-988 (0.01 – 1.0 mg kg^{-1}, i.p.) potentiated or suppressed this inhibitory action of morphine.

5 In conclusion, the results of the present study indicate that CCK_A and CCK_B receptors modulate different properties of morphine. Thus, whilst a selective CCK_A receptor antagonist blocked the rewarding properties of morphine, a selective CCK_B receptor antagonist potentiated the antinociceptive action. However, neither compound displayed a potential for modulating the influence of morphine on gastro-intestinal motility. It is suggested that these findings may have important implications for development of CCK receptor antagonists as analgesic adjuncts to the therapeutic use of morphine.

Keywords: Antinociception; formalin; tailflick; tolerance; dependence; conditioned place preference; cholecystokinin; gastro-intestinal

Introduction

Cholecystokinin (CCK) is a neuropeptide widely distributed in the CNS and has been suggested to play a role in anxiety and panic disorders, control of appetite, modulation of dopaminergic pathways and in the transmission of nociceptive information (Woodruff & Hughes, 1991). CCK interacts with at least two distinct types of receptor (Innis & Snyder, 1980; Moran *et al.*, 1986; Wank *et al.*, 1992): CCK_B receptors, which represent the predominant form in the rodent CNS (Pélaprat *et al.*, 1987; Hill & Woodruff, 1990) and CCK_A receptors, the abundant form in peripheral tissues (Zetler, 1984; Van Dijk *et al.*, 1984; Chang & Lotti, 1986) but are also found in discrete nuclei of the CNS (Hill *et al.*, 1987a; 1988). Molecular cloning and pharmacological studies have demonstrated a close relationship between CCK_B and gastrin receptors found mainly in the parietal cells of the gastric mucosa (Chang *et al.*, 1989; Pisegna *et al.*, 1992; Hunter *et al.*, 1993).

The implication of a modulator role for CCK in the control of nociception has been suggested by a number of observations; the distribution of CCK (Stengaard-Pedersen & Larsson, 1981; Beinfeld & Palkovits, 1982; Fuji *et al.*, 1985; Gall *et al.*, 1987) and its receptors (Van Dijk *et al.*, 1984; Hill & Woodruff, 1990) closely parallels that of the enkephalin family of opioid peptides within regions of the rodent brain (e.g. cortex, thalamus, periaqueductal grey, medullary nuclei) and spinal cord associated with the control of nocicpetive information. In behavioural studies, CCK has been shown to modulate noxious stimuli in a complex manner with higher 'pharmacological' doses producing a naloxone-reversible antinociception (Barbaz *et al.*, 1989; Hill *et al.*, 1987b) whilst lower, more 'physiological' doses reverse the antinociception produced by exogenous, mainly morphine and endogenous opioids (Faris *et al.*, 1983; Barbaz *et al.*, 1989; Dourish *et al.*, 1990). Moreover, in rodents, CCK_B antagonists potentiate opioid-mediated antinociception suggesting that the anti-opioid effect of CCK exists primarily as a tonic modulation of the opioid system by the endogenous neuropeptide (Watkins *et al.*, 1985a, b; Dourish *et al.*, 1988; 1990; Wiesenfeld-Hallin *et al.*, 1990; Maldonado *et al.*, 1993). This apparent physiological antagonism can be clearly demonstrated following an acute noxious stimulus. However, recent evidence suggests that when there is an accompanying inflammatory response, usually associated with tissue injury, there is a decreased availability of

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endogenous CCK in the spinal cord and, consequently, CCK_B antagonists appear no longer effective at modulating the morphine responses (Stanfa & Dickenson, 1993).

In neurochemical studies, CCK has been shown to exert a biphasic effect on mesolimbic dopaminergic function (Marshall *et al.*, 1991; Crawley, 1992) with CCK_A mediating an increase and CCK_B receptors a decrease in dopamine release from the nucleus accumbens. Furthermore, CCK antagonists have been found in rodents to prevent the development of tolerance (Dourish *et al.*, 1988, 1990; Kellstein & Mayer, 1991) and/or the re-inforcing properties (Higgins *et al.*, 1992) of morphine suggesting that there may be a tonic modulation of the effect of morphine on dopaminergic transmission by endogenous CCK.

The aims of the present study were three fold. The first aim was to investigate the effects of selective CCK_A and CCK_B receptor antagonists on morphine-induced antinociception in both acute and prolonged (tonic) models of nociception. The second aim was to investigate the potential interaction between the CCK receptor antagonists and morphine in rodent place preference and drug discrimination models of opiate dependence. The final aim was to evaluate the effect of both types of CCK receptor antagonist on morphine-induced gastrointestinal dysfunction to determine whether the opioid-CCK interaction was specific to the CNS or whether it extended to peripheral effects of morphine.

Methods

Animals

Male Sprague Dawley rats (70–90 g and 180–250 g) and male Wistar rats (240–300 g) were obtained from Bantin and Kingman (Hull, U.K.). Male Hooded Lister rats (200–250 g) were obtained from Interfauna, (Huntingdon, U.K.). Animals were housed in groups of 6–10 under a 12 h light/dark cycle (lights on at 07 h 00 min) with food and water *ad libitum*, except Hooded Lister rats who were maintained at 80–85% of their free feeding body weight. All behavioural tests were carried out between 09 h 00 min and 17 h 00 min.

Drug administration

Drugs were administered either i.p. or s.c. in a volume of 1 ml kg^{-1} for rats weighing over 100 g and 2 ml kg^{-1} for those under 100 g. The charcoal meal was given as a 1.0 ml suspension orally by gavage. All experiments were carried out by an observer unaware of the drug treatments.

Radiant heat tailflick model

Male Sprague Dawley rats (180–250 g) were used in the radiant heat tail flick test. Baseline latencies (BL) were measured (mean of 2/3 trials within 30–40 min inter-trial interval) before drug administration. Radiant heat was adjusted to attain a mean baseline of 2.5 to 3.5 s and exposure to the noxious stimulus was terminated at 10 s to avoid tissue damage. Test latencies (TL) were determined in the same group of animals at 20, 40 and 60 min after morphine administration and were expressed as % of maximum possible effect (%MPE) calculated as: $\%MPE = [(TL - BL)/(10 - BL)] \times 100$. CCK receptor antagonists were administered s.c. 20 min before a subthreshold dose of morphine (5 mg kg^{-1}, i.p.). For clarity only the data obtained from the test carried out at 40 min after morphine administration is shown.

Rat paw formalin test

Male Sprague Dawley rats (70–90 g) were habituated to perspex observation chambers (24 cm × 24 cm × 24 cm) for at least 15 min prior to testing. Formalin-induced hindpaw licking and biting was initiated by a 50 μl subcutaneous injection of a 5% formalin solution (5% formaldehyde in isotonic saline) into the plantar surface of the left hindpaw. Immediately following the formalin injection, licking/biting of the injected hindpaw was scored in 5 min bins for 60 min. A keypad linked to an IBM compatible computer running a behavioural testing program was used to score the animals, where key pressing recorded the length of licking observed over 5 min. Formalin produced a biphasic response. The results are expressed as mean combined licking/biting time for the early (acute) phase (0–10 min) and late (tonic) phase (20–35 min). Morphine was administered s.c. 20 min before formalin. CCK receptor antagonists were administered s.c. 20 min before a threshold dose of morphine. All experiments were carried out on the morning of the test day.

The ability of CI-988 to block development of tolerance to the antinociceptive action of morphine was examined in the rat formalin test of nociception. Groups of male Sprague Dawley rats (60–70 g at start of experiments) were subjected to an incremental twice daily dosing of either saline (controls) or morphine, beginning with 1 mg kg^{-1} (i.p.) on day 1 and culminating in 16 mg kg^{-1} on both days 5 and 6 (1, 2, 4, 8, 16 and 16 mg kg^{-1}, i.p.). Animals received a co-administration of either saline or CI-988 (0.001–0.1 mg kg^{-1}, s.c.) before each injection of saline/morphine. On the morning of day 7 animals were challenged with either saline or morphine (4 mg kg^{-1} s.c.) 20 min before the injection of formalin (5%, i.pl.). Immediately following the formalin injection, licking/biting of the injected paw was scored following the same procedure described above.

Morphine conditioned place preference

Male Wistar rats (240–300 g) were given morphine or saline s.c. and placed immediately into the appropriate conditioning chamber for 45 min. Each conditioning chamber consisted of two distinct compartments of equal size (34 cm × 25 cm × 34 cm). One compartment was grey with a smooth perspex floor and the other white with a rough perspex floor. Hence only visual and tactile discriminative cues were used. The two compartments were inter-linked by a short tunnel (3 cm × 8 cm × 8 cm). A central partition coloured to match each appropriate compartment allowed two rats to be simultaneously conditioned to each chamber. Each compartment was illuminated by a 60 W red light bulb and the chambers housed under dim white light. To establish place conditioning to morphine 4 saline and 4 drug conditioning sessions were carried out (one of each daily), over 4 days. Immediately after morphine or saline was administered the animals were confined to the appropriate conditioning compartments. The duration of each conditioning session was 45 min and the sessions were spaced at least 5 h, but not more than 24 h, apart. On the test day each rat was individually placed in a compartment in a counter-balanced manner and allowed free exploration of the entire box for 15 min. The cumulative amount of time spent by each rat in each compartment was determined. No pretreatment was given to the rats on this day. For antagonism studies, the drugs were administered intraperitoneally 60 min before morphine. All treatments were counter-balanced between compartments.

Morphine drug discrimination

Male Hooded Lister rats were trained to discriminate between the administration of morphine (3.0 mg kg^{-1}, i.p.) and saline (i.p.) by a two-lever, operant drug discrimination paradigm with a fixed ratio FR10 schedule of food reinforcement as previously described (Tricklebank *et al.*, 1989). Training sessions were of 15 min duration and commenced 30 min after drug administration. Lever selection was considered correct if the animal made five or fewer responses on the inappropriate lever before completing 10 presses on the appropriate one.

Tests of stimulus generalisation and antagonism

Generalisation and antagonism tests of 15 min duration were carried out in the presence of food reinforcement. The lever selected for reinforcement was the one on which the animal first emitted 10 responses. A maximum of two tests per week were carried out with at least 48 h between tests. CCK receptor antagonists were given 60 min before test.

Assessment of gastrointestinal motility

Male Sprague Dawley rats (180–250 g) were given 1.0 ml of a suspension of charcoal meal (10% charcoal in 5% gum acacia) orally by gavage and were killed by cervical dislocation after 15 min. The abdomen was opened and the intestine was dissected out from the pyloric end up to the ileocaecal junction. Gastrointestinal transit was expressed as the distance travelled by the charcoal as a percentage of the total length of the small intestine. Morphine was administered s.c. 25 min before charcoal. All antagonists were given i.p. 20 min before a submaximal dose of morphine (5.0 mg kg^{-1}, s.c.) except naloxone (1.0 mg kg^{-1}, s.c.) which was given 5 min before morphine.

Statistics

All results were analysed by use of significant ANOVA followed by a Dunnett's *t* test, with the exception of the conditioned place preference data in which a paired Student's *t* test analysis was performed.

Drugs

PD 140548 (benzenebutanoic acid, β-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl] amino]propyl] amino],-[S-(R*, S*)] and CI-988 (4-{[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy) carbony]amino]propyl]amino] - 1 - phenylethyl]amino} - 4 - oxo-[R-(R*, R*)]-butanoate N-methyl-D-glucamine) were synthesized at Parke-Davis Neuroscience Research Centre, Cambridge. Morphine sulphate was obtained from Savory and Moore, Cambridge. Charcoal was obtained from Sigma Chemical Co. (Poole, U.K.). All drugs were dissolved in 0.9% w/v NaCl except charcoal which was suspended in 5% gum acacia.

Results

Effect of CCK receptor antagonists on morphine analgesia

Radiant heat tailflick model: Morphine (2.5–20 mg kg^{-1}, i.p.), as expected, produced a dose-dependent increase in tail-flick latency to the thermal noxious stimulus (Figure 1a) with a MPE_{50} (50% of the maximum possible effect) of 7.8 (5.5–10.8) mg kg^{-1} from at least 10 animals per group. A threshold dose (5 mg kg^{-1}, i.p.) of morphine (approx. 20% of the MPE) which induced significant antinociception ($F(4,45)=5.74$, $P<0.001$) was then chosen for subsequent studies involving combination with the CCK receptor antagonists CI-988 and PD 140548.

CI-988 (0.001–10 mg kg^{-1}, s.c.), administered 20 min before morphine, caused a dose-dependent potentiation of the antinociceptive effect of morphine (Figure 1b), the response reaching the level of statistical significance at both 0.05 ($F(6,195)=5.74$, $P<0.01$) and 0.1 ($F(7,195)=5.74$, $P<0.01$) mg kg^{-1}. Higher doses of 1 and 10 mg kg^{-1} CI-988, however, were ineffective at modulating the response to morphine (Figure 1b). In contrast, PD 140548 (0.0001–10 mg kg^{-1}, s.c.) following a similar pretreatment, was found to be completely ineffective ($F(6,138)=0.20$, $P=0.97$), at all dose levels, at modulating morphine antinociceptive activity against the radiant heat noxious stimulus (Figure 1c). These interaction studies were carried out over a number of days but there was no significance between session variation in morphine control groups (CI-988 study $F(5,52)=1.87$, $P=0.12$; PD140548 study ($F(1,16)=2.39$, $P=0.11$).

Rat paw formalin test: Morphine (1.0–6.0 mg kg^{-1}, s.c.) dose-dependently inhibited both early (acute), and late (tonic), phases of the formalin-induced hindpaw licking/biting response reaching the level of statistical significance at 6.0 mg kg^{-1} ($F(3,24)=10.05$, $P<0.01$) against the early phase and at 3.0 and 6.0 mg kg^{-1} ($F(3,24)=21.2$, $P<0.01$) against the late phase (Figure 2a). Threshold doses of 1.0 and 3.0 mg kg^{-1} (s.c.) of morphine for the tonic and acute phases of the formalin response respectively, were chosen for the subsequent investigation involving combination with CCK antagonists.

CI-988 (0.001–1.0 mg kg^{-1}, s.c.) potentiated the antinociceptive effect of morphine during the tonic phase of the formalin response at doses of 0.01 and 0.1 mg kg^{-1} ($F(4,27)=5.66$, $P<0.05$) (Figure 2b) but was ineffective at

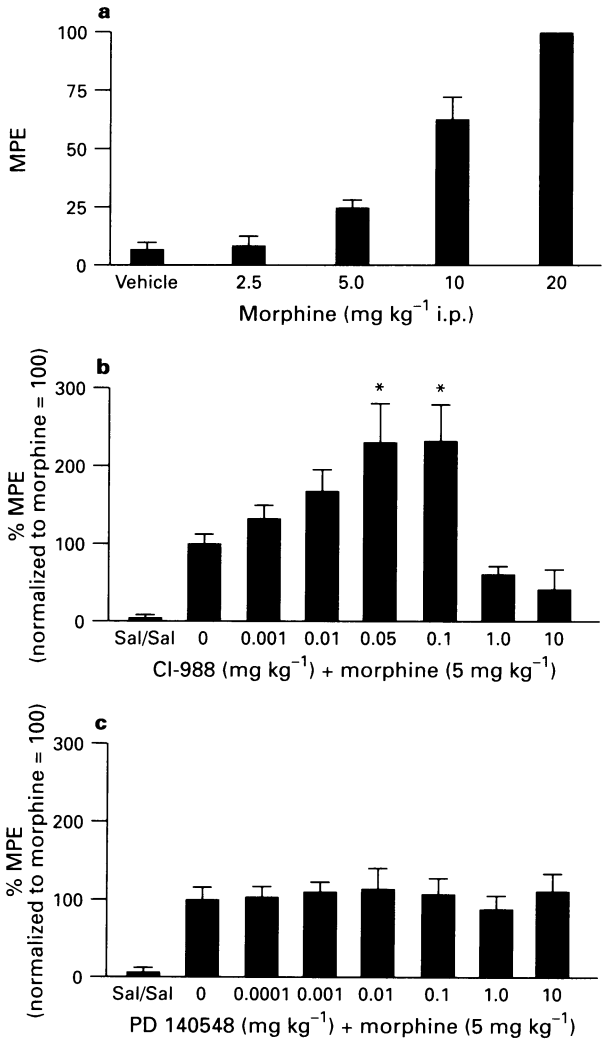

Figure 1 Effect of CI-988 (s.c.) and PD 140548 (s.c.) on the antinociceptive action of morphine in the rat radiant heat tail flick test. Data represent the dose-response relationship for morphine alone (a), followed by the effect of increasing doses of CI-988 (b) and PD 140548 (c), 20 min before a threshold dose of morphine (5 mg kg^{-1}, i.p.). Test latencies (TL) are expressed as % of the maximum possible effect (%MPE = [(TL − BL)/(10 − BL)] × 100) with a baseline of approx. 3 s and a maximum cut-off of 10 s. Results are shown as the mean (vertical bars represent s.e.mean) of at least 10 animals per group. $^{*}P<0.05$, $^{**}P<0.01$, significantly different from vehicle + morphine treated controls (ANOVA followed by Dunnett's *t* test).

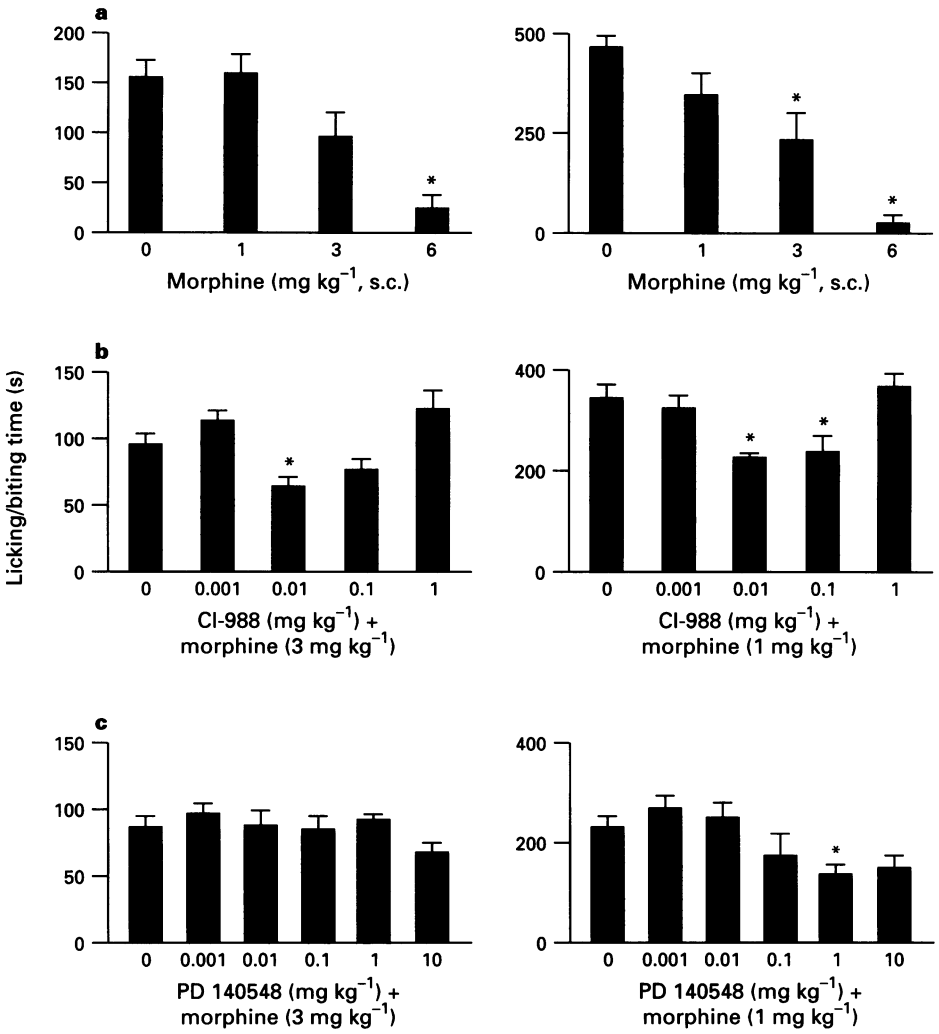

Figure 2 Effect of CI-988 and PD 140548 on the antinociceptive action of morphine in the rat paw formalin test. For the dose-response study morphine was administered s.c. (a). For the potentiation studies CI-988 (b) or PD 140548 (c) was given s.c. 20 min before a threshold dose of morphine (3 mg kg^{-1}, s.c. Acute phase; 1 mg kg^{-1}, s.c. Tonic phase). Formalin (50 μl of 5% solution) was administered into the intraplantar surface of the left hindpaw 20 min after morphine. Left hand panels represent time spent licking/biting of the injected paw during the acute phase (0–5 min post formalin) of the response. Right hand panels represent time spent licking/biting of the injected paw during the tonic phase (20–35 post formalin) of the response. Results are shown as the mean (vertical bars represent s.e.mean) of at least 10 animals per group. $^{*}P<0.05$, significantly different from vehicle + morphine treated controls (ANOVA followed by Dunnett's t test).

1.0 mg kg^{-1}. CI-988, also potentiated morphine suppression of the acute phase of the response at 0.01 mg kg^{-1} ($F(4,45)=6.6$, $P<0.05$) (Figure 2b). Similar pretreatment with PD 140548, at a dose of 1 mg kg^{-1}, potentiated the antinociceptive action of morphine on the formalin-induced tonic phase ($F(5,46)=4.25$, $P<0.05$) but was ineffective at both lower (0.001–0.1 mg kg^{-1}) and higher (10 mg kg^{-1}) doses (Figure 2c). However, no potentiation was observed of the acute phase at any dose of PD 140548 ($F(5,54)=1.26$, $P>0.05$). These interaction studies were carried out over a number of days, but there was no significance between session variation in morphine control groups (CI-988 study $F(2,5)=0.097$, $P=0.91$; PD 140548 study $F(2,3)=0.55$, $P=0.63$).

In the morphine tolerance study, a dose of 0.01 mg kg^{-1} (s.c.) CI-988 was chosen for it represented the effective dose at potentiating morphine antinociception in the acute administration study. Animals that received a chronic treatment of saline plus morphine exhibited profound tolerance displaying minimal antinociception during the tonic phase of the formalin licking/biting response following the acute challenge with morphine (4 mg kg^{-1}, s.c.) ($F(5,37)=12.3$, $P<0.01$) (Figure 3). In contrast, chronic pretreatment with CI-988 (0.01 mg kg^{-1}, s.c.) before each morphine injection appeared to prevent the development of morphine tolerance as shown by the reduced intensity of the tonic phase licking/biting response to the morphine challenge (4 mg kg^{-1}, s.c.). This was not significantly different to the group of naive rats receiving the single, acute dose of morphine following chronic treatment with saline/saline (Figure 3). In contrast to the acute data, 0.001 and 0.1 mg kg^{-1} doses of CI-988 were ineffective (data not shown). However, CI-988 (0.001–0.1 mg kg^{-1}, s.c.) had no significant effect on morphine tolerance of the acute phase of the response ($F(5,37)=3.54$, $P>0.05$) (Figure 3, data for 0.001, 0.1 mg kg^{-1}, not shown). Chronic treatment with CI-988, before twice daily injection of saline, produced no intrinsic antinociceptive activity.

Effect of CCK receptor antagonists on morphine conditioned place preference

Morphine (0.1–3.0 mg kg^{-1}, s.c.) dose-dependently induced place preference when administered over 4 trials, with a minimum effective dose of 1.0 mg kg^{-1} ($t(8)=1.788$, $P=0.05$) (Figure 4a). In contrast, 4 trials with saline alone produced no preference to either compartment ($t(11)=0.5148$, $P>0.05$)

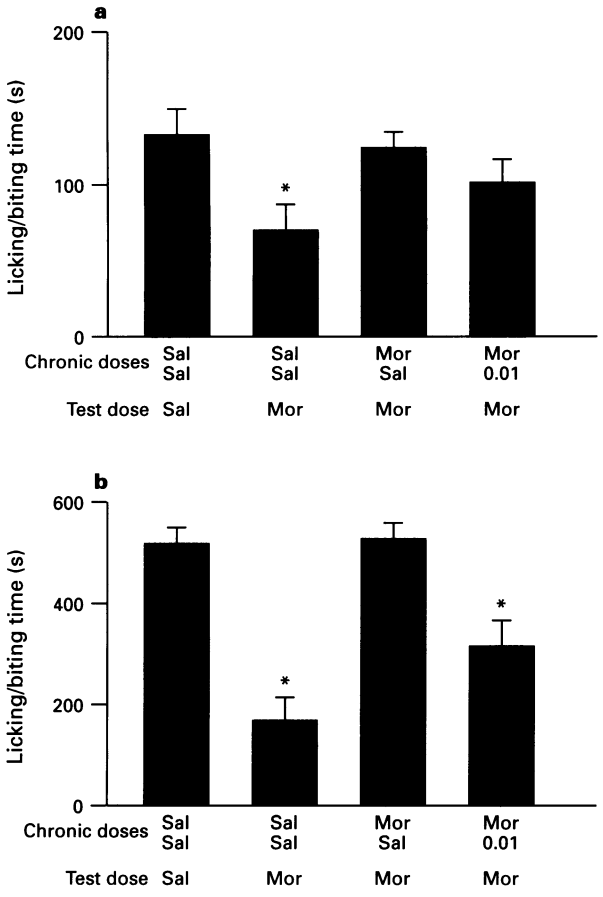

Figure 3 Effect of CI-988 on the development of tolerance to the antinociceptive action of morphine in the rat paw formalin test. Animals were subjected to incremental twice daily dosing of either saline or morphine (Mor) with prior co-administration of either saline (Sal) or CI-988 (0.01 mg kg^{-1}, s.c.) for 6 days. On the morning of day 7 animals were challenged with either saline or morphine (4 mg kg^{-1}, s.c.), 20 min before the injection of formalin. Formalin (50 μl of 5% solution) was administered into the intraplantar surface of the left hindpaw 20 min after morphine. (a) Represents time spent licking/biting of the injected paw during the acute phase (0–10 min post formalin) of the response, whilst (b) represents time spent licking/biting of the injected paw during the tonic phase (15–35 min post formalin) of the response. Results are shown as the mean (vertical bars represent s.e.mean) of at least 10 animals per group. *$P<0.05$, significantly different from chronic morphine + vehicle treated controls (ANOVA followed by Dunnett's t test).

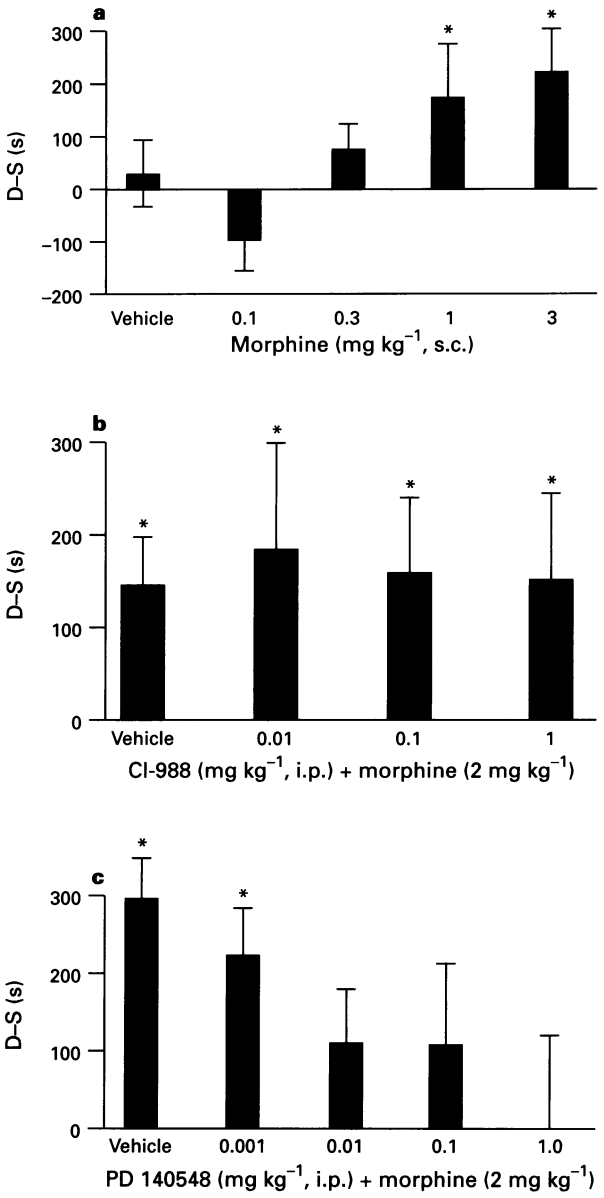

Figure 4 Effect of CI-988 and PD 140548 on morphine conditioned place preference in the rat. For the dose-response study, groups of rats were administered saline or morphine (Mor) s.c. and confined immediately to the appropriate compartment for 45 min (a). CI-988 (b) and PD 140548 (c) were administered i.p. 60 min before morphine. Data are expressed as mean (of at least 8 animals per group ± s.e.mean) time spent (s) in the drug paired minus vehicle paired side (D–S) during the subsequent 15 min test session. *$P<0.05$, significantly different from the time spent on the vehicle paired side (Student's paired t test).

(Figure 4a), indicating the unbiased nature of the apparatus. The daily administration of PD 140548 (0.001–1.0 mg kg^{-1}, i.p.) 1 h before the sub-maximal dose of morphine (2 mg kg^{-1}, s.c.) dose-dependently decreased the development of place preference to the μ-receptor agonist ($t(12)=5.556$, $P<0.0001$ for saline, $t(8)=3.591$, $P<0.01$ for 0.001 mg kg^{-1}, PD 140548, $t(7)=1.586$, $P>0.05$ for 0.01 mg kg^{-1}, $t(11)=1.005$, $P>0.05$ for 0.1 mg kg^{-1}, and $t(9)=0.003019$, $P>0.05$ for 1.0 mg kg^{-1}) (Figure 4c). In contrast, similar administration of CI-988 (0.01–1.0 mg kg^{-1}, i.p.) did not affect the morphine conditioned place preference (Figure 4b). PD 140548 or CI-988 administered (0.01–1.0 mg kg^{-1}, i.p.) alone did not produce place preference or aversion (data not shown).

Effect of CCK receptor antagonists on morphine drug discrimination

Under the conditions of the test of stimulus generalisation, morphine dose-dependently (0.3–3.0 mg kg^{-1}, i.p.) induced drug lever responding (Table 1). At the training dose of 3.0 mg kg^{-1} morphine, 75% of the rats responded on the drug appropriate lever ($F(5,24)=5.4$, $P<0.01$) (Table 1). Naloxone when administered immediately before the training dose of morphine, dose-dependently (0.01–0.3 mg kg^{-1}, s.c.), antagonised the discriminative stimulus ($F(4,59)=3.6$, $P<0.01$). In contrast, s.c. administration of PD 140548 (0.01–10.0 mg kg^{-1}) or CI-988 (0.01–1.0 mg kg^{-1}) before morphine failed to reduce drug lever responding (Table 1). Furthermore, similar administration of either compound failed to induce drug lever responding in the absence of morphine (Table 1).

Effect of CCK receptor antagonists on morphine induced gastrointestinal dysfunction

The oral administration of 1 ml of charcoal via gavage in control animals, travelled approximately 75% of the length of

Table 1 Effect of CI-988 and PD 140548 on the morphine discriminative stimulus

Drug	Dose ($mg\,kg^{-1}$)	No. responding[a]	No. choosing drug lever[b]	%[c]	Error[d] score	Responses per 15 min[e]
Generalization to stimulus						
Saline	0.0	9/9	1/9	11.1	8.7±0.9	1320.8±80.3
Morphine	0.3	9/9	1/9	11.1	8.6±1.0	1453.4±91.6
	0.5	6/6	1/6	16.7	8.3±1.7	1202.7±130.3
	0.75	7/7	3/7	42.9	5.9±2.0	1389.3±67.4
	1.0	9/9	8/9	88.9	2.2±1.1**	1334.8±68.6
	3.0	8/8	6/8	75.0	2.5±1.4**	1285.4±87.0
PD 140548	0.01	5/5	1/5	20.0	8.2±1.8	1199.6±52.5
	0.1	6/6	2/6	33.3	7.2±1.8	1584.3±105.0
	1.0	6/6	1/6	16.7	8.3±1.7	1436.5±98.4
CI-988	0.01	6/6	0/6	0.0	10.0±0.0	1522.0±128.6
	0.1	6/6	1/6	16.7	8.7±1.3	1449.0±48.7
	1.0	6/6	0/6	0.0	10.0±0.0	1441.2±97.8
Antagonism of stimulus						
Saline	0.0	32/32	26/32	81.3	2.1±0.6	1116.0±52.7
Naloxone	0.01	8/8	6/8	75.0	3.1±1.5	1179.2±93.2
	0.03	8/8	5/8	62.5	4.9±1.6	1114.1±122.9
	0.1	8/8	4/8	50.0	5.3±1.6	1280.6±44.1
	0.3	8/8	2/8	25.0	7.5±1.6**	1209.0±68.3
PD 140548	0.01	6/6	4/6	66.7	3.0±1.9	1095.3±135.9
	0.1	13/13	11/13	84.6	2.1±1.0	1123.5±75.5
	1.0	13/13	9/13	69.2	3.1±1.2	1253.2±54.1
	10.0	6/6	6/6	100.0	0.8±0.8	1142.5±59.1
CI-988	0.01	8/8	7/8	87.5	1.4±1.2	1187.5±120.7
	0.1	8/8	8/8	100.0	0.5±0.3	1057.6±132.9
	1.0	8/8	7/8	87.5	1.3±1.3	1123.0±156.3

[a] Number of animals making at least 10 responses on either the drug or saline lever. [b] Number of animals making fewer responses on the saline lever before completing 10 on the drug lever. [c] The percentage (%) of animals responding on the drug lever. [d] Mean number of responses on the saline lever when the number on the drug lever is 10 (animals showing no preference for the drug lever were given error score of 10). [e] Response rate is expressed as the total number of lever presses during the 15 min test session. **$P<0.01$, significantly different from morphine + vehicle treated controls (ANOVA followed by Dunnett's t test).

the intestine. Morphine (1.0–10.0 mg kg^{-1}, s.c.) dose-dependently decreased the distance travelled by the charcoal meal in the intestine ($F(3,16)=19.754$, $P<0.0001$) (Figure 5a). The effect of a submaximal dose of 5.0 mg kg^{-1} morphine was completely antagonized by naloxone (1.0 mg kg^{-1}, s.c.) ($F(5,34)=15.732$, $P<0.0001$) when administered 30 min before the charcoal meal. In contrast, CI-988 (0.01–1.0 mg kg^{-1}, i.p.) and PD 140548 (0.01–1.0 mg kg^{-1}, i.p.) were both ineffective at modulating the action of the μ-opioid receptor agonist (Figure 5b, c).

Discussion

There are three main findings of the present study. Firstly, the selective CCK_A receptor antagonist PD 140548 was found to antagonize the acquisition of morphine conditioned place preference. In addition, the selective CCK_B receptor antagonist CI-988 potentiated morphine-induced antinociception in both acute and tonic models of nociception and prevented the development of morphine tolerance. Finally, neither of the CCK receptor antagonists affected morphine-induced inhibition of gut motility.

The ability of PD 140548 to block the development of morphine conditioned place preference was consistent with a recent study demonstrating that devazepide (formerly L-364,718 and MK-329), a chemically dissimilar but selective CCK_A receptor antagonist (Chang & Lotti, 1986), antagonized acquisition of morphine conditioned place preference (Higgins *et al.*, 1992). Like devazepide, PD 140548 administered alone failed to induce place aversion in this paradigm. It has been suggested that the conditioned place preference paradigm measures the reinforcing properties of substances of abuse (Carr *et al.*, 1989). Thus, the data of the present study support the hypothesis that CCK_A antagonists can antagonize the positive reinforcing properties of morphine (Higgins *et al.*, 1992). It remains to be seen, however, whether such antagonists will also block the rewarding properties of other drugs of abuse.

Convincing evidence indicates that the positive reinforcing behaviour of morphine involves activation of the mesolimbic A10 dopaminergic pathway (Spyraki *et al.*, 1983; Phillips *et al.*, 1984). Immunohistochemical studies have shown that CCK coexists with dopamine in the projection of this pathway from the ventral tegmental area (VTA) to the nucleus accumbens (Hokfelt *et al.*, 1980), an area widely thought to be involved in mediating drug-induced reinforcement behaviour (Vaccarino & Koob, 1984; White *et al.*, 1991). Moreover, neurochemical studies have shown that CCK, through an interaction with CCK_A receptors, appears to facilitate dopamine release in the posterior nucleus accumbens (Marshall *et al.*, 1991). CCK_A receptor antagonist prevention of the overactivation of the mesolimbic pathway caused by morphine may therefore explain the blockade of its reinforcing behaviour by PD 140548 and devazepide. The ineffectiveness of PD 140548 alone would imply, that there is no tonic modulation of the system by CCK. However, while the performance of CCK_A receptor antagonists in the conditioned place preference paradigm would suggest that such an action might limit the onset of opiate dependence, it has been previously demonstrated that devazepide has no effect on the intensity of the characteristic symptoms that appeared following naloxone-precipitated withdrawal after chronic morphine (Dourish *et al.*, 1988). At present, it is difficult to interpret this apparently anomalous situation but it may be related to simple differences in the respective experimental models.

The inability of CI-988 and L-365,260 (Higgins *et al.*, 1992) to block morphine conditioned place preference would argue against CCK_B receptors playing a role in the positive reinforcing properties of morphine. In this respect, it is interesting to note that CI-988, unlike L-365,260 (Higgins *et al.*, 1992) did not potentiate morphine conditioned place preference or by

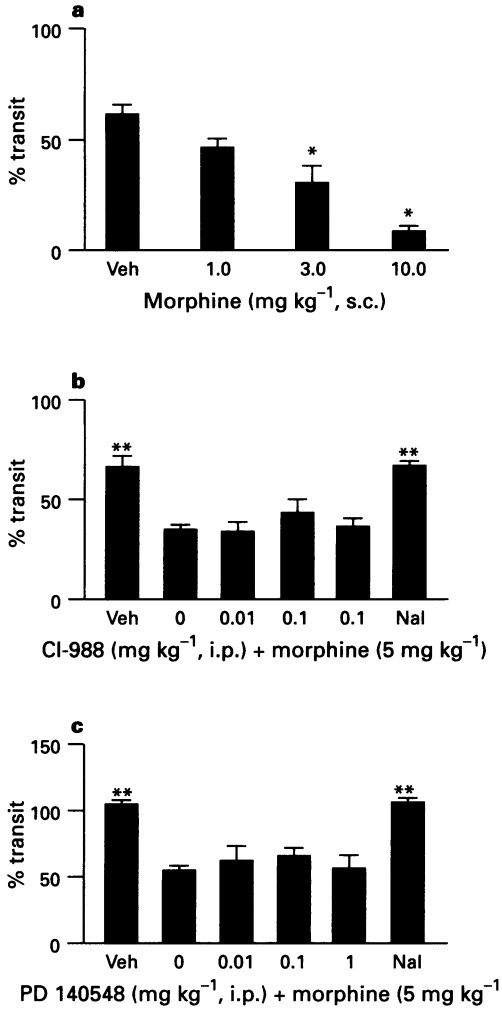

Figure 5 Effect of (b) CI-988 and (c) PD 140548 on (a) morphine-induced inhibition of gastrointestinal motility in the rat following oral administration of a charcoal meal 15 min before test. Morphine was administered s.c. 25 min before the charcoal meal. Naloxone (Nal; 1 mg kg^{-1}, s.c.) or CCK antagonists were administered 5 and 20 min before morphine, respectively. The results are shown as the mean (vertical bars represent s.e.mean) % gastrointestinal transit in at least 6 animals per group. $^{*}P<0.01$, significantly different from vehicle (Veh) control group or ** from morphine-treated controls (ANOVA followed by Dunnett's t test).

itself appear to induce positive reinforcement in animals. This would suggest that CI-988 may have little potential for abuse which is further supported by the findings that cessation of chronic administration of CI-988 does not lead to withdrawal (Hughes *et al.*, 1990; Singh *et al.*, 1992).

The results of the present study and those of previous studies show that CCK_A receptor antagonists are unable to antagonize the discriminative stimulus and self-administration properties of morphine (Higgins *et al.*, 1994; Jackson *et al.*, 1994) which would again appear to be inconsistent with the ability of such compounds to antagonize the morphine conditioned place preference. However, in a similar manner to the situation involving naloxone-precipitated withdrawal, there are major differences between drug discrimination, self-administration and conditioned place preference tests that may explain the different data obtained with CCK_A receptor antagonists in these models. Firstly, in the conditioned place preference paradigm, CCK_A receptor antagonists have been shown to block the development of morphine place preference but it is not known whether they would affect the conditioned place preference once it has been established. In drug discrimination and self-administration studies the ability of CCK receptor antagonists to modify the opioid receptor mediated effects was examined against established behaviour (Higgins *et al.*, 1994; Jackson *et al.*, 1994). No study to date has been published where the ability of CCK receptor antagonists was examined to block the acquisition of the discriminative stimulus and self-administration properties of morphine. Secondly, while there is wide agreement that conditioned place preference measures the rewarding properties of the conditioning compound (Carr *et al.*, 1989), what drug discrimination measures may be open to some dispute, especially in cases where the training compound has more than one action. Furthermore, it is known that some compounds which are not abused induce strong discriminative stimuli, for example, the convulsant pentylenetetrazol (Lal, 1979). It is possible, therefore, that the morphine discriminative stimulus involves a property other than its positive reinforcing action as in case of the conditioning place preference paradigm.

In both acute and chronic models of nociception CI-988 potentiated the antinociceptive response to a threshold dose of morphine and was considerably more potent than the CCK_A selective antagonist PD 140548. The effectiveness of CI-988 in the rat tail flick is consistent with previous studies which showed that the chemically dissimilar compounds L-365,260 (CCK_B selective) and high doses of devazepide (CCK_A selective), potentiate morphine analgesia (Dourish *et al.*, 1990). This supports the predominant involvement of CCK_B receptors in the mediation of endogenous CCK modulation of opiate antinociception in the rat CNS. However, while much of this information has been obtained in thermal (Dourish *et al.*, 1990; Wiesenfeld-Hallin *et al.*, 1990; Lavigne *et al.*, 1992) and mechanical (Dourish *et al.*, 1990) models of nociception employing an acute, high threshold noxious stimulus in rodents, clinical pain elicited by either tissue/nerve injury or disease is a tonic phenomenon with a persistent, lower intensity that may also have an associated inflammatory component. The present study has now extended the investigation of CCK-opiate interactions to include modulation of morphine antinociception in the formalin model of prolonged rather than acute nociception. The exact site of CCK-opiate interaction is unclear but it may involve supraspinal sites (Noble *et al.*, 1993), for example, in the midbrain and/or brainstem. However, the effect of CI-988 in the formalin model cannot exclude the possibility that the CCK-opiate interaction may take place at a spinal level. Thus, endogenous CCK released from cells in the superficial dorsal horn may exert a tonic inhibition of opiate antinociception by acting on the terminals of the descending inhibitory fibres activated by morphine in the PAG/medulla.

Irrespective of the site of this interaction, the results indicate that CCK_B receptor antagonists can potentiate morphine antinociception during acute and tonic nociception and lend support to the hypothesis that CCK antagonists may have a potential use as adjuncts to opiate therapy for the treatment of chronic pain conditions. In this regard, the potential ability to prevent the development of opitae tolerance would be of considerable benefit. The effectiveness of CI-988 at preventing morphine tolerance while having little influence on the onset of dependence was consistent with the hypothesis that independent molecular mechanisms may be responsible for mediating opiate tolerance and dependence (Ling *et al.*, 1984; Panerai *et al.*, 1987). A note of caution is necessary, however, over the choice of CCK antagonist involved in any possible combination therapy with an opiate drug, for although the CCK_B receptor predominates in rodent spinal cord it is the CCK_A receptor that appears to be the major type in primate species (Hill *et al.*, 1988). A mixed $CCK_{A/B}$ antagonist, such as PD 142898 (Boden *et al.*, 1993), may therefore offer the best compromise.

It is noteworthy that in both nociception models, CI-988 displayed similar bell-shaped dose-response curves to those previously documented for L-365,260 (Dourish *et al.*, 1990). However, in other behavioural tests the effects of CI-988 and L-365,260 have been observed over a much wider dose range (Singh *et al.*, 1991). The reason for the bell-shaped dose-re-

sponse curves is unclear but they may make selection of the dose difficult in the clinic. However, it is interesting to note that in a previous study, CI-988 potentiated morphine analgesia over a wide dose-range (Wiesenfield-Hallin *et al.*, 1990).

Electrophysiological recordings of single dorsal horn nociceptive neurones have shown that CCK receptor antagonists do not enhance the antinociceptive action of spinally injected morphine in a carrageenan-induced inflammation model (Stanfa & Dickenson, 1993). In the same study, morphine was found to be considerably more potent in the inflamed animal when compared to normal rats, suggesting that there appears to be a reduced availability of CCK in the spinal cord following carrageenan-inflammation (Stanfa *et al.*, 1992; Stanfa & Dickenson, 1993). Both the failure of CCK antagonists to potentiate the action of morphine and the increased potency of morphine in the carrageenan inflammation model are in disagreement with the observations of the present study. Thus, we have found little difference in the potency of morphine between the tail flick test and both phases of the formalin model and have demonstrated the ability of CI-988 to potentiate morphine antinociception during the tonic phase of the formalin test which also involves inflammation. There are important differences between the two models which may help to explain the inconsistent data. Thus, in the carrageenan-induced inflammation model the animals are under halothane anaesthesia, whereas the animals in the present study were fully conscious. It is possible that anaesthesia may affect some inflammatory mechanism, e.g. decrease the release of CCK. It has also been suggested that CCK receptor antagonists do not enhance morphine analgesia in a familiar environment but do so in animals under stress that may be caused by exposure to novel surroundings (Lavigne *et al.*, 1992). It could be argued therefore that the present experiments were conducted under conditions to which the animals were not fully acclimatised. In contrast, no such stress would exist in animals under anaesthesia. Finally, in the present study, morphine was administered systemically, whereas in the electrophysiological study it was applied locally to the dorsal horn nociceptive neurones. If the CCK-opiate interaction were to involve predominantly a supraspinal, rather than spinal site of action then it may not be detectable by recordings from the dorsal horn cells.

In conclusion, the results of the present study have demonstrated that the selective CCK_A receptor antagonist PD 140548 blocked the positive reinforcing properties of morphine in the conditioned place preference paradigm while the CCK_B receptor selective antagonist CI-988 was ineffective. In contrast, low doses of CI-988 potentiated the antinociceptive actions of morphine in both acute and tonic nociception models the latter with an associated inflammation. CI-988 and PD 140548 did not appear to influence morphine-induced gastrointestinal dysfunction. This suggests that unlike the CNS, there is no interaction between CCK and opiate systems within the gut. Collectively, this study would suggest that a selective CCK_A receptor antagonist may reduce the abuse potential of morphine while a selective CCK_B and/or CCK_A receptor (primate species) antagonist may be able to potentiate its analgesic action, without a concomitant increase in tolerance, dependence and constipation which are considered to be clinically limiting adverse events associated with opiate maintenance therapy in the treatment of chronic pain. Thus, the therapeutic ratio for morphine may be considerably enlarged through a reduction in the dose of the μ-opioid receptor agonist resulting from combination therapy with a CCK receptor antagonist. However, in view of the differential modulation of morphine behaviour by CCK_A and CCK_B receptor antagonists and possible species variation, a mixed $CCK_{A/B}$ receptor antagonist (e.g. PD 142898; Boden *et al.*, 1993), might therefore represent the preferred profile for clinical evaluation.

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