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Modulation of the in vivo actions of morphine by the mixed $CCK_{A/B}$ receptor antagonist PD 142898

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Abstract

The ability of a mixed $CCK_{A/B}$ receptor antagonist PD 142898 (benzenebutanic acid, ß-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]carbonyl]amino]-1-oxopropyl]amino]-[1*S*-[1α[*S**(*R**)]-2ß]]) to modulate the antinociceptive, positive reinforcing and gastrointestinal actions of morphine was investigated in the rat. PD 142898 antagonised the development and maintenance of morphine (2.0 mg/kg, s.c.) induced conditioned place preference at 0.1 mg/kg, i.p. However, it potentiated the antinociceptive action of a subthreshold dose of morphine in the radiant tail flick model at doses of 0.001 and 0.01 mg/kg, s.c. Furthermore, PD 142898 (0.0001–1.0 mg/kg, s.c.) also potentiated the antinociceptive action of morphine (1.0 mg/kg, s.c.) against the late phase of formalin response associated with inflammation at the dose of 0.001 mg/kg. PD 142898 (0.001 mg/kg, s.c.) blocked the development of tolerance to morphine in the formalin test. It failed (0.001–1.0 mg/kg, i.p.) to modulate the inhibitory action of morphine (5.0 mg/kg, s.c.) on gastrointestinal transit as measured using the charcoal meal test. It is argued that the effect of PD 142898 in the conditioned place preference test involves antagonism of CCK_A receptors, whilst the potentiation of the antinociceptive action of morphine is mediated via blockade of CCK_B receptors. These results suggest that the mixed $CCK_{A/B}$ receptor antagonist may potentiate the analgesic action of morphine, block the development of tolerance without a concomitant increase in constipation and may also reduce the abuse potential of the opiate.

Keywords: Conditioned place preference; Tail-flick test; Formalin paw test; Charcoal meal

1. Introduction

Cholecystokinin (CCK) is a neuropeptide widely distributed in the central nervous system (CNS). It is thought to play a role in control of appetite, the modulation of dopaminergic pathways and in the transmission of nociceptive information. More recent data suggests that CCK also modulates anxiety and panic related behaviours (Woodruff and Hughes, 1991). CCK interacts with at least two distinct types of receptor (Innis and Snyder, 1980; Moran et al., 1986; Wank et al., 1992): CCK_A receptors, the abundant form in peripheral tissues (Zetler, 1984; Van Dijk et al., 1984; Chang and Lotti, 1986) but also found in discrete nuclei of the CNS (Hill et al., 1987a,1988), and CCK_B receptors, which represent the predominant form in the rodent CNS (Pélaprat et al., 1987; Hill and Woodruff, 1990).

A modulatory role for CCK in the control of nociception has been suggested by a number of observations; the distribution of CCK (Stengaard-Pedersen and Larsson, 1981; Beinfeld and Palkovits, 1982; Fuji et al., 1985; Gall et al., 1987) and its receptors (Van Dijk et al., 1984; Hill and 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 nociception. Whole animal studies have shown that CCK modulates the response to noxious stimuli in a complex manner. Higher, 'pharmacological', doses produce a naloxone-reversible antinociception (Barbaz et al., 1989; Hill et al., 1987b) but lower, more 'physiological', doses reverse the antinociception produced by morphine and endogenous opioids (Faris et al., 1983; Barbaz et al., 1989; Dourish et al., 1990). Further studies in rodents have shown that CCK_B receptor antagonists potentiate the antinociceptive actions of morphine and endogenous opioids (Watkins et al., 1985a,b; Dourish et al., 1988, 1990;

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Wiesenfeld-Hallin et al., 1990; Maldonado et al., 1993) and block the development of tolerance to this action of the μ-receptor agonist (Dourish et al., 1988, 1990; Kellstein and Mayer, 1991).

Immunohistochemical studies have shown that CCK coexists with dopamine in the mesolimbic A10 dopaminergic pathway, that projects from the ventral tegmental area to the nucleus accumbens (Hökfelt et al., 1980). This pathway is widely thought to be involved in mediation of reinforcement behaviour (Spyraki et al., 1983; Phillips et al., 1984). Neurochemical studies have shown that activation of CCK_A receptors in the posterior nucleus accumbens facilitates dopamine release, whilst activation of CCK_B receptors leads to a decrease in the catecholamine release (Marshall et al., 1991; Crawley, 1992). Behavioural studies have shown that the selective CCK_A receptor antagonist devazepide can block the development of the rewarding properties of morphine in the rat conditioned place preference paradigm (Higgins et al., 1992).

Taken together, these observations suggest that selective CCK_A receptor antagonists may reduce the abuse potential of morphine whilst selective CCK_B receptor antagonists may potentiate its analgesic action. It has been suggested that combination therapy with a selective CCK_B receptor antagonist would increase the therapeutic ratio of morphine. The present study investigates the ability of a mixed $CCK_{A/B}$ receptor antagonist PD 142898 (Boden et al., 1993; Singh et al., 1995), to modulate the in vivo actions of morphine. Here we report that the individual effects of selective CCK_A and CCK_B receptor antagonists on the actions of morphine are all displayed in the profile of the mixed antagonist.

2. Methods and materials

2.1. 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, UK). Animals were housed in groups of 6–10 under a 12 h light/dark cycle (lights on at 07 h 00 min) with free access to food and water. All behavioural tests were carried out between 09:00 h and 17:00 h.

2.2. Models of nociception

2.2.1. Radiant heat tail flick 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 with 30–40 min inter-trial interval) prior to drug administration. Radiant heat was adjusted to attain a mean baseline of 2.5–3.5 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 percent of maximum possible effect (% MPE), calculated as: % MPE $= [(TL - BL)/(10 - BL)] \times 100$. PD 142898 was administered s.c. 20 min before a subthreshold dose of morphine (5 mg/kg, i.p.). For clarity only the data obtained from the test carried out at 40 min after morphine administration is shown.

2.2.2. 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 hind paw 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 hind paw. Immediately following the formalin injection, licking/biting of the injected hind paw was scored in 5 min bins for 60 min. Formalin produced a typical biphasic response. The results are expressed as mean combined licking/biting time for the early phase (0–10 min) and late phase (20–35 min). Threshold doses of morphine, 3 mg/kg for the early and 1 mg/kg for the late phase, were administered s.c. 20 min before formalin. PD 142898 (0.0001–1 mg/kg) was administered s.c. 20 min before morphine. Threshold doses of morphine were chosen from dose-response analysis. Separate groups of animals were used for the early and late phases of the formalin response.

The ability of PD 142898 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 morphine, beginning with 1 mg/kg (i.p.) on day 1 and culminating to 16 mg/kg on days 5 and 6 (1, 2, 4, 8, 16 and 16 mg/kg, i.p.). Animals received a co-administration of either saline or PD 142898 (0.001 mg/kg, s.c.) prior to each injection of saline/morphine. On the morning of day 7 animals were challenged with either saline or morphine (4 mg/kg, s.c.), 20 min prior to the injection of formalin. Immediately following the formalin injection, licking/biting of the injected paw was scored following the same procedure described above.

2.3. Effect of PD 142898 on 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 × 25 × 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 × 8 × 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, four saline and four drug conditioning sessions were carried out 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 in each compartment was determined. No pretreatment was given to the rats on this day. For antagonism studies, PD 142898 was administered i.p. 60 min before morphine.

To examine the effect of PD 142898 on maintenance of morphine conditioned place preference, rats received four saline and four drug conditioning sessions over 4 days as described above. The animals then received one additional saline and drug session on day 5 during which PD 142898 was administered i.p. 60 min before morphine. Subjects were then tested for place preference (test 1) on day 6, as described above. These animals received a further four saline and four drug conditioning sessions with PD 142898 being administered i.p. 60 min before morphine during the drug conditioning sessions. The day after the last conditioning sessions subjects were again tested for morphine conditioned place preference (test 2). All treatments were counter-balanced between compartments.

2.4. Effect of PD 142898 on morphine induced inhibition of gastrointestinal transit

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. PD 142898 was given i.p. 20 min before a submaximal dose of morphine (5.0 mg/kg, s.c.). Naloxone (1.0 mg/kg, s.c.) was administered 5 min before morphine.

2.5. Drugs

PD 142898 (benzenebutanic acid, ß-[[3-(1*H*-indol-3-yl)-2-methyl-2-[[[(2-methylcyclohexyl)oxy]carbonyl]amino]-1-oxopropyl]amino]-[1*S*-[1α[*S**(*R**)]-2ß]]) was synthesised at Parke-Davis Neuroscience Research Centre, Cambridge, UK. Morphine sulphate was obtained from Savory and Moore, Cambridge, UK. Charcoal and naloxone were obtained from Sigma Chemical Co. (Poole, UK). All drugs were dissolved in 0.9% w/v NaCl except charcoal which was suspended in 5% gum acacia.

Drugs were administered either i.p. or s.c. in a volume of 1 ml/kg for rats weighing over 100 g and 2 ml/kg 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.

2.6. Statistics

All results were analysed using one-way analysis of variance (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.

3. Results

3.1. Effect of PD 142898 on the antinociceptive action of morphine

3.1.1. Radiant heat tail flick model

A threshold dose (5 mg/kg, i.p.) of morphine (approximately 20% of the MPE) which induced significant antinociception was chosen from dose-response analysis (data not shown) for combination studies with PD 142898. PD 142898 (0.0001–1 mg/kg, s.c.), administered 20 min prior to morphine, caused a dose-dependent potentiation of the antinociceptive effect of morphine (Fig. 1a), the response reaching the level of statistical significance at both 0.001 and 0.01 mg/kg. Higher doses of 0.1 and 1 mg/kg PD 142898, however, were ineffective at modulating the response to morphine (Fig. 1a).

3.1.2. Rat paw formalin test

Threshold doses of morphine not inducing significant reduction of the early (3 mg/kg, s.c.) and late phase (1 mg/kg, s.c.) of the formalin response and were chosen from dose-response analysis (data not shown) for interaction studies with PD 142898. PD 142898 (0.0001–1.0 mg/kg, s.c.) potentiated the antinociceptive effect of morphine during the late phase of the formalin response at the dose of 0.001 mg/kg but was ineffective at higher doses (Fig. 1c). PD 142898 demonstrated a similar effect in the early phase of the formalin response but the potentiation at 0.001 mg/kg failed to reach significance (Fig. 1b).

In the morphine tolerance study, a dose of 0.001 mg/kg (s.c.) PD 142898 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 early (Fig. 2a) and late (Fig. 2b) phases of the formalin licking/biting response following the acute challenge with morphine (4 mg/kg, s.c.). In contrast, chronic pretreat-

ment with PD 142898 (0.001 mg/kg, s.c.) prior to each morphine injection appeared to prevent the development of morphine tolerance as shown by the reduced intensity of the late phase licking/biting response to the morphine challenge (4 mg/kg, s.c.) (Fig. 2b). This was not significantly different to the group of naive rats receiving the single, acute dose of morphine following chronic treatment with saline/saline (Fig. 2b). PD 142898 (0.001 mg/kg, s.c.), appeared to have a small nonsignificant effect on morphine tolerance in the early phase of the response (Fig. 2a). Chronic treatment with PD 142898, prior to a twice daily injection of saline, produced no intrinsic antinociceptive activity.

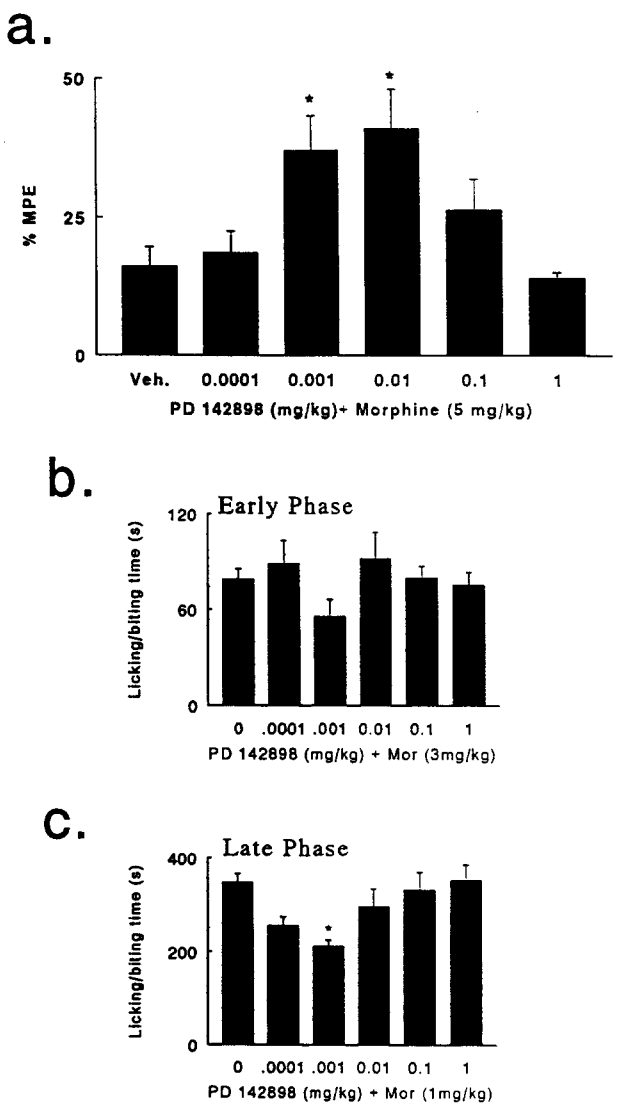

Fig. 1. Effect of PD 142898 on the antinociceptive action of morphine in the rat radiant heat tail flick test and formalin paw test. PD 142898 was administered (s.c.), 20 min before a threshold dose of morphine. For the tail flick test (a), test latencies (TL) were expressed as percent of the maximum possible effect (%MPE = [(TL − BL)/(10 − BL)] × 100). For the formalin test, formalin (50 μl of 5% solution) was administered into the intraplantar surface of the left hind paw 20 min after morphine. The duration of licking/biting of the injected paw was determined for the early phase (b) and the late phase (c). Results are shown as the mean (vertical bars represent ± S.E.M.) of at least ten animals per group. * $P < 0.05$, significantly different from vehicle + morphine treated controls (ANOVA followed by Dunnett's t-test).

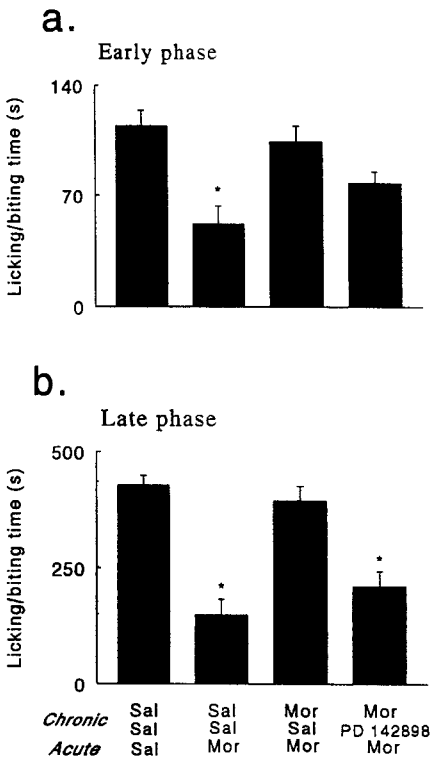

Fig. 2. Effect of PD 142898 on the development of tolerance to the antinociceptive action of morphine in the rat paw formalin test. Animals were subjected to an incremental twice daily dosing of either saline (Sal) or morphine (Mor) with prior co-administration of either saline or PD 142898 (0.001 mg/kg, s.c.) for 6 days. On the morning of day 7 animals were challenged with either saline or morphine (4 mg/kg s.c.), 20 min prior to the injection of formalin. Formalin (50 μl of 5% solution) was administered into the intraplantar surface of the left hind paw 20 min after morphine. The top panel (a) represents time spent licking/biting of the injected paw during the early phase (0–10 min post formalin) of the response, whilst the bottom panel (b) represents time spent licking/biting of the injected paw during the late phase (15–35 post formalin) of the response. Results are shown as the mean (vertical bars represent ± S.E.M.) of at least ten animals per group. * $P < 0.05$, significantly different from chronic Mor/Sal + Acute Mor group (ANOVA followed by Dunnett's t-test).

3.2. Effect of PD 142898 on morphine conditioned place preference

Four trials with saline alone produced no preference to either compartment indicating the unbiased nature of the apparatus. The daily administration of PD 142898 (0.001–1.0 mg/kg, i.p.) 1 h before the sub-maximal dose of morphine (2 mg/kg, s.c.) decreased the development of place preference to the μ-opioid receptor agonist at 0.1 mg/kg (Fig. 3a). However, PD 142898 at the higher dose of 1 mg/kg failed to reduce the effect of morphine (Fig. 3a). PD 142898 administered (0.01–1.0 mg/kg, i.p.) alone did not produce place preference or aversion (data not

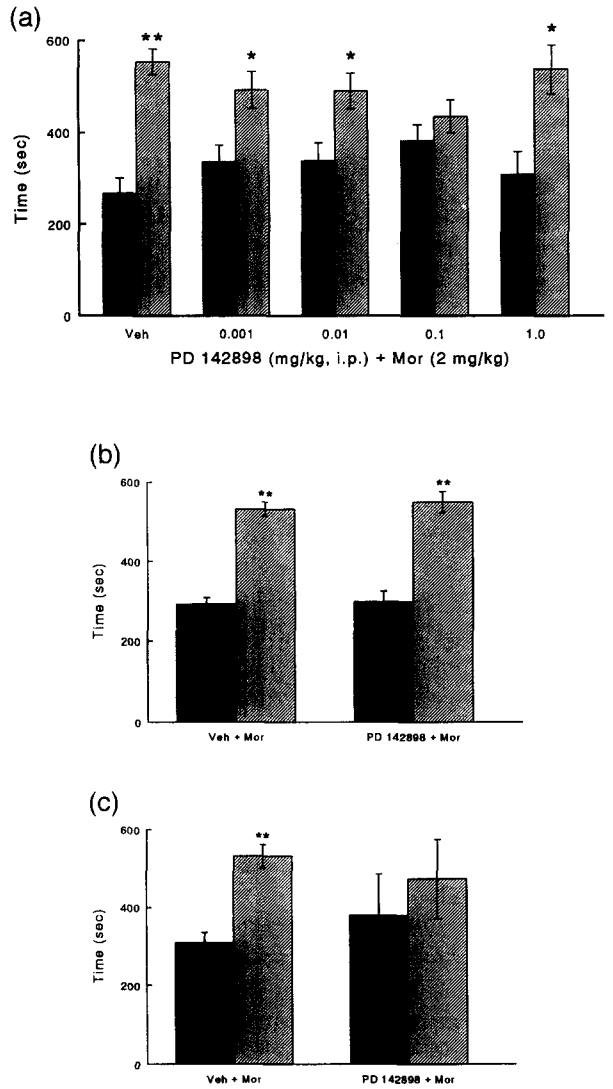

Fig. 3. Effect of PD 142898 on development and maintenance of morphine conditioned place preference in the rat.
a: *Development.* Rats were given four saline and four morphine (Mor; 2 mg/kg, s.c.) conditioning sessions over 4 days. Immediately afterwards, animals were confined to the appropriate conditioning compartment for 45 min. PD 142898 was administered i.p. 60 min before each of the morphine conditioning sessions. Conditioned place preference was measured by allowing the animal free exploration of the entire box for 15 min. The cumulative amount of time spent in each compartment was determined.
b, c: *Maintenance.* Animals received four saline and four drug conditioning sessions over 4 days as described above. The animals then received one additional saline and drug session on day 5 during which PD 142898 was administered i.p. 60 min before morphine. Subjects were then tested for conditioned place preference (b) on day 6. These animals received further four saline and four drug conditioning sessions with PD 142898 being administered i.p. 60 min before morphine during the drug conditioning sessions. The day after the last conditioning session subjects were again tested for morphine conditioned place preference (c). Data are expressed as mean (of at least six animals per group ± S.E.M.) time spent (s) in the vehicle paired (black columns) or drug paired (hatched columns) side during the 15 min test session. * $P < 0.05$, ** $P < 0.01$, significantly different from the time spent on the vehicle paired side (Student's paired *t*-test).

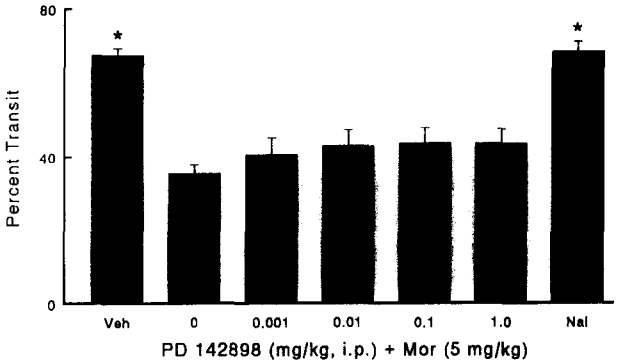

Fig. 4. Effect of PD 142898 on morphine-induced inhibition of gastrointestinal transit in the rat. Morphine (Mor) was administered s.c. 25 min before the charcoal meal. The animals were killed 15 min after charcoal and the gastrointestinal transit was determined. PD 142898 was administered 20 min and naloxone (Nal; 1 mg/kg, s.c.) 5 min before mor. The results are shown as the mean (vertical bars represent S.E.M.) percent gastrointestinal transit in at least six animals per group. * $P < 0.05$ significantly different from morphine treated controls (ANOVA followed by Dunnett's *t*-test).

shown). In a separate group of animals, administration of a single dose of PD 142898 (0.1 mg/kg, i.p.) 1 h before the last drug conditioning session did not decrease the morphine conditioned place preference (Fig. 3b). This established place preference to morphine was maintained following an additional four conditioning trials (Fig. 3c). However, the daily administration of PD 142898 (0.1 mg/kg, i.p.) 1 h before each of the further four morphine conditioning sessions decreased the established conditioned place preference (Fig. 3c).

3.3. Effect of PD 142898 on morphine induced inhibition of gastrointestinal transit

The oral administration of 1 ml of charcoal via gavage in control animals, travelled approximately 75% of the length of the intestine in 15 min. Morphine (1.0–10.0 mg/kg, s.c.), dose-dependently decreased the distance travelled by the charcoal meal in the intestine (data not shown). The effect of a submaximal dose of 5.0 mg/kg morphine was completely antagonised by naloxone (1.0 mg/kg, s.c.) when administered 5 min before morphine. In contrast, PD 142898 (0.001–1.0 mg/kg, i.p.) was ineffective at modulating the effect of the μ-opioid receptor agonist (Fig. 4).

4. Discussion

It has been reported that PD 142898 possesses nanomolar affinity for CCK_A and CCK_B binding sites (CCK_B $IC_{50} = 4.2$ nM, CCK_A $IC_{50} = 3.8$ nM) and is a functional antagonist at both of these receptor types (Boden et al., 1993; Singh et al., 1995). Other studies have shown that PD 142898 shows anxiolytic-like action in animal models

(Singh et al., 1995). The present results show that PD 142898 is able to modulate the in vivo actions of morphine. The main findings of the present study are that PD 142898 can block the development and maintenance of morphine-induced conditioned place preference and is able to potentiate the antinociceptive action of the μ-opioid receptor agonist. However, it does not affect the inhibitory action of morphine on gut transit.

The ability of PD 142898 to block the development of morphine-induced conditioned place preference is likely to be mediated via the CCK_A receptor. It has been previously demonstrated that the selective CCK_A receptor antagonist devazepide can block the development of morphine conditioned place preference (Higgins et al., 1992). It has been reported that the CCK_B receptor has little or no effect on opiate reinforcement behaviour in rats (Higgins et al., 1992). The present study extends these findings by showing that PD 142898 can also block the maintenance of morphine conditioned place preference. This suggests that PD 142898 may be able to block morphine reinforcement behaviour after it becomes established and this may be more relevant to the clinical situation. The nucleus accumbens is widely thought to be involved in mediation of reinforcement behaviour (Spyraki et al., 1983; Phillips et al., 1984). Neurochemical studies have shown that activation of CCK_A receptors in the posterior nucleus accumbens facilitates dopamine release (Marshall et al., 1991; Crawley, 1992). The CCK_A receptor mediated blockade of overactivation of mesolimbic pathway induced by morphine may be responsible for the antagonism of the positive reinforcing properties of the opiate by PD 142898. The failure of PD 142898 to induce place aversion suggests that there is no tonic modulation of this pathway by CCK.

PD 142898 potentiated the antinociceptive action of a subthreshold dose of morphine in both acute and tonic models of nociception. Previously, it has been reported that selective CCK_B receptor antagonists L-365,260 and CI-988 are more potent than the CCK_A receptor antagonist devazepide at potentiating morphine antinociception (Dourish et al., 1988, 1990; Wiesenfeld-Hallin et al., 1990). The present results show that the magnitude of the effect of PD 142898 is not greater than that reported with selective CCK_B receptor antagonists. This is consistent with the suggestion that CCK modulates morphine analgesia predominantly via interactions at CCK_B receptors in the rat. Most of the information regarding the CCK modulation of μ-opioid receptor mediated analgesia has been obtained using thermal and mechanical models of nociception employing an acute, high threshold stimulus in rodents (Dourish et al., 1990; Wiesenfeld-Hallin et al., 1990). However, clinical pain elicited by either tissue or nerve injury is a tonic event with persistent but low intensity that may also have an associated inflammatory component. The present study shows that a mixed $CCK_{A/B}$ receptor antagonist can also potentiate morphine antinociception during prolonged noxious stimulation associated with inflammation. The ability of PD 142898 to block the development of tolerance to the antinociceptive action of morphine in the formalin test would also be beneficial during the treatment of chronic pain.

It is interesting to note that CCK antagonists show bell-shaped dose response curves in studies involving CCK and morphine interactions. It has been reported that the CCK_B receptor antagonist L-365,260 potentiates morphine analgesia with a bell-shape dose response curve (Dourish et al., 1990). Other studies have shown that devazepide blocks development of morphine conditioned place preference also with a bell-shape dose response curve (Higgins et al., 1992). In this regard the results presented here show that PD 142898 potentiated morphine analgesia and blocked morphine conditioned place preference over a narrow dose range with the effects disappearing at higher doses. However, it has been reported that CCK_B receptor antagonists potentiate the antinociceptive action of endogenous enkephalins over a much wider dose range than that observed in combination with morphine (Valverde et al., 1994). Furthermore, in these studies the magnitude of potentiation induced by CCK_B receptor antagonist was much greater than that seen with morphine (Valverde et al., 1994). The reasons for these observations are not understood but it suggests that care must be taken in selecting the dose of CCK antagonists in combination therapy with morphine.

In conclusion, the results presented here show that the mixed $CCK_{A/B}$ receptor antagonist PD 142898 appears to potentiate the analgesic action of morphine, without a concomitant increase in constipation. It may also block the development of tolerance and reduce the abuse potential of the opiate. Tolerance, dependence and especially constipation are considered to be clinically limiting side effects associated with opiate maintenance therapy in the treatment of chronic pain. The combination therapy with a CCK antagonist and morphine should lead to a reduction in constipation as a lower dose of the μ-receptor agonist will be required to induce analgesia. Therefore, combination therapy with a CCK receptor antagonist will increase the therapeutic ratio of morphine. The central site involved in mediating the interaction between CCK and opiate analgesia is not known but the spinal cord may play an important role. In this regard it should be noted that the CCK_B receptor predominates in the rodent species whilst the CCK_A receptor is the major type in primates. Therefore a mixed $CCK_{A/B}$ receptor antagonist, such as PD 142898 may represent an optimal compound for evaluation as adjunct to opiate therapy for the treatment of chronic pain conditions.

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