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CI988, a selective antagonist of cholecystokinin$_B$ receptors, prevents morphine tolerance in the rat

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1 The effect of chronic treatment with CI988, a recently developed selective antagonist of cholecystokinin type-B receptors (CCK_B receptors) on the tolerance to morphine analgesia was studied in rats with the hot plate test.

2 Morphine tolerance was induced with the use of two paradigms. Morphine was injected i.p. either in a schedule of increasing doses (1–32 mg kg^{-1}) twice daily for 6 days or at a fixed dose (3 mg kg^{-1}) daily for 29 days.

3 In both series of experiments, tolerance to the analgesic effect of morphine was prevented by simultaneous treatment with i.p. CI988. Chronic treatment with only CI988 daily for up to 29 days did not reduce the analgesic effect of a weekly injection of morphine.

4 CI988 did not diminish the physical dependence to morphine, as examined with naloxone precipitated withdrawal.

5 The present results provide evidence that chronic treatment with a selective CCK_B receptor antagonist could prevent tolerance to the analgesic effect of morphine without affecting morphine-induced physical dependence. Application of CCK antagonists may be clinically important in treating chronic pain patients by preventing morphine tolerance and by eliminating the need to increase morphine doses to unacceptable levels.

Keywords: Morphine; tolerance; dependence; cholecystokinin$_B$ receptor; hot plate test

Introduction

The peptide cholecystokinin (CCK) occurs in many areas of the CNS, primarily as the sulphated octapeptide CCK-8 (Vanderhaeghen *et al.*, 1975; Dockray, 1976; Rehfeld, 1978; Williams *et al.*, 1987). It fulfils many of the criteria for a neurotransmitter and may have a role in various CNS functions, including pain modulation (see Vanderhaeghen & Crawley, 1985; Baber *et al.*, 1989 for review). It has been suggested that CCK is an endogenous opioid antagonist. Thus, exogenously applied CCK antagonizes the analgesic effect of morphine and endogenous opioids (Itoh *et al.*, 1982; Faris *et al.*, 1983; Han *et al.*, 1985; Watkins *et al.*, 1985; Wiesenfeld-Hallin & Duranti, 1987), CCK antagonists potentiate morphine analgesia (Watkins *et al.*, 1984; 1985; Katsuura & Itoh, 1985; Dourish *et al.*, 1988; 1990) and reverse or prevent morphine tolerance (Watkins *et al.*, 1984; Tang *et al.*, 1984; Panerai *et al.*, 1987; Dourish *et al.*, 1988; 1990).

Two subtypes of CCK receptors exist, which have been denoted as CCK_A and CCK_B (Innis & Snyder, 1980; Saito *et al.*, 1980; Moran *et al.*, 1986). CCK_A receptors are located principally in the peripheral nervous system of rodents, whereas the majority of CCK receptors in brain and spinal cord of rodents belong to the B type (Moran *et al.*, 1986; Hill & Woodruff, 1990). Recently, selective antagonists for CCK_A (MK-329, L-365,031) and CCK_B (L-365,260, CI988) receptors have been developed (Evans *et al.*, 1986; Hughes *et al.*, 1990), making it possible to examine the subtypes of CCK receptor involved in CCK-opioid interaction. Thus, Dourish *et al.* (1988, 1990) have shown that MK-329, L-365,031 and L-365, 260 potentiate morphine analgesia and prevent morphine tolerance in rats with the rank order of potency L-365, 260 > MK-329 > L-365,031, which corresponds to their potency in blocking the central CCK_B receptors, indicating a critical role for these receptors in modulation of the opioid system. We have also recently demonstrated that CI988 (formerly PD 134308), another highly selective and potent CCK_B receptor antagonist (Hughes *et al.*, 1990), strongly enhances the analgesic effect of morphine, as examined in both electrophysiological and behavioural studies (Wiesenfeld-Hallin *et al.*, 1990).

The object of the present study was to examine the effect of chronic treatment with CI988 on the development of tolerance to the analgesic effect of morphine. We used two experimental paradigms to induce morphine tolerance, during 6 and 29 days respectively, in order to evaluate the short and long term effect of CCK_B receptor blockade on morphine analgesia. The latter is particularly interesting since Kellstein & Mayer (1990) have recently reported that chronic administration of the weak and non-selective CCK receptor antagonists, proglumide and lorglumide, reverses the enhancement of morphine analgesia induced by acute pretreatment with the antagonists. Furthermore, the possible effect of chronic treatment with CI988 on the physical dependence induced by chronic morphine was also examined by means of naloxone-precipitated withdrawal. Since it has been shown previously that repeated nociceptive testing could induce behavioural tolerance to morphine analgesia (Advokat, 1981; Sherman *et al.*, 1982; Advokat & Isaac, 1983; Milne *et al.*, 1989), we also tested whether behavioural tolerance developed in our experimental paradigm over long term, repeated nociceptive testing.

Methods

Animals

Eighty male Sprague-Dawley rats weighing 200 g at the beginning of the experiments were used (Alab AB, Stockholm, Sweden) and housed and maintained in groups of five in standard translucent plastic laboratory cages under a 12 h light/dark cycle (on at 06 h 00 min). Water and laboratory chow were available *ad libitum*.

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Analgesia testing

The analgesic effect of morphine was tested with a hot plate (IITC, Woodland Hills, CA, U.S.A.) at 54.0 ± 0.2°C. The latency to licking a hindpaw was determined to an accuracy of 0.1 s. Before any drugs were administered, the rats were habituated by being tested on the hot plate for 5 days to obtain a stable control response value (Figure 1). Our previous experience indicated this procedure is very important to obtain a stable baseline response since the lick latency of rats decreased as a factor of repeated testing in the hot plate test. On the day of testing for analgesia, the rats were pre-tested three times on the hot plate. The values from the last two pre-tests were averaged and taken as baseline latency (BL). The animals were tested on single trials at 10 min intervals for 60 min after 1 mg kg^{-1} morphine i.p., or at 20 or 30 min intervals for 150 min after 3 mg kg^{-1} morphine i.p., and the postdrug latency (DL) was recorded. The drug effect was calculated as the percentage change in reaction time from baseline latency values by use of the formula [(DL-BL)/BL] × 100%.

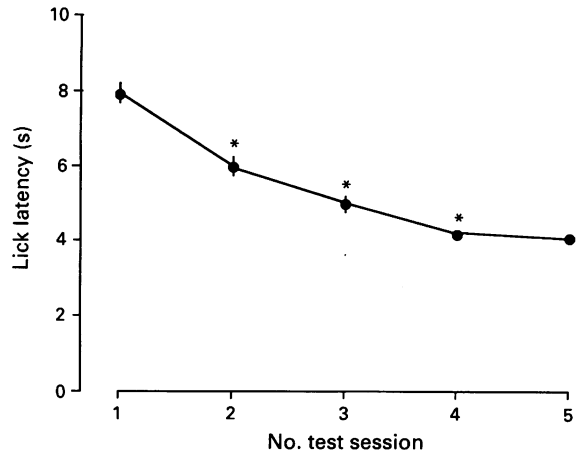

Figure 1 The effect of habituation on the lick latency of rats in the hot plate test in 5 test sessions performed daily at the same time each day. Each test session consists of two or three trials and the lick latencies in each session were averaged. ANOVA indicated a significant difference in the response latency across test sessions ($F_{4,395}$ = 134.437, $P < 0.0001$). The Newman-Keuls test indicated that up to the 4th test session, the lick latency is significantly shorter than in the previous session. * $P < 0.01$ compared to latency on the previous day.

Experimental design

Two series of experiments were conducted. In series I, thirty rats were randomly divided into 4 treatment groups as indicated in Table 1. Morphine tolerance was induced by a 6 day schedule of twice daily injection of incremental doses of morphine, beginning at 1 mg kg^{-1} and culminating with 32 mg kg^{-1} on the morning prior to the analgesia test (see Table 1 for details). Animals were injected daily with i.p. saline, CI988 (0.5 mg kg^{-1}), saline plus morphine or CI988 plus morphine. On the afternoon of day 6 the rats were challenged with 1 mg kg^{-1} morphine and the analgesic effect was evaluated.

In series II, opioid tolerance was induced with a daily i.p. injection of 3 mg kg^{-1} morphine. Fifty rats were divided into five groups, as indicated in Table 2. The analgesic effect of 3 mg kg^{-1} i.p. morphine was assessed on day 1 in four groups. The rats were then injected daily with saline plus saline, CI988 (1 mg kg^{-1}) plus saline, CI988 plus morphine, or saline plus morphine (see Table 2 for details). The analgesic effect of morphine was tested in these four groups of rats on days 8, 15, 22 and 29 to follow the development of tolerance. In group 5, ten rats received daily i.p. injection of saline and weekly analgesic testing without morphine. On day 29, these rats were injected i.p. with 3 mg kg^{-1} morphine and its analgesic effect was examined.

All testing was carried out in a 'blind' fashion.

Naloxone-precipitated withdrawal

The method of naloxone-precipitated withdrawal was used to assess the effect of CI988 on opioid dependence. On day 7 of series I and day 30 of series II, all rats were injected with 1 mg kg^{-1} i.p. naloxone and the appearance of various characteristic withdrawal symptoms were noted and recorded during the following 20 min.

Drugs

CI988 (Parke-Davis, Cambridge, U.K., see Hughes *et al.*, 1990 for structure), morphine hydrochloride (Kabi, Stockholm, Sweden) and naloxone (Dupont, Wilmington, Del., U.S.A.)

Table 1 Chronic treatment regime used in series I to study the effects of CI988 on morphine tolerance and dependence

	Treatment (twice daily)			
Day	*Group 1*	*Group 2*	*Group 3*	*Group 4*
1	Morphine 1 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
2	Morphine 2 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
3	Morphine 4 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
4	Morphine 8 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
5	Morphine 16 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
6 (08 h 00 min)	Morphine 32 mg kg^{-1} + saline	Saline + saline	CI988 + morphine	CI988 + saline
6 (14 h 00 min)	All groups challenged with 1 mg kg^{-1} morphine i.p.			
7	All groups challenged with 1 mg kg^{-1} naloxone i.p.			

n = 5–10 animals in each group. The dose of CI988 used was 0.5 mg kg^{-1}.

Table 2 Chronic treatment regime used in series II

	Treatment (daily)				
Day	*Group 1*	*Group 2*	*Group 3*	*Group 4*	*Group 5*
1	Morphine + test + saline	Morphine + test + CI988	Morphine + test + CI988	Morphine + test + saline	Saline + test
2–7	Saline + morphine	CI988 + morphine	CI988 + saline	Saline + saline	Saline
8	Morphine + test + saline	Morphine + test + CI988	Morphine + test + CI988	Morphine + test + saline	Saline + test
9–14	Saline + morphine	CI988 + morphine	CI988 + saline	Saline + saline	Saline
15	Morphine + test + saline	Morphine + test + CI988	Morphine + test + CI988	Morphine + test + saline	Saline + test
16–21	Saline + morphine	CI988 + morphine	CI988 + saline	Saline + saline	Saline
22	Morphine + test + saline	Morphine + test + CI988	Morphine + test + CI988	Morphine + test + saline	Saline + test
23–28	Saline + morphine	CI988 + morphine	CI988 + saline	Saline + saline	Saline
29	Morphine + test + saline	Morphine + test + CI988	Morphine + test + CI988	Morphine + test + saline	Morphine + test
30	All groups of rats were challenged with $1\,mg\,kg^{-1}$ naloxone				

$n = 10$ animals in each group. The dose of morphine was $3\,mg\,kg^{-1}$ and CI988 was $1\,mg\,kg^{-1}$. The temporal order of treatments is shown in the table.

were dissolved in 0.9% saline and injected i.p. in a volume of $1\,ml\,kg^{-1}$.

Statistics

All data are expressed as mean ± s.e.mean. Statistics were calculated with analysis of variance (ANOVA) followed by Dunnett's test or Newman-Keuls test.

Results

Effect of CI988 on morphine tolerance in series I

Morphine $1\,mg\,kg^{-1}$, i.p., elicited moderate analgesia for 50 min in group 2, which received twice daily injections of saline (Figure 2). The effect was similar to that seen in normal rats (Wiesenfeld-Hallin *et al.*, 1990). In contrast, in group 1, which received incremental doses of morphine for 6 days, the challenge dose of $1\,mg\,kg^{-1}$ morphine did not evoke an analgesic effect (Figure 2). Morphine caused analgesia in rats in group 3, which received chronic treatment with CI988 prior to injection with morphine (Figure 2). Chronic administration of CI988 by itself (group 4) did not reduce morphine-induced analgesia (Figure 2). ANOVA with repeated measures indicated that the analgesic effect of morphine was significantly different when all 4 groups were compared ($F_{3,130} = 9.669$, $P < 0.0001$), but there was no difference among groups 1, 3 and 4 ($F_{2,110} = 2.856$, $P > 0.05$), indicating a lack of morphine tolerance in rats of group 3, which received CI988 along with morphine.

Effect of CI988 on morphine tolerance in series II

Morphine at a dose of $3\,mg\,kg^{-1}$, i.p., had a strong analgesic effect for about 2 h on the first testing occasion. Daily injection of i.p. saline and weekly analgesic testing for 4 weeks (group 5) did not reduce the analgesic effect of morphine on day 29. After daily i.p. injection of $3\,mg\,kg^{-1}$ morphine (group 1), the analgesic effect of morphine was significantly diminished on day 8 and totally disappeared by day 15 and thereafter (Figure 3). In the other three groups of rats in which morphine plus CI988, CI988 or saline were injected daily (groups 2, 3 and 4 respectively), the analgesic effect of morphine was unchanged on day 8. A significant reduction of morphine's analgesic effect was observed in all three groups of rats on day 15 (Figure 3). The effect of morphine in rats in these three groups were still, however, significantly stronger than that in group 1, which received daily injections of morphine, and no difference between these three groups of rats was found ($F_{2,27} = 0.399$, $P > 0.05$). There was no further reduction of the analgesic effect of morphine in these three groups of rats up to 29 days, when the experiments were terminated. Two factor ANOVA (treatment groups vs time) indicated that the difference in the analgesic effect of morphine

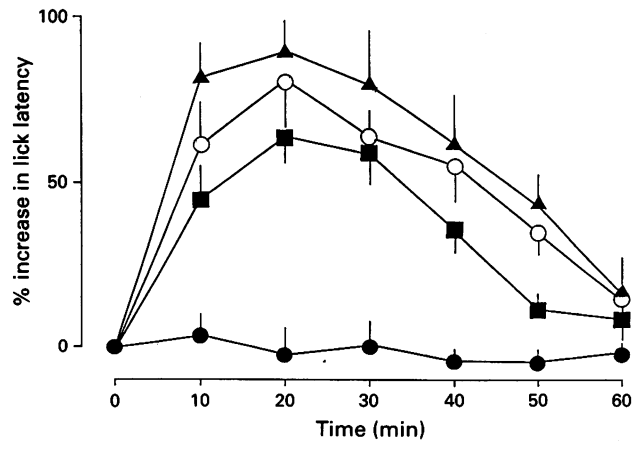

Figure 2 Effect of i.p. morphine ($1\,mg\,kg^{-1}$) on the lick latency of rats on the hot plate on day 6 of series I in groups of rats which received twice daily i.p. injection of increasing doses of morphine (●), saline (○), morphine plus CI988 ($0.5\,mg\,kg^{-1}$) (■) or CI988 (▲). The challenge dose of morphine was injected at time 0 and vertical bars represent s.e.mean $n = 5$–10 in each group. ANOVA with repeated measures indicated that the analgesic effect of morphine was significantly different when all 4 groups were compared ($F_{3,130} = 9.669$, $P < 0.0001$), but there was no difference in groups which received chronic saline, CI988 plus morphine or CI988 ($F_{2,110} = 2.865$, $P > 0.05$).

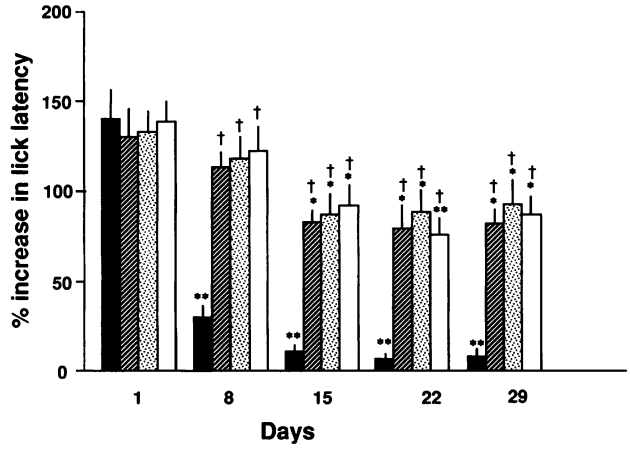

Figure 3 Effect of i.p. morphine ($3\,mg\,kg^{-1}$) on the lick latency on the hot plate in groups of rats in series II which received daily injection of morphine (solid columns), morphine plus CI988 ($1\,mg\,kg^{-1}$) (hatched columns), CI988 (stippled columns) or saline (open columns). The time elapsed from the beginning of the chronic treatment is indicated under the columns. The maximal increase of lick latency for each rat, which is usually achieved 40 or 60 min after injection of morphine, is presented. The vertical lines indicate s.e.mean. $n = 10$ in each group. † $P < 0.005$ compared to the group which received daily injection of morphine; * $P < 0.05$ and ** $P < 0.01$ compared to the respective group on day 1.

among the four groups over the entire experimental period was significant ($F_{3,180} = 38.176$, $P < 0.001$). The reduction of the analgesic effect of morphine over time was also significant ($F_{4,180} = 30.688$, $P < 0.001$). ANOVA applied to data from rats in groups 2, 3 and 4 revealed that there was no significant difference in the analgesic effect of morphine ($F_{2,135} = 0.645$, $P > 0.5$), but the decrease of the analgesic effect of morphine over time was significant ($F_{4,135} = 10.731$, $P < 0.001$), among these three groups (Figure 3).

There was no significant change in baseline reaction latency in all groups of rats over the entire period of observation (not shown).

Effect of chronic treatment with CI988 on morphine dependence

Severe characteristic withdrawal symptoms were induced in group 1 of series I after i.p. injection of $1\,mg\,kg^{-1}$ naloxone (Table 3). Chronic treatment with CI988 had no effect on the appearance or intensity of these symptoms (Table 3). Very slight withdrawal symptoms were observed in some animals that only received daily injection of CI988, which on average were not significantly different from saline controls (Table 3).

The expression of withdrawal symptoms was much weaker in group 1 of series II, which may be related to the administration of a lower dose of morphine ($3\,mg\,kg^{-1}$) during chronic treatment (Table 4). CI988 again failed to prevent the occurrence of these symptoms and in rats which received a daily injection of CI988 and weekly injection of morphine, slight withdrawal symptoms were also present, which again were not significantly different from the saline group (Table 4). There were no detectable withdrawal symptoms in rats which received a daily injection of saline and a weekly injection of morphine.

Discussion

The present study presents clear evidence which indicates that chronic treatment with CI988, a highly selective antagonist of

Table 3 Effects of CI988 on naloxone-precipitated morphine withdrawal in rats in series I

	Groups			
Withdrawal symptoms	*Morphine + saline*	*Saline + saline*	*Morphine + CI988*	*Saline + CI988*
Ptosis	100	0	90	20
Irritability	100	0	100	20
Diarrhoea	80	0	60	10
Teeth chattering	100	0	100	20
Writhing	60	0	40	0
Wet dog shakes	8.0 ± 1.4*	0	6.7 ± 1.1*	2.4 ± 0.6

Rats were injected twice daily with increasing doses of morphine (group 1), saline (group 2), morphine plus CI988 (group 3) and CI988 (group 4) for 6 days (see Table 1 for details). Naloxone-precipitated withdrawal was assessed on day 7. All rats were injected i.p. with naloxone $1\,mg\,kg^{-1}$ prior to a 20 min observation period. The frequency of wet dog shakes (mean ± s.e.mean) during 20 min is indicated, other values are the percentages of animals in each group showing the symptom during the test ($n = 5$–10 in each group). ANOVA indicated that the frequency of wet dog shakes is significantly different among 4 groups ($F_{3,26} = 12.785$, $P < 0.001$). * $P < 0.01$ compared to group 2 and group 4 with Newman-Keuls test.

Table 4 Effects of CI988 on naloxone-precipitated morphine withdrawal in series II

	Groups			
Withdrawal symptoms	*Morphine + saline*	*Morphine + CI988*	*Saline + CI988*	*Saline + saline*
Ptosis	70	70	20	0
Irritability	60	60	20	0
Diarrhoea	0	0	0	0
Teeth chattering	80	80	0	0
Writhing	10	0	0	0
Wet dog shakes	3.9 ± 1.4*	3.3 ± 0.9*	1.7 ± 0.6	0.2 ± 0.2

Rats were injected daily with morphine (group 1), CI988 plus morphine (group 2), CI988 (group 3) and saline (group 4) for 29 days (see Table 2 for details). Naloxone precipitated withdrawal was assessed on day 30. All animals were injected i.p. $1\,mg\,kg^{-1}$ naloxone prior to a 20 min observation. Wet dog shakes are frequency (mean ± s.e.mean) during the test, other values are the percentages of animals in each group showing the symptom during the test ($n = 10$ in each group). ANOVA indicated that the frequency of wet dog shakes is significantly different among the 4 groups ($F_{3,36} = 3.457$, $P < 0.05$). * $P < 0.05$ compared with group 4.

the CCK_B receptor, prevents the development of tolerance to the analgesic effect of morphine in rats. These data support the results from previous studies in which a number of selective and non-selective CCK antagonists were shown to prevent or reverse tolerance to morphine (Tang *et al.*, 1984; Watkins *et al.*, 1984; Panerai *et al.*, 1987; Dourish *et al.*, 1988; 1990). Since CI988 is a highly selective and potent CCK_B antagonist, our results also support the notion that the CCK-opioid interaction occurs through CCK_B receptors in rodents (Dourish *et al.*, 1990).

In series II of the present study, we observed that the analgesic effect of morphine is reduced over time in rats that received daily injection of CI988 plus morphine. Since similar phenomena also occur in rats which receive daily injection of saline, it is clear that this effect does not reflect the inefficacy of CI988 in preventing morphine tolerance over a long period of time, but some other factors. A number of previous studies have shown that repeated nociceptive testing could induce behavioural tolerance to morphine analgesia (Advokat, 1981; Sherman *et al.*, 1982; Advokat & Isaac, 1983; Milne *et al.*, 1989). We tested for the occurrence of behavioural tolerance by including another group of rats which received daily injection of saline and a weekly nociceptive test and were challenged with morphine only once, after 4 weeks. The observation that the analgesic effect of morphine is similar in this group to that in rats examined at the beginning of the experiments fails to support the hypothesis of behavioural tolerance to nociceptive testing in rats which were injected with morphine weekly, but not daily. Thus, it is possible that a weekly injection of morphine causes the development of some tolerance, albeit significantly less than that caused by daily injection. Tolerance development was not prevented by CI988, as on the day of nociceptive testing, CI988 was injected 2–3 h later than morphine to avoid the acute potentiation of morphine's analgesic effect by the CCK_B antagonist (Wiesenfeld-Hallin *et al.*, 1990). However, we cannot exclude the possibility that the single morphine injection (group 5) had a discriminative stimulus property, which led to the full analgesic effect of the opioid. Nonetheless, the analgesic effect of morphine was not totally diminished in rats which received a weekly injection of morphine and was significantly stronger than in rats which received a daily injection during the entire 29 day experimental period (Figure 3). It should be noted that our procedure of habituating the animals on the hot plate test for five days before data collection may have contributed to the increased sensitivity of this assay of morphine's analgesic effect, since unhabituated animals would respond with a longer latency at the first testing session than in later sessions, which may be incorrectly interpreted as tolerance.

The observation that there is no difference between groups of rats which receive daily CI988, saline and CI988 plus morphine therefore indicates that (1) tolerance induced by daily morphine injection is prevented by CI988 and (2) chronic treatment with CI988 *per se* does not reduce morphine analgesia. The latter conclusion is at variance with the recent report by Kellstein & Mayer (1990), who showed that chronic treatment with the CCK antagonists proglumide and lorglumide reduces the analgesic effect of morphine. Although difference in route of drug administration (intrathecal vs systemic) or in nociceptive tests used (tail flick vs hot plate) may contribute to this difference, it should be noted that the use of proglumide and its analogues as tools to study the function of CCK has been questioned because of the poor potency and low selectivity of such drugs for the CCK_B receptor (Makovec *et al.*, 1986; Rezvani *et al.*, 1987; Gaudreau *et al.*, 1990).

The mechanism for the prevention of morphine tolerance by CCK antagonists has not been studied in detail. Since exogenously applied CCK antagonizes morphine-induced analgesia and injection of morphine increases the release of CCK in the spinal cord *in vivo* (Tang *et al.*, 1984), it is possible that chronic treatment with morphine may induce up-regulation of the endogenous CCK system, which antagonizes the action of morphine, and tolerance occurs. Chronic treatment with CCK antagonists may suppress such an up-regulation, thus preventing the development of morphine tolerance. Neurochemical studies examining changes in CCK levels in CNS after injection of morphine have given inconsistent results. While Faris *et al.* (1986) reported that acute or chronic morphine increases the level of CCK in the hypothalamus in rats, Rosén & Brodin (1989) failed to observe significant changes in CCK content in brain and spinal cord after acute morphine injection. However, it should be pointed out that examination of changes in CCK synthesis after acute and chronic morphine treatment may be more relevant in this context, since changes in tissue CCK content may have been masked by increased release of this neuropeptide.

There are, however, other possibilities by which a CCK_B antagonist may prevent morphine tolerance. For example, it has been suggested that tolerance to the analgesic effects of morphine may be related to classical conditioning (Siegel, 1976; Siegel & MacRae, 1984; Dafters & Odber, 1989). According to this theory, the administration of morphine can be viewed as a conditioning trial, with environmental cues present at the time of drug administration constituting the conditioned stimulus, and the acute pharmacological stimulation constituting the unconditioned stimulus. Tolerance is interpreted in this theory as a manifestation of the acquisition of a compensatory conditioned response between the pharmacological effects of the drug and those environmental cues which always precede these pharmacological effects. Since CI988 is a very strong anxiolytic agent (Hughes *et al.*, 1990), it is therefore possible that it may depress the reaction and memory of animals to negative environmental cues, resulting in the prevention of tolerance.

Chronic treatment with the CCK_B antagonist did not diminish naloxone-precipitated withdrawal symptoms induced by chronic morphine, indicating that CCK receptor blockade is ineffective in preventing morphine dependence. A similar conclusion has been reached by Panerai *et al.* (1987) and Dourish *et al.* (1988, 1990). It seems likely that the development of morphine dependence is different from that of tolerance and does not involve changes in the endogenous CCK system (see Ling *et al.*, 1984). The weak withdrawal symptoms observed in rats that were daily injected only with CI988 supports our previous results that CI988 causes a weak depression of the spinal flexor reflex in a naloxone-reversible manner. Since CI988 has negligible affinity for all three subtypes of opioid receptors (Hughes *et al.*, 1990), it is unlikely that this effect of CI988 results from a direct action on opioid receptors, but suggests a tonic CCK-ergic inhibition of the endogenous opioid system by the CCK_B antagonist. Nonetheless, the withdrawal symptoms seen in CI988-treated rats were weak, not significantly different from animals which received chronic treatment with saline and were even less apparent after long term than after short term treatment (Tables 3 and 4).

In conclusion, the present results demonstrate that CI988 effectively prevents morphine tolerance in rats over an extended testing period. Provided that similar mechanisms operate in man, this and other CCK receptor antagonists may be clinically useful in managing chronic pain by preventing morphine tolerance. It should be noted, however, that although the dominant receptor subtype for CCK is the B-type in rat CNS, recent studies have reported the existence of a substantial level of CCK_A binding sites in primate brain and spinal cord (Hill *et al.*, 1990; Hill & Woodruff, 1990). Therefore, one cannot rule out the possibility that a CCK_A antagonist may be more relevant in man.

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