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In mononeuropathic rats, the enhancement of morphine antinociception by L-365,260, a selective CCK_B receptor antagonist, depends on the dose of systemic morphine and stimulus characteristics

Juhana J. Idänpään-Heikkilä [a,*], Serge Perrot [b], Gisèle Guilbaud [c], Valérie Kayser [c]

[a] *Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki, FIN-00014 Helsinki, Finland*
[b] *Service de Rhumatologie, Hôpital Cochin, 27 Rue du Faubourg St. Jacques, 75014 Paris, France*
[c] *Unité de Recherches de Physiopharmacologie du Système Nerveux, I.N.S.E.R.M. U161, 2 Rue d'Alésia, 75014 Paris, France*

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Abstract

The ability of the selective cholecystokinin$_B$ (CCK_B) receptor antagonist L-365,260 (0.2 mg/kg s.c.) to modulate the antinociceptive action of relatively low doses of systemic morphine (0.1, 0.3 and 1.0 mg/kg i.v.) was evaluated using a well established rat model of peripheral unilateral mononeuropathy. Behavioural tests based on both mechanical (vocalization threshold to paw pressure) and thermal (struggle latency after immersion of the paw into a cold (10°C), warm (44°C) or hot (46°C) water bath) stimuli were used. Experiments were performed 2 weeks after the surgery when the pain-related behaviour has fully developed. We demonstrated a differential effect of L-365,260 depending both on the dose of morphine and the test used. Pretreatment with the CCK_B receptor antagonist (0.2 mg/kg) inverted the ineffectiveness of the lowest dose (0.1 mg/kg i.v.) of morphine against the noxious (46°C) thermal stimulus, and the effect of the combination was equal to that seen after the dose 0.3 mg/kg of morphine alone. Likewise, in the mechanical test, the already enhanced effect of this dose (0.1 mg/kg) of morphine on the nerve-injured paw was further increased (by 4-fold) after L-365,260 pretreatment. These effects were abolished by naloxone (0.01 mg/kg i.v.). However, the effects of the higher doses (0.3 and 1.0 mg/kg i.v.) of morphine against the mechanical or noxious thermal stimuli were not significantly enhanced by pretreatment with the CCK_B receptor antagonist. Further, L-365,260 was found to be completely ineffective in modulating the responses to morphine at 10°C and at 44°C.

Keywords: Antinociception; Mononeuropathic rat; CCK_B receptor antagonist; L-365,260; Morphine; Naloxone; Vocalization threshold; Struggle latency

1. Introduction

Cholecystokinin (CCK) is an endogenous neuropeptide, widely distributed in the brain and in the spinal cord (Vanderhaeghen et al., 1975; Beinfeld et al., 1981). The central nervous system distribution of CCK parallels that of the endogenous opioids within the pain processing areas, such as laminae I and II of the spinal cord, the periaqueductal gray matter and the dorsomedial thalamus (Stengaard-Pedersen and Larsson, 1981; Beinfeld and Palkovits, 1982), providing anatomical evidence that a functional relationship may exist between these two transmitter systems. In fact, along with its other physiological functions, CCK has been shown to modulate pain transmission and the anti-opioid effects of this peptide are well documented (Itoh et al., 1982; Faris et al., 1983; O'Neill et al., 1989).

The effects of CCK are mediated by CCK_A and CCK_B receptors both of which have recently been cloned (Kopin et al., 1992; Wank et al., 1992; Lee et al., 1993). The majority of the CCK receptors in the central nervous system are of the CCK_B subtype, whereas CCK_A receptors predominate in peripheral tissues (Moran et al., 1986). In agreement with these results, both CCK_A receptor antagonists (Dourish et al., 1988; Rattray et al., 1988) and CCK_B receptor antagonists (Dourish et al., 1990; Wiesenfeld-Hallin et al., 1990) have been reported to enhance morphine-induced antinociception in the rat, but the CCK_B receptor antagonist L-365,260 has been shown to be 40

* Corresponding author. Present address: Unité de Recherches de Physiopharmacologie du Système Nerveux, I.N.S.E.R.M. U161, 2 Rue d'Alésia, 75014 Paris, France. Tel.: (33-1) 4589-3662; Fax: (33-1) 4588-1304.

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times more potent than the CCK_A receptor antagonist L-365,031 in this effect (Dourish et al., 1990).

Nerve damage that affects peripheral nerves leads to abnormal pain states referred to as neuropathic pain. Neuropathic pain may be long-lasting and the individuals show a marked sensitivity to nociceptive stimuli (hyperalgesia). There is also a perception of normally innocuous stimuli being nociceptive, a state referred to as allodynia (Payne, 1986). Neuropathic pain is poorly controlled by currently available medications (see Ollat, 1992 for a review) and the effectiveness of opioids remains debatable.

Various animal models have been developed to investigate neuropathic pain. Of these, the well established rat model of peripheral mononeuropathy produced by persistent moderate constriction of the common sciatic nerve (Bennett and Xie, 1988; Attal et al., 1990), has extensively been studied in our laboratory. We have previously shown that morphine produces dose dependent anti-allodynic effects against mechanical stimuli and has an enhanced effect on the nerve-injured side (Attal et al., 1991; Kayser et al., 1995b; Catheline et al., 1996). In contrast, we have shown that morphine is unable to relieve thermal allodynia (10°C and 44°C), but is effective against noxious (46°C) thermal stimuli (Lee et al., 1994).

The aim of the study was to investigate whether L-365,260 is able to enhance the effect of morphine against mechanical allodynia and noxious thermal stimuli in this model of mononeuropathy. We also wanted to find out whether the selective CCK_B receptor antagonist is able to reinstate the effect of morphine against thermal allodynia.

2. Materials and methods

The Committee for Research and Ethical Issues of the IASP (1983) ethical guidelines were adhered to in these studies. In particular, the duration of the experiments was as short as possible and the number of animals used was kept to a minimum.

2.1. Animals

Male Sprague-Dawley rats (Charles River, France), $n = 226$, weighing 175–200 g on arrival were used. The rats were housed 5 in a cage on a 12 h light/12 h dark cycle. The ambient temperature was kept at 22°C, and the rats had free access to standard laboratory food and tap water. The animals were allowed to habituate to the housing facilities for at least one week before the experiments were begun.

2.2. Surgical procedure

The unilateral peripheral mononeuropathy was produced on the right hind paw according to the method described by Bennett and Xie (1988) and Attal et al. (1990). In brief, the animals were anaesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.). The common sciatic nerve was exposed by blunt dissection at the level of the mid-thigh and 4 loose ligatures (5-0 chromic catgut, about 1 mm spacing) were placed around the nerve taking care not to interrupt the epineural circulation. To minimize the discomfort and possible painful mechanical stimulation, the rats were housed in large cages with saw dust bedding after the surgery. The neuropathic rats were able to eat and drink unaided.

2.3. Behavioral testing

Neuropathic rats were used 2 weeks after surgery. At this time, as described previously, the abnormal pain behaviour is at a stable maximum (Bennett and Xie, 1988; Attal et al., 1990; Desmeules et al., 1995).

All experiments were carried out in a quiet room between 8:00 a.m. and 2:00 p.m. The animals were randomly assigned in groups of 5 (mechanical test) or 10 (thermal tests) for a given series of tests and were not acclimatized to the test situations before. The experiments were performed by two different experimenters both unaware of the drug combinations used.

2.3.1. Thermal tests

Thermal nociception and allodynia-like behaviour were tested by measuring the struggle latency elicited by immersion of the nerve-injured hind paw into a 10°C (non-noxious range, Guilbaud et al., 1990), 44°C (at the noxious threshold, Zimmermann, 1979; Guilbaud et al., 1990) or 46°C (clearly in the noxious range) water bath (Ministat MHUB 11, Bioblock Scientific, France) as extensively described elsewhere (Attal et al., 1990; Lee et al., 1994; Kayser et al., 1995a).

For each rat, a control latency (mean of two consecutive trials 20 min apart, expressed in seconds) was determined before injecting the drugs. After drug administration, struggle latencies were measured every 20 min until they had returned to baseline. The long interval (20 min) between successive measurements was necessary, as in this model, abnormal reactions lasting for more than 15 min following a thermal stimulus have been reported (Bennett and Xie, 1988; Attal et al., 1990; Guilbaud et al., 1990). For the same reason, we could not test the contralateral hind paw as it would have meant a testing interval of 40 min for each paw. We could have used another group of rats to test the paw without nerve injury, but this would not have permitted comparing the injured and non-injured sides either, since this would have concerned different rats.

2.3.2. Mechanical test

The antinociceptive action was similarly determined by measuring the vocalization threshold elicited by pressure on the nerve-injured and the contralateral paw, using the Ugo Basile (Comerio, Italy) analgesymeter. This instru-

ment generates a linearly increasing mechanical force applied by a dome shaped plastic tip (diameter = 1 mm) into the dorsal surface of the rat's hind paw. The tip was positioned between the 3rd and 4th metatarsus (into the sciatic nerve territory) and force applied until the rat squeaked. This centrally integrated response is especially sensitive to analgesic compounds, particularly in this model of mononeuropathy (Attal et al., 1991; Ardid and Guilbaud, 1992; Desmeules et al., 1993; Kayser et al., 1995a). For each rat, a control threshold (mean of 2 consecutive stable thresholds expressed in grams) was determined before injecting the drugs. During the first 30 min following the drug administration, the vocalization thresholds were measured every 5 min and thereafter every 10 min, until they had returned to the level of the control values.

2.4. Drugs and doses

The following drugs were used: morphine hydrochloride (Meram, Paris, France), naloxone hydrochloride (Narcan, Du Pont Pharma, Paris, France), L-365,260 (kindly donated by Dr. R. Hill, Merck Sharp and Dohme) and saline (0.9% NaCl). Morphine and naloxone were diluted in saline and administered i.v. in a volume of 1 ml/kg into the lateral tail vein. The doses of morphine used in this study were from 0.1 to 1.0 mg/kg. These doses have induced significant effects on the vocalization threshold to paw pressure test in this model of neuropathic pain (Attal et al., 1991). Naloxone was injected i.v. at a dose 0.01 mg/kg, that prevented the effect of 0.1 mg/kg i.v. of morphine in normal and arthritic rats (Kayser and Guilbaud, 1983). L-365,260 was dissolved in 10% Tween and alcohol, diluted with saline and administered s.c. in a volume of 1 ml/kg 20 min before morphine. The 0.2 mg/kg dose of L-365,260 was chosen, as it has represented the most effective dose to potentiate morphine antinociception in a previous study of Dourish et al. (1990). In an additional group of neuropathic rats ($n = 64$), we evaluated the effectiveness of various doses of L-365,260 (0.05, 0.1, 0.2 and 0.5 mg/kg) in potentiating the effect of morphine in the vocalization to paw pressure test. In each group, the control rats received the same volume of i.v. or s.c. saline.

2.5. Statistical procedures

Data are expressed as means ± S.E.M. Values in grams (vocalization thresholds) or seconds (struggle latencies) were used for the statistical analyses. Student's *t*-test was used to determine the differences between two means. With three or more means, analysis of variance (ANOVA) was used first. The observed significances were then confirmed with Tukey's HSD test. The statistical procedures were carried out using a statistical computer program (Statgraphics Plus, Manugistics, Rockville, MD, USA). The observed differences were regarded as being significant when the *P*-values were lower than 0.05.

3. Results

3.1. General results

As reported earlier (Lee et al., 1994; Desmeules et al., 1995), 2 weeks after the nerve ligature, struggle latencies to cold, warm and hot stimuli from the nerve-injured paw were decreased by several seconds. At this time, the mean struggle latency at 10°C was 3.8 ± 0.2 s ($P < 0.001$, Tukey's test vs. the preconstriction value 14.7 ± 0.2 s, $n = 32$, Table 1) and 8.7 ± 0.3 s at 44°C ($P < 0.001$ vs. the preconstriction value 14.8 ± 0.1 s, $n = 46$, Table 1). These decreases in struggle latencies, which were observed after stimuli that are in the non-noxious range (10°C) or just at level of the noxious threshold (44°C), were considered to reflect thermal allodynia (Merskey, 1986). The mean struggle latency at the frankly noxious temperature of 46°C (Bennett and Xie, 1988; Attal et al., 1990) was 4.8 ± 0.1 s ($P < 0.001$ vs. the preconstriction value 8.2 ± 0.2 s, $n = 103$, Table 2).

In further agreement with previous studies (Attal et al., 1990; Desmeules et al., 1993; Kayser et al., 1995a), the vocalization thresholds to paw pressure were markedly

Table 1
Maximal mean struggle latencies elicited by immersion of the nerve-injured paw into a cold (10°C) or warm (44°C) water bath before and after injection of saline + morphine or L-365,260 (0.2 mg/kg s.c.) + morphine

Treatment	10°C		44°C	
	Before injection	After injection	Before injection	After injection
Saline + saline	4.2 ± 0.3	4.0 ± 0.4 ($n = 5$)	8.5 ± 1.0	9.1 ± 1.5 ($n = 8$)
L-365,260 + saline	3.8 ± 0.3	3.8 ± 0.5 ($n = 5$)	8.4 ± 0.7	8.8 ± 1.2 ($n = 5$)
Saline + morphine 0.1	4.4 ± 0.3	4.5 ± 0.2 ($n = 6$)	7.8 ± 0.7	8.4 ± 1.8 ($n = 5$)
L-365,260 + morphine 0.1	4.3 ± 0.4	4.6 ± 0.5 ($n = 6$)	7.2 ± 0.3	7.7 ± 1.4 ($n = 6$)
Saline + morphine 1.0	3.5 ± 0.5	4.2 ± 0.6 ($n = 5$)	8.6 ± 0.8	12.1 ± 1.2 [b] ($n = 10$)
L-365,260 + morphine 1.0	3.9 ± 0.3	5.0 ± 1.5 ($n = 5$)	10.1 ± 0.7	13.3 ± 0.9 [a] ($n = 12$)

The results (in seconds) are expressed as mean ± S.E.M., [a] $P < 0.05$, [b] $P < 0.01$ vs. before injection (Tukey's test). The mean preoperative latency was 14.7 ± 0.2 s (at 10°C, $n = 32$) and 14.8 ± 0.1 s (at 44°C, $n = 46$).

Table 2
Maximal mean struggle latencies elicited by immersion of the nerve-injured paw into a hot (46°C) water bath before and after injection of saline + morphine or L-365,260 (0.2 mg/kg s.c.) + morphine

Treatment	46°C	
	Before injection	After injection
Saline + saline	4.5 ± 0.1	4.8 ± 0.2 ($n = 11$)
L-365,260 + saline	4.4 ± 0.4	4.6 ± 0.2 ($n = 10$)
Saline + morphine 0.1	4.8 ± 0.2	5.0 ± 0.4 ($n = 12$)
L-365,260 + morphine 0.1	5.1 ± 0.2	6.9 ± 0.4 [a] ($n = 15$)
Saline + morphine 0.3	4.8 ± 0.2	6.2 ± 0.3 [b] ($n = 11$)
L-365,260 + morphine 0.3	4.9 ± 0.2	7.0 ± 0.6 [b] ($n = 17$)
Saline + morphine 1.0	4.6 ± 0.2	9.0 ± 1.0 [b] ($n = 12$)
L-365,260 + morphine 1.0	5.1 ± 0.2	9.7 ± 0.9 [b] ($n = 15$)

The results (in seconds) are expressed as mean ± S.E.M., [a] $P < 0.05$, [b] $P < 0.01$ vs. before injection (Tukey's test). The mean preoperative latency was 8.2 ± 0.2 s ($n = 103$).

decreased 2 weeks after the nerve ligature. At this time, the mean threshold for the nerve-injured paw was 193 ± 5 g ($P < 0.001$ vs. the preconstriction value 280 ± 8 g, $n = 68$, Table 3). This decreased threshold was considered to reflect mechanical allodynia (Merskey, 1986). As described earlier (Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995a), although diminished for some rats, the mean vocalization threshold for the contralateral paw 273 ± 5 g was not significantly decreased (N.S. vs. the mean preconstriction value 285 ± 6 g, $n = 68$, Table 3).

Morphine, saline or L-365,260 alone or any of the combinations of the drugs had no effect on the general behaviour of the animals.

3.2. Thermal stimuli

3.2.1. Cold (10°C) test

Saline or L-365,260 alone had no effect in this test (Table 1). Similarly, the two doses (0.1 and 1.0 mg/kg) of morphine alone did not modify the struggle latencies in the saline-pretreated rats (Table 1). Morphine was still ineffective after pretreatment with L-365,260 (Table 1).

3.2.2. Warm (44°C) test

Saline or L-365,260 alone had no effect in this test (Table 1). Similarly, the 0.1 mg/kg dose of morphine was ineffective in both pretreatment groups (Table 1). By contrast, a significant increase in the mean struggle latency ($F(9,95) = 3.5$, ANOVA: $P < 0.01$) was observed in the saline-pretreated group after the 1.0 mg/kg dose of morphine. The effect was maximal at 60 min after the morphine injection. At this time, the struggle latency was 140% (of the pre-injection value, $P < 0.01$, Tukey's test, $n = 10$). Recovery was observed by 140 min. L-365,260 pretreatment was ineffective in enhancing the response to this dose (1.0 mg/kg) of morphine. Administration of the combination resulted in a significant overall effect ($F(8,107) = 2.6$, ANOVA: $P < 0.05$), which reached a maximum (132%) at 60 min and lasted for 120 min. The AUCs of these two treatments did not differ significantly ($P = 0.16$).

3.2.3. Hot (46°C) test

Saline, L-365,260 (0.2 mg/kg) alone (Table 2) or morphine (0.1 mg/kg) alone (Fig. 1A and Table 2) were unable to significantly increase the struggle latencies. The combination of L-365,260 (0.2 mg/kg) with the 0.1 mg/kg dose of morphine produced a significant overall effect ($F(7,111) = 3.3$, ANOVA: $P < 0.01$) lasting for 140 min with a maximum at 40 min (Fig. 1A and Table 2).

Morphine (0.3 mg/kg) alone resulted in a significant overall effect ($F(6,70) = 7.6$, ANOVA: $P < 0.001$) lasting for 100 min with a maximum at 40 min (Fig. 1B and Table 2). Similarly, the combination of L-365,260 (0.2 mg/kg) and morphine (0.3 mg/kg) produced a significant effect ($F(6,70) = 7.6$, ANOVA: $P < 0.001$) lasting for 120 min and peaking at 80 min (Fig. 1B and Table 2).

Likewise, both morphine (1.0 mg/kg) and the combination of L-365,260 (0.2 mg/kg) with morphine (1.0 mg/kg) increased the struggle latencies significantly ($F(9,108) = 4.7$, ANOVA: $P < 0.001$ and $F(9,141) = 3.9$, ANOVA: $P < 0.001$, respectively, Fig. 1C and Table 2).

Table 3
Maximal mean vocalization thresholds from the nerve-injured and contralateral paws in the paw pressure test before and after injection of saline + morphine or L-365,260 (0.2 mg/kg s.c.) + morphine

Treatment	Nerve-injured paw		Contralateral paw	
	Before injection	After injection	Before injection	After injection
Saline + saline	156 ± 9	167 ± 15	252 ± 23	255 ± 26 ($n = 8$)
L-365,260 + saline	228 ± 21	225 ± 24	292 ± 14	288 ± 22 ($n = 8$)
Saline + morphine 0.1	189 ± 11	262 ± 17 [b]	284 ± 11	335 ± 18 ($n = 9$)
L-365,260 + morphine 0.1	187 ± 10	372 ± 14 [b]	271 ± 7	357 ± 8 [b] ($n = 11$)
Saline + morphine 0.3	171 ± 6	250 ± 14 [b]	250 ± 8	320 ± 16 [a] ($n = 8$)
L-365,260 + morphine 0.3	170 ± 7	283 ± 28 [b]	242 ± 7	319 ± 23 [b] ($n = 8$)
Saline + morphine 1.0	207 ± 7	388 ± 12 [b]	298 ± 5	453 ± 22 [b] ($n = 8$)
L-365,260 + morphine 1.0	217 ± 12	420 ± 24 [b]	296 ± 23	461 ± 38 [b] ($n = 8$)

The results (in grams) are expressed as mean ± S.E.M., [a] $P < 0.05$, [b] $P < 0.01$ vs. before injection (Tukey's test). The mean preoperative thresholds were 285 ± 6 g and 280 ± 8 g for the two paws, respectively ($n = 68$).

As shown in Fig. 1D, a clear dose-effect relationship for increasing doses of morphine was observed ($F(2,74) = 15.5$, ANOVA: $P < 0.001$). The overall effect of the combination of L-365,260 (0.2 mg/kg) with morphine was found to be significantly different from the effect of morphine alone ($F(1,74) = 4.7$, ANOVA: $P < 0.05$, Fig. 1D). The AUC of the combination of L-360,260 (0.2 mg/kg) and the 0.1 mg/kg dose of morphine was significantly larger than that of morphine (0.1 mg/kg) alone ($F(2,35) = 10.1$, ANOVA: $P < 0.001$ and $P < 0.05$ Tukey's test, Fig. 1D). By contrast, the AUCs after combining the CCK_B receptor antagonist with higher doses (0.3 mg/kg and 1.0 mg/kg) of morphine did not differ significantly from the AUCs of morphine (0.3 an 1.0 mg/kg) alone (Fig. 1D).

3.3. Mechanical stimulus

3.3.1. Nerve-injured paw

Saline and L-365,260 (0.2 mg/kg) alone were both devoid of effects in this test (Table 3). Both morphine (0.1 mg/kg) alone and the combination of L-365,260 (0.2 mg/kg) with morphine (0.1 mg/kg) had an increasing overall effect on the vocalization thresholds ($F(7,63) = 2.7$, ANOVA: $P < 0.05$ and $F(11,93) = 4.3$, ANOVA: $P < 0.001$, respectively, Fig. 2A). The effect of morphine (0.1 mg/kg) alone lasted up to 30 min and peaked (137 ± 5%) at 10 min, whereas that of the combination lasted up to 70 min and peaked (204 ± 13%) at 20 min (Fig. 2A, Table 3).

Both morphine (0.3 mg/kg) alone and the combination of L-365,260 with this dose of morphine resulted in a significant overall elevation of the vocalization thresholds ($F(8,63) = 7.0$, ANOVA: $P < 0.001$ and $F(9,70) = 5.5$, ANOVA: $P < 0.001$, respectively, Fig. 2B). The effect of morphine (0.3 mg/kg) alone lasted up to 50 min and peaked (145 ± 6%) at 10 min. The effect of the combination lasted up to 60 min and peaked at 5 min (Fig. 2B, Table 3).

The vocalization thresholds increased significantly after administration of both morphine (1.0 mg/kg) alone ($F(9,90) = 7.2$, ANOVA: $P < 0.001$) and L-365,260 (0.2 mg/kg) in combination with this dose of morphine ($F(10,108) = 7.7$, ANOVA: $P < 0.001$, Fig. 2C, Table 3). The antinociceptive effect of both treatments lasted up to 60 min and peaked (189 ± 8% and 194 ± 9% for morphine alone and for the combination, respectively) at 20 min (Fig. 2C, Table 3).

A clear-cut dose-effect relationship for morphine was observed in the mechanical test of the nerve-injured paw ($F(2,49) = 22.7$, ANOVA: $P < 0.001$, Fig. 2D). The overall effect of the combination of morphine and L-365,260 was significantly different from that of morphine alone

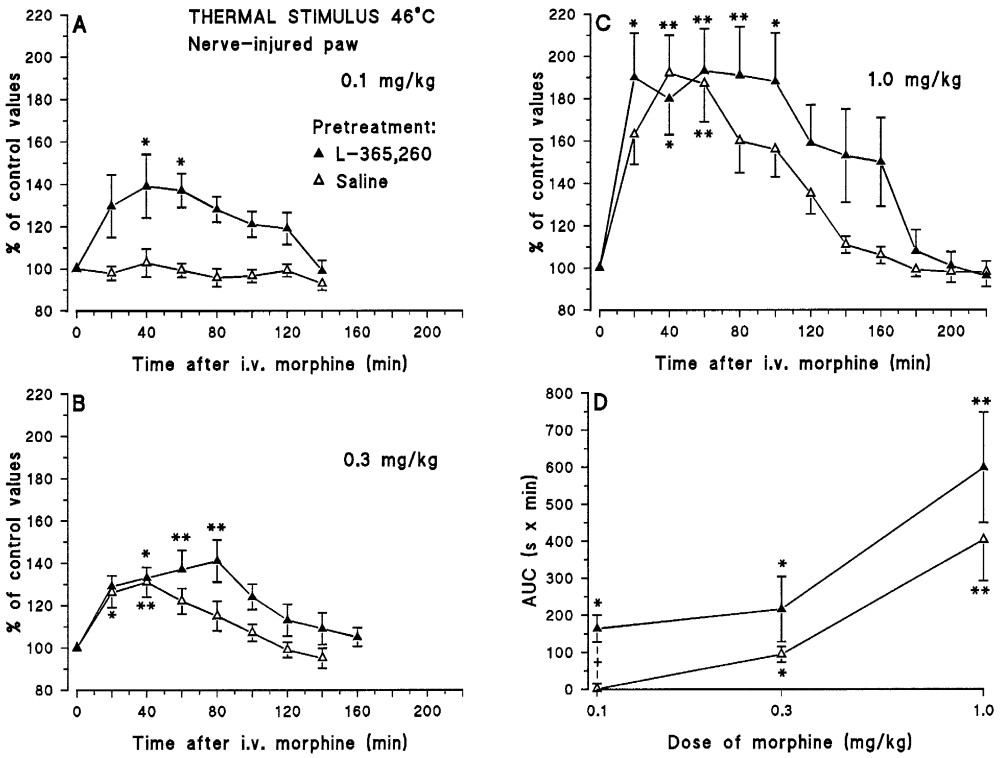

Fig. 1. The antinociceptive time curve of morphine after pretreatment with saline or the CCK_B receptor antagonist L-365,260 in the noxious thermal (46°C) test of the nerve-injured paw (A–C). A, morphine 0.1 mg/kg; B, morphine 0.3 mg/kg; C, morphine 1.0 mg/kg; D, AUCs (s × min) of the respective time curves. Solid triangles = pretreatment with 0.2 mg/kg of L-365,260, open triangles = pretreatment with saline. The values (means ± S.E.M.) in A–C are represented as percentage of the control values. Statistics were calculated with struggle latencies expressed in seconds: * $P < 0.05$, ** $P < 0.01$ vs. control; $^{+}$ $P < 0.05$ vs. pretreatment with saline, Tukey's test.

($F(1,49) = 13.8$, ANOVA $P < 0.001$, Fig. 2D). The AUC of the combination of L-365,260 (0.2 mg/kg) and morphine (0.1 mg/kg) was significantly larger than that of morphine (0.1 mg/kg) alone ($P < 0.01$, Tukey's test, Fig. 2D). By contrast, the AUCs of the combinations of the CCK_B receptor antagonist and the doses 0.3 mg/kg and 1.0 mg/kg of morphine did not differ significantly from the AUCs of morphine (0.3 and 1.0 mg/kg) alone (Fig. 2D).

3.3.2. Contralateral paw

Saline, L-365,260 (0.2 mg/kg, Table 3), or the 0.1 mg/kg dose of morphine alone (Fig. 3A, Table 3) had no effect on the vocalization thresholds. The combination of L-365,260 (0.2 mg/kg) with morphine (0.1 mg/kg) resulted in a moderate, but significant overall effect ($F(8,72) = 2.7$, ANOVA: $P < 0.05$) that peaked ($132 \pm 2\%$) at 10 min (Fig. 3A, Table 3).

Both morphine (0.3 mg/kg) alone and the combination of L-365,260 (0.2 mg/kg) with morphine (0.3 mg/kg) resulted in an overall increase in vocalization thresholds of the contralateral paw ($F(6,49) = 4.2$, ANOVA: $P < 0.01$ and $F(5,42) = 6.6$, ANOVA: $P < 0.001$, respectively, Fig. 3B, Table 3). Similarly, both the dose of 1.0 mg/kg of morphine and the combination of the CCK_B receptor antagonist with morphine (1.0 mg/kg) resulted in a significant overall effect ($F(9,90) = 5.5$, ANOVA: $P < 0.001$ and $F(10,108) = 6.0$, ANOVA: $P < 0.001$, respectively) which lasted up to 50 min (Fig. 3C, Table 3).

A significant dose-effect relationship was observed for morphine ($F(2,49) = 13.9$, ANOVA: $P < 0.001$, Fig. 3D). Pretreatment with L-365,260 significantly modified the overall effect of morphine ($F(1,49) = 4.7$, ANOVA: $P < 0.05$, Fig. 3D). The AUC of the combination of L-365,260 (0.2 mg/kg) and morphine (0.1 mg/kg) was significantly larger than that of morphine (0.1 mg/kg) alone ($P < 0.05$, Tukey's test, Fig. 3D). By contrast, there were no significant differences between the AUCs of the doses of 0.3 and 1.0 mg/kg of morphine alone and the AUCs of the combinations of the respective doses with L-365,260 (Fig. 3D).

3.4. Effect of naloxone

Both in the thermal (46°C, $n = 8$) and mechanical ($n = 8$) tests, the effect of morphine (0.1 mg/kg) combined with L-365,260 (0.2 mg/kg) was abolished by naloxone (0.01 mg/kg i.v.) coinjected with morphine (not shown).

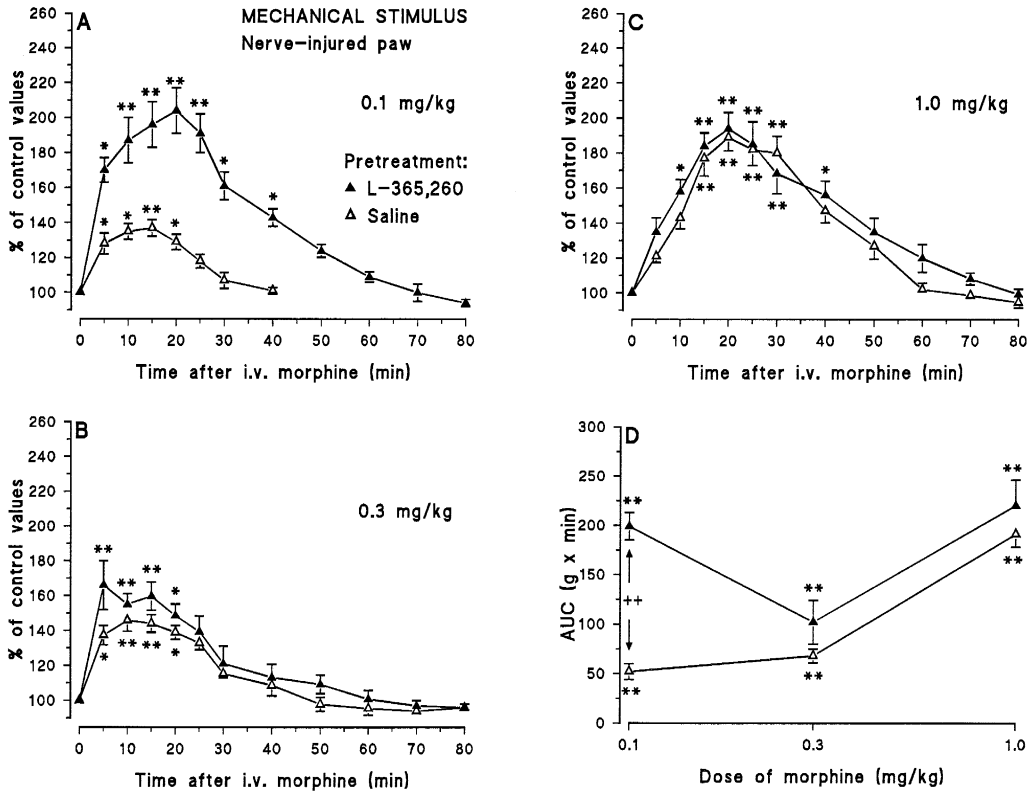

Fig. 2. The anti-allodynic time curve of morphine after pretreatment with saline or the CCK_B receptor antagonist L-365,260 in the mechanical pressure test of the nerve-injured paw of the mononeuropathic rats (A–C). A, morphine 0.1 mg/kg; B, morphine 0.3 mg/kg; C, morphine 1.0 mg/kg; D, AUCs (g × min) of the respective time curves (A–C). For further details, see Fig. 1. Statistics were calculated with vocalization thresholds expressed in grams: * $P < 0.05$, ** $P < 0.01$ vs. control; $^{++}$ $P < 0.01$ vs. pretreatment with saline, Tukey's test.

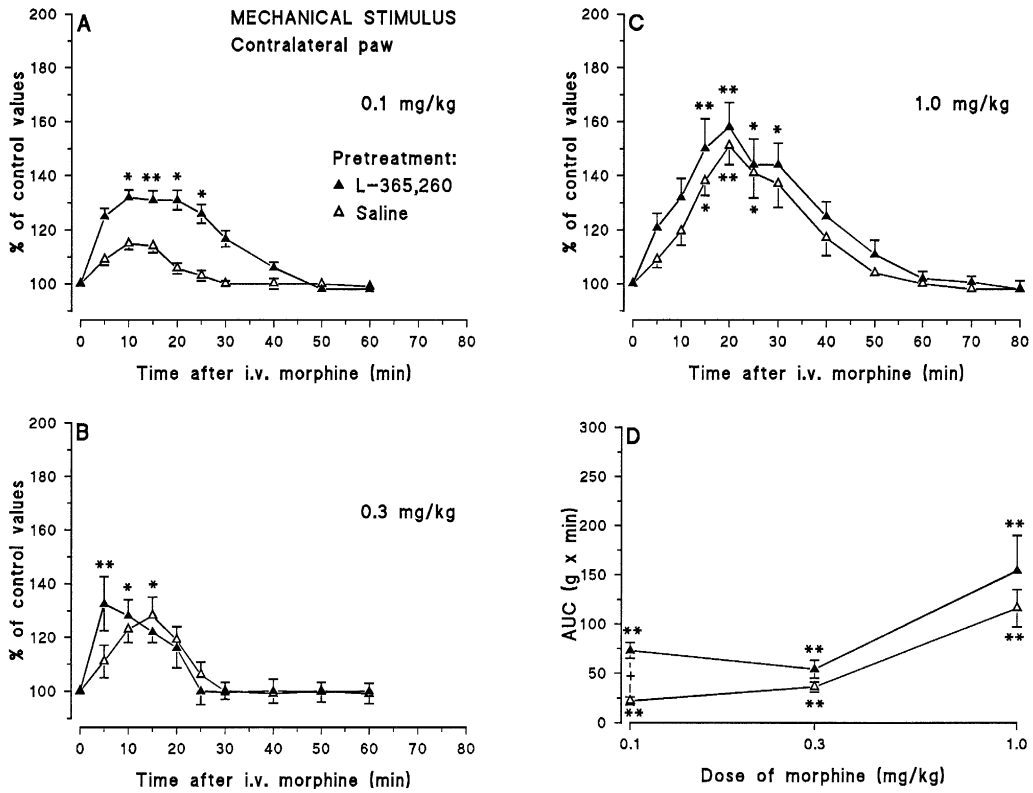

Fig. 3. The time curve of the effect of morphine after pretreatment with saline or the CCK_B receptor antagonist L-365,260 in the mechanical pressure test of the contralateral paw of the mononeuropathic rats (A–C). A, morphine 0.1 mg/kg; B, morphine 0.3 mg/kg; C, morphine 1.0 mg/kg; D, AUCs (g × min) of the respective time curves (A–C). For further details see Fig. 1. Statistics were calculated with vocalization thresholds expressed in grams: * $P < 0.05$, ** $P < 0.01$ vs. control; $^{+}$ $P < 0.05$ vs. pretreatment with saline, Tukey's test.

3.5. Various doses of L-365,260

We further assessed the effect of increasing doses (0.05, 0.1, 0.2 and 0.5 mg/kg) of L-365,260 on the effect of morphine (0.1 mg/kg i.v.) in the vocalization to paw pressure test (Fig. 4). The effect of the CCK_B receptor antagonist was found to be dose-dependent and bell-shaped. In the nerve-injured paw, the 0.2 mg/kg dose of L-365,260 resulted in maximal effect. The highest dose (0.5 mg/kg) resulted already in an inferior effect. In the contralateral paw, only the 0.2 mg/kg dose of the CCK_B receptor antagonist resulted in a significantly greater effect than morphine (0.1 mg/kg) alone (Fig. 4).

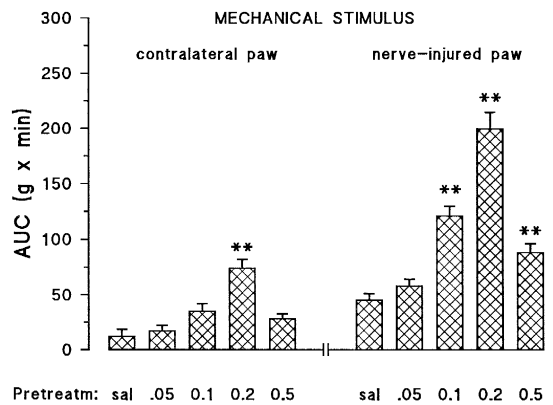

Fig. 4. The overall effect (AUC g×min) of the i.v. dose of 0.1 mg/kg of morphine in the vocalization to paw pressure test of the contralateral and nerve-injured paw of mononeuropathic rats. The rats were pretreated (pretreatm:) s.c. with saline (sal) or with increasing doses (0.05 (.05), 0.1, 0.2 or 0.5 mg/kg) of L-365,260. Means ± S.E.M. ($n = 6$–10). * $P < 0.05$, ** $P < 0.01$ vs. saline + morphine, Tukey's test.

3.6. Normal rats

In the vocalization to paw pressure test of an additional group ($n = 8$) of unoperated rats, the AUCs of the two hind paws (58 ± 5 g × min and 55 ± 8 g × min, respectively), after administration of the combination of L-365,260 (0.2 mg/kg) with the dose of 0.1 mg/kg of morphine, were roughly comparable to the AUC (73 ± 8 g × min) found in the contralateral paw of the neuropathic rats.

4. Discussion

Before receiving any drug injections, the rats with a chronic constriction injury of the sciatic nerve clearly exhibited abnormal pain sensitivity, with decreased thresh-

olds to mechanical stimulation and decreased latencies to thermal stimulation, as shown previously (Attal et al., 1990; Lee et al., 1994). In these rats, morphine was unable to alleviate allodynia-like behaviour to a non-noxious cold stimulus (10°C). At the noxious heat threshold (44°C), only the highest dose (1 mg/kg) of morphine was effective in increasing the struggle latencies and diminishing the signs of allodynia-like behaviour. At the noxious thermal range (46°C), a dose-dependent effect of increasing doses of morphine was found. Similarly, in the vocalization to paw-pressure test, morphine dose-dependently increased the vocalization thresholds confirming the efficacy of relatively low i.v. doses of morphine in this rat model of peripheral mononeuropathy (Neil et al., 1990; Attal et al., 1991; Jazat and Guilbaud, 1991; Kayser et al., 1995b; Catheline et al., 1996). In further accordance with our previous data, the effect of morphine was evidently enhanced in the nerve-injured paw compared with the contralateral paw.

It has been suggested that CCK may have a physiological role in neuropathic pain (see Stanfa et al., 1994 for a review), since the synthesis of CCK has been shown to be increased in the rat spinal cord after peripheral sciatic nerve section (Xu et al., 1993). In the present study, we showed that 2 weeks after the nerve injury, L-365,260 alone has no effect against allodynic cold, warm or mechanical stimuli or against clearly noxious thermal stimuli. Likewise, intrathecal L-365,260 alone has been shown not to alleviate mechanical allodynia of neuropathic rats 1–3 weeks after the neuropathic injury (Nichols et al., 1995). However, it has been shown that CI988, another antagonist of the CCK_B receptors, is able to relieve mechanical allodynia-like symptoms when administered i.p. to spinally injured rats 3–10 months after the lesion (Xu et al., 1994). It is interesting that in a previous study of Yamamoto and Nozaki-Tguchi (1995), where they used the same model of neuropathy as in the present study, the CCK_B receptor antagonist YM022 administered alone had no effect in the thermal paw-withdrawal test of the nerve-injured paw 1 week after the lesion, but an antinociceptive effect was observed 5 weeks after the lesion. These results suggest that the antinociceptive effect of CCK_B receptor antagonists alone in neuropathic rats is probably time-dependent and no antinociceptive effect is found during the first 2–3 weeks, when the abnormal pain sensitivity in the present model has already reached a maximum. The activity and importance of the CCK mechanisms in the modulation of neuropathic pain may thus vary with time.

The results obtained in this study indicate that, irrespective of the fact that the drug alone is ineffective, pretreatment with L-365,260 enhances the effect of morphine in neuropathic rats. The effect of the CCK_B receptor antagonist was dual: the peak effect of morphine was increased in both the thermal and mechanical tests of the nerve-injured paw and the duration of the effect of morphine was also expanded. In the thermal test, the ineffectiveness of morphine (0.1 mg/kg i.v.) against hyperalgesia was inverted by the CCK_B receptor antagonist, and a significant antinociceptive effect of morphine was observed. The overall effect of the combination was greater than that of morphine 0.3 mg/kg alone. Similarly, in the mechanical test, after pretreatment with L-365,260, an enhanced antiallodynic effect of the 0.1 mg/kg dose of morphine was observed. In this mechanical test, the overall effect of the combination was close to the effect of the 1.0 mg/kg dose of morphine alone. This enhanced effect, in both tests, was mediated by opioid receptors, since it was abolished by naloxone. However, it should be noted that the enhancing effect was limited to mechanical and noxious thermal (46°C) stimuli and no such effect could be detected against allodynic warm (44°C) or cold (10°C) stimuli. As previously discussed in detail (Lee et al., 1994), the dissociation of the effect of morphine suggests that fibres and/or dorsal horn neurons mediating the abnormal reactions to noxious and innocuous thermal stimuli in neuropathic rats are different. Indeed, cold allodynia has been considered as a significant clinical sign of sympathetic dysfunction and it is used in humans to assess sympathetically maintained pain (Frost et al., 1988). Accordingly, there is evidence that in the mononeuropathic rat, the development of allodynia-like behaviour to cold stimulation can be prevented by sympatholytic treatments or surgical sympathectomy (Neil et al., 1991; Perrot et al., 1993; Desmeules et al., 1995) and the α_2-adrenoceptor agonist clonidine is highly effective against cold stimuli (Kayser et al., 1995a). The present findings give further evidence of the involvement of systems other than the CCK system in the cold allodynia of mononeuropathic rats.

The systemic administration of drugs represents a common route of administration and delivers drugs to tissues naturally via the circulation. This is a major advantage over other routes of administration, and in the present study, the drugs were therefore administered either i.v. or s.c. However, with systemic administration, the site of drug actions is not precisely determined and supraspinal as well as spinal mechanisms should be considered. CCK_B receptors are found in the brain within the pain processing areas and interactions at this level are highly plausible. The rat spinal cord has also been shown to be an important site for the interaction of morphine and CCK_B receptor antagonists. In normal rats, both i.t. and s.c. administration of L-365,260 has enhanced the antinociceptive effect of both s.c. and i.t. morphine (Zhou et al., 1993) and this enhancement, by either route, has been antagonized by the δ-opioid receptor antagonist naltrindole (Ossipov et al., 1994). Similarly, in neuropathic rats, the enhanced antinociceptive effect of the combination of i.t. morphine and i.t. L-365,260 was abolished by i.t. naltrindole (Nichols et al., 1995), stressing the importance of spinal δ-opioid receptor-mediated mechanisms in the interaction of morphine and CCK.

In contrast to the results obtained with the dose of 0.1 mg/kg of morphine, no enhancement of the antinocicep-

tive effect could be seen when L-365,260 was combined with higher doses (0.3 and 1.0 mg/kg i.v.) of morphine. The enhancing effect of the CCK_B receptor antagonist thus seems to vanish with increasing doses of morphine. Both systemically (Zhou et al., 1993) and spinally (Benoliel et al., 1994) administered morphine has been shown to increase the release of CCK from the spinal cord. This effect of morphine has been shown to be dose dependent with low doses of morphine decreasing and high doses increasing the release of CCK-like material (Benoliel et al., 1994). It has been proposed that this upregulation of CCK may constitute a self-limiting process for the antinociceptive effects of opioids (Zhou et al., 1993). The gradual increase in the release of CCK may also gradually override the effect of the CCK_B receptor antagonist.

Another reason for the observed ineffectiveness of L-365,260 to enhance the effect of higher doses of morphine may be the fact that with the i.v. dose of 1 mg/kg of morphine, 50% of the mechanical anti-allodynic effect on the nerve-injured paw has been shown to result from a peripheral action (Kayser et al., 1995b; Catheline et al., 1996). Another study (Singh et al., 1996) recently showed that the effect of the CCK_B receptor antagonists does not extend to the peripheral effects of morphine.

For L-365,260, the optimal s.c. dose in the vocalization to paw pressure test of the neuropathic rats is 0.2 mg/kg. This result is in line with the study of Dourish et al. (1990), who reported the same dose to be the most effective in enhancing the antinociceptive effects of i.p. morphine in the tail-flick test of normal rats. In further accordance with this previous study, we found a bell-shaped dose-response curve for the CCK_B receptor antagonist in the mechanical test of both the nerve-injured and contralateral paw. The mechanism of this decrease in the enhancing effect with higher doses of L-365,260 is obscure and should be investigated in detail.

In conclusion, the present series of experiments confirm that under neuropathic conditions, endogenous CCK may oppose the effects of morphine. Even though pretreatment with L-365,260 does not modulate the activity of morphine against non-noxious cold stimuli (10°C) or against stimuli at the noxious heat threshold (44°C), the administration of L-365,260 inverted the ineffectiveness of the low dose of morphine against noxious thermal (46°C) stimulation and enhanced the anti-allodynic effect of the same dose of morphine against mechanical stimulation. This enhancing effect seems to be more prominent against mechanical than thermal stimuli, and in both cases, it disappears with increasing doses of morphine. From the clinical point of view, it is interesting that CCK_B receptor antagonists increase the effectiveness of already low doses of morphine thereby enabling the use of doses of opioids that give rise to fewer undesirable side-effects. It should be noted, however, that the enhancing effect of CCK_B receptor antagonists may be limited, and seen only against mechanical and noxious thermal stimuli. These drugs are thus likely to serve as adjuvant drugs in the treatment of patients suffering from peripheral neuropathic pain where the triggering stimuli are of either kind.

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