Prevention of Tolerance to the Antinociceptive Effects of Systemic Morphine by a Selective Cholecystokinin-B Receptor Antagonist in a Rat Model of Peripheral Neuropathy

JUHANA J. IDÄNNPÄÄN-HEIKKILÄ, GISELÉ GUIBAUD and VALE ´RIE KAYSER

Unité de Recherches de Physiopharmacologie du Système Nerveux, INSERM U 161, 75014 Paris, France (J.J.I.-H.; G.G.; V.K.) and The Institute of Biomedicine, Department of Pharmacology and Toxicology, 00014-University of Helsinki, Helsinki, Finland (J.J.I.-H.)

ABSTRACT

The ability of pretreatment by the selective cholecystokinin-B (CCKB) receptor antagonist L-365,260 (0.2 mg/kg s.c.) to prevent the development of tolerance to the antinociceptive action of morphine was evaluated by a well-established rat model of unilateral peripheral mononeuropathy. The 4-day pretreatment regimens (saline, L-365,260 or morphine alone, or with the combination of L-365,260 and morphine) were begun on post-operative day 12. The experiments were performed on day 16, when the pain-related behavior reached a stable maximum. Behavioral test based on a mechanical stimulus (vocalization threshold to paw pressure) and relatively low acute doses of systemic morphine (0.1, 0.3 and 1.0 mg/kg i.v.) were used. On day 16, the base-line vocalization threshold to paw pressure values of the groups pretreated with one of the four regimens were similar, which suggests that the pretreatments had no effect on the development of mechanical allodynia. Pretreatment with morphine alone (10 mg/kg s.c., two times a day during 4 days) induced a complete tolerance to the antinociceptive effect of acute morphine (0.1–1.0 mg/kg i.v.). However, pretreatment with the combination of L-365,260 with morphine completely prevented the development of tolerance to the antinociceptive effect of acute morphine. The effect of acute morphine in this latter pretreatment group was dose dependent, naloxone reversible and similar to the effect of acute morphine seen in the saline-pretreated group. Our results suggest that in this well-characterized model of neuropathic pain, the development of tolerance to the antinociceptive effect of systemic morphine can be prevented by systemic coadministration of the CCKB antagonist L-365,260. We further show, that in contrast to a tonic activity of the endogenous opioidergic system, a tonic activity of the endogenous CCK system cannot be revealed in this rat model of neuropathic pain.

The endogenous neuropeptide and neurotransmitter CCK is widely distributed in the brain and spinal cord (Vanderhaeghen et al., 1975; Beinfeld et al., 1981) and it mediates important physiological functions in the somatosensory system (for a review, see Wiesenfeld-Hallin and Xu, 1996). The central nervous system distribution of CCK parallels that of the endogenous opioids and opioid receptors within pain processing areas, such as the superficial laminae of the spinal cord and the periaqueductal gray matter of the brain (Saito et al., 1980; Stengaard-Pedersen and Larsson, 1981; Beinfeld and Palkovits, 1982), which provides anatomical evidence that a functional relationship may exist between these two transmitter systems. In fact, considerable experimental evidence suggests that CCK modulates pain transmission and the antiopioid effects of this peptide are well documented (Itoh et al., 1982; Faris et al., 1983; O’Neill et al., 1989). In accordance with these findings, behavioral studies on normal rats have demonstrated that CCKB-receptor antagonists potentiate the antinociceptive actions of both morphine and endogenous opioids (Watkins et al., 1985a, b; Dourish et al., 1988, 1990; Wiesenfeld-Hallin et al., 1990; Maldonado et al., 1993; Singh et al., 1996) and block the development of tolerance to the antinociceptive action of morphine (Dourish et al., 1988, 1990; Kellstein and Mayer, 1991).

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 spontaneous pain and a marked sensitivity to nociceptive stimuli (hyperalgesia). There is also a perception of normally innocuous stimuli being noxious, a state referred to as allodynia (Payne, 1986). Neuropathic pain is usually poorly controlled by currently available medications, and the effectiveness of opioids remains debatable (for a review, see Dray et al., 1994).

ABBREVIATIONS: AUC, area under the dose-response curve; CCK, cholecystokinin; IASP, International Association for the Study of Pain; i.v., intravenous; s.c., subcutaneous; VTPP, vocalization threshold to paw pressure; ANOVA, analysis of variance.
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 been studied extensively in our laboratory. We have previously shown that in this model systemic morphine and selective opioid receptor agonists produce dose-dependent antiallodynic effects against mechanical stimuli and have an enhanced effect on the nerve-injured side (Neil et al., 1990; Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995b; Catheline et al., 1996a). Tolerance to the antinoceptive effect of acute morphine against mechanical stimuli develops rapidly (Catheline et al., 1996b). However, we have also shown that systemic morphine and other selective agonists of the opioid receptors have no antinoceptive effects against thermal allodynia in these rats (Lee et al., 1994; Idån­pään-Heikkilä et al., 1997). Similar results have been obtained in other recent studies with the same model of neuropathy and thermal stimuli (Mao et al., 1995).

The synthesis of CCK has been shown to be increased in the rat spinal cord ganglia after peripheral nerve section (Verge et al., 1993; Xu et al., 1993) and it has been suggested that CCK may have a physiological role in neuropathic pain (review in Stanfa et al., 1994). Even though the interaction between CCK-antagonists and opioids has been studied to some extent on unoperated animals, the experimental evidence on neuropathic rats is still sparse (Nichols et al., 1995, 1996; Xu et al., 1994). We have recently demonstrated that in these rats pretreatment with a single dose (0.2 mg/kg s.c.) of the CCKB-receptor antagonist L-365,260 can enhance the antinoceptive effect of low doses of morphine against mechanical allodynia (Idån­pään-Heikkilä et al., 1997). This finding indicates that a relationship between cholecystokininergic and opioid systems may also exist in this model of clinical pain. Similar results have recently been obtained in another model of experimental neuropathy, in which the ineffectiveness of i.t. morphine was reversed by i.t. L-365,260 (Nichols et al., 1995).

The present experiments were undertaken to investigate whether chronic interaction of L-365,260 (0.2 mg/kg) with CCKB-receptors can prevent the development of tolerance to the antinoceptive effect of acute morphine in mononeuropathic rats. We have already shown that this dose of the CCKB-receptor antagonist is optimal in enhancing the effect of acute morphine as measured by the VTPP test in this model of neuropathic pain (Idån­pään-Heikkilä et al., 1997).

**Materials and Methods**

The recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP) Ethical Guidelines (1988) 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.

**Animals.** Male Sprague-Dawley rats (Charles River, Saint-Aubin-Lès-Elbeuf, France), n = 106, weighing 175 to 200 g on arrival, were used. The rats were housed five 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 1 week before the experiments were begun.

The unilateral peripheral mononeuropathy was produced on the right hind limb according to the method described by Bennett and Xie (1988) and Attal et al. (1990). The animals were anesthetized 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 four loose ligatures (5–0 chronic cugtue, 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.

**Surgery.** The unilateral peripheral mononeuropathy was produced on the right hind limb according to the method described by Bennett and Xie (1988) and Attal et al. (1990). The animals were anesthetized 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 four loose ligatures (5–0 chronic cugtue, 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.

**Morphine pretreatment.** Groups of five rats were pretreated with two consecutive s.c. injections of the following combinations of drugs: 1) saline + saline, 2) saline + morphine (10 mg/kg), 3) L-365,260 (0.2 mg/kg) + morphine (10 mg/kg), 4) L-365,260 (0.2 mg/kg) + saline. This morphine pretreatment has been shown to induce a complete tolerance to the antinoceptive effects of acute morphine in these rats (Catheline et al., 1996b). The drugs were injected in a volume of 1 ml/kg. The injections were given twice daily (at 9:30 A.M. and 5:30 P.M.) for 4 days, beginning on day 12 after the surgery. The effect of the acute treatments was tested at 17 h after the last pretreatment injection, on day 16 after the surgery. At this time, the abnormal pain behavior is at a stable maximum (Bennett and Xie, 1988; Attal et al., 1990).

**Behavioral testing.** All experiments were carried out in a quiet room between 8:00 A.M. and 12:00 P.M. The animals were randomly assigned in groups of 5 for a given series of tests and were not acclimatized to the test situations beforehand. The experimenter was unaware of the drug combinations used. The antinoceptive action was determined by measuring the vocalization threshold elicited by pressure on both the nerve-injured and the contralateral paw, with use of the Ugo Basile (Como­rio, Italy) algysymeter. This instrument generates a linearly increasing mechanical force applied by a dome-shaped plastic tip (diameter = 1 mm) placed on the dorsal surface of the hind paw. The tip was positioned between the third and fourth metatarsus (into the sciatic nerve territory) and force was 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). On day 16, a control threshold (mean of two consecutive stable thresholds expressed in g) was determined just before the injection of morphine or saline. During the first 30 min after the drug administration, VTPP values were measured every 5 min thereafter every 10 min, until they had returned to the level of the control values.

**Drugs.** The following drugs were used: Morphine hydrochloride (Meram, Paris, France), naltrexone 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 naltrexone were diluted in saline and administered in the acute experiments i.v. in a volume of 1 ml/kg into the lateral tail vein. The doses of acute morphine used in this study were from 0.1 to 1.0 mg/kg. These doses had induced significant effects on the vocalization threshold to paw pressure in this model of neuropathic pain (Attal et al., 1991). Acute naltrexone was injected i.v. at a dose of 0.1 mg/kg, that had been shown to prevent the dose effect of 1.0 mg/kg i.v. morphine in unoperated and neuropathic rats (Kayser and Guilbaud, 1989; Attal et al., 1991). L-365,260 was dissolved in a drop of ethanol and diluted in 10% Tween in saline. The 0.2 mg/kg dose of L-365,260 was chosen, because it has represented the most effective dose to potentiate acute morphine antinoception in unoperated (Dourish et al., 1990) and in neuropathic (I­dan­pään-Heikkilä et al., 1997) rats. The control rats received i.v. saline.

**Statistics.** Data are expressed as means ± S.E.M. The overall effects of various treatments (AUCs) were calculated by use of the trapezoidal rule. Student’s t test was used to determine the differences between two means. With three or more means, ANOVA was used first. The observed significances were then confirmed with
Tukey’s test. The statistical procedures were carried out with a statistical computer program (Statgraphics Plus, Manugistics, Rockville, MD). The observed differences were regarded as significant when the P values were lower than .05.

Results

General results. The mean VTPPs of the unoperated rats were 263 ± 2 g and 256 ± 4 g (n = 106) for the left and right hind paws, respectively. In agreement with previous studies (Attal et al., 1990; Desmeules et al., 1993; Kayser et al., 1995a; Idänpää-Heikkilä et al., 1997), the VTPPs of the nerve-injured paw were markedly decreased at day 16 after the nerve ligation. At this time, the mean VTPP for the nerve-injured paw was 160 ± 2 g (P < .001 vs. the preconstriction value). This decreased threshold was considered to reflect mechanical allodynia (Merskey, 1986; Idänpää-Heikkilä et al., 1997). As described earlier (Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995a), the mean VTPP for the contralateral paw 263 ± 2 g was not significantly modified (N.S. vs. the mean preconstriction value). The mean VTPPs in the various pretreatment groups (given below) were similar, which indicated that the pretreatments by themselves were devoid of effects on the development of mechanical allodynia.

Effect of acute morphine in saline-pretreated rats. In this group, the control values were 259 ± 2 and 158 ± 3 (n = 25) for the contralateral and nerve-injured paws, respectively. In the nerve-injured paw, the effect of the 0.1 mg/kg dose of morphine peaked at 10 min (283 ± 10 g, n = 6, P < .01 vs. control) and lasted up to 25 min. The effects of the higher doses (0.3 and 1.0 mg/kg) of morphine both peaked at 15 min (333 ± 10 g, n = 6 and 416 ± 24 g, n = 8, respectively, P < .001 vs. control) and lasted for 40 and 60 min, respectively (fig. 1A).

Similarly, in the contralateral paw, all three doses of morphine resulted in significant antinociception. The effects of the 0.1 and 0.3 mg/kg doses of morphine both peaked at 10 min (310 ± 9 g and 365 ± 13 g, respectively, P < .01 vs. control) and lasted for 20 and 30 min, respectively. The effect of the 1.0 mg/kg dose of morphine peaked at 15 min (446 ± 21 g, P < .001 vs. control) and lasted up to 40 min (fig. 1B). Saline had no effect on either hind paw (n = 5).

The effect of morphine was dose-dependent in both the nerve-injured (F2,17 = 33.8, ANOVA P < .001, fig. 2A) and in the contralateral paw (F2,17 = 27.7, ANOVA P < .001, fig. 2B). The overall effect of morphine was clearly enhanced in the nerve-injured paw compared with the contralateral paw (F1,36 = 42.3, ANOVA P < .001, fig. 2, A and B).

Effect of acute morphine in morphine-pretreated rats. The control values of this group were 267 ± 5 and 161 ± 3 (n = 30) for the contralateral and nerve-injured paws, respectively. In both hind paws, all doses (0.1–1.0 mg/kg) of morphine failed to produce any antinociception (fig. 2, A and B). Saline had no effect on either hind paw (n = 6).

Effect of acute morphine in morphine and L-365,260-pretreated rats. In this group, the control values were 266 ± 4 and 160 ± 3 (n = 37) for the contralateral and nerve-injured paws, respectively. In the nerve-injured paw, the effects of the 0.1 and 0.3 mg/kg doses of morphine both peaked at 15 min (265 ± 19 g, n = 6 and 309 ± 22 g, n = 8, P < .01 vs. control) and lasted up to 25 min. The effect of the 1.0 mg/kg dose of morphine peaked at 20 min (374 ± 38 g, n = 11, P < .001 vs. control) and lasted for 60 min (fig. 3A).

Similarly, in the contralateral paw, the effects of the 0.1 and 0.3 mg/kg doses of morphine both peaked at 15 min (322 ± 13 g and 351 ± 24 g, respectively, P < .01 vs. control) and lasted for 25 min. The effect of the 1.0 mg/kg dose of morphine peaked at 20 min (436 ± 27 g, P < .001 vs. control) and lasted up to 40 min (fig. 3B). Saline had no effect on either hind paw (n = 6).

The effect of morphine was dose-dependent and similar to that observed in the saline-pretreated group in both the nerve-injured (F2,22 = 13.4, ANOVA: P < .001, fig. 2A) and in the contralateral paw (F2,22 = 10.8, ANOVA: P < .001, fig. 2B). Further, the overall effect of morphine was clearly enhanced in the nerve-injured paw compared with the con-
trateral paw \((F_{1,46} = 11.1, \text{ANOVA: } P < .01, \text{fig. 2, A and B}).\)

**Effect of acute naloxone.** In the morphine and L-365,260-pretreated rats, the effect of the acute dose 1.0 mg/kg of morphine on both hind paws was completely antagonized by coadministration of naloxone (0.1 mg/kg i.v., \(n = 6\), fig. 3, A and B). In the contralateral paw, in addition to antagonizing the effect of morphine, naloxone produced a significant decrease in the VTPP between 20 and 30 min (fig. 3B). Some rats showed a similar decrease also on the nerve-injured side, but the mean value was not significantly different from the base line (fig. 3A).

**Effect of acute morphine in L-365,260-pretreated rats.** The control values of this group were 256 ± 3 and 162 ± 3 (\(n = 14\)) for the contralateral and nerve-injured paws, respectively. The effect of morphine (1.0 mg/kg i.v.) was similar to the effect observed in the saline-pretreated group. In the nerve-injured paw, the effect of morphine peaked at 15 min (343 ± 7 g, \(n = 8\), \(P < .001 \text{ vs. control}) and lasted up to 50 min, whereas in the contralateral paw, the effect peaked at 15 min (345 ± 8 g, \(P < .001 \text{ vs. control}) and lasted for 30 min. Saline produced no effect on either hind paw (\(n = 6\)).

**Discussion**

On day 16 after the surgery, the rats with the chronic constriction injury of the sciatic nerve clearly exhibited abnormal pain sensitivity, with decreased thresholds to mechanical stimulation, as shown previously (Attal *et al.*, 1990; Lee *et al.*, 1994; Ida¨npa¨a¨¨n-Heikkil¨a *et al.*, 1997). We have also previously shown that, at this time, both the decrease in the VTPPs and the pain-related behavior reach a stable maximum (Attal *et al.*, 1990; Desmeules *et al.*, 1995). In the saline-pretreated group, 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.*, 1996a). In further accordance with our previous data, the overall effect of morphine was enhanced in the nerve-injured paw compared with the contralateral paw.
Even though the maximal effect of morphine was equal on both paws, the statistical analysis revealed a significant difference between the overall effects (AUCs) of morphine on the two paws at all dose levels. This was caused by both the decreased base-line thresholds of the nerve-injured paw and the increased duration of the effect of morphine on this paw. Antiallodynic effects of both systemic (s.c.) and intracerebroventricular (i.c.v.) morphine against mechanical stimuli have also been shown recently in another model of peripheral neuropathy (Bian et al., 1995).

However, we have also shown previously that the antinociceptive effect of systemic morphine is limited to mechanical and clearly noxious thermal (46°C) stimuli of the nerve-injured paw, and no such effect could be detected against allodynic warm (40 and 42°C) or cold (10°C) stimuli (Lee et al., 1994; Idänpään-Heikkilä et al., 1997). We also found a modulating effect of L-365,260, which was different according to the quality of the stimulus (Idänpään-Heikkilä et al., 1997). Taken together, these studies suggest, that even within the same model of neuropathic pain, the pathophysiological mechanisms mediating the abnormal reactions to noxious and innocuous stimuli are different, as discussed in detail previously (Lee et al., 1994; Desmeules et al., 1995; Kayser et al., 1995a). The various animal models may have fundamentally different pathophysiologicals, and the sensitivity to opiates and CCK may vary accordingly.

In the morphine-pretreated (10 mg/kg s.c., two times per day) rats, the acute doses of i.v. morphine had no effect on the VTPPs of neither hind paw, which confirms that with this pretreatment regimen a complete tolerance to the antinociceptive effects of morphine had occurred (Catheline et al., 1996b). Chronic coadministration of L-365,260 with morphine completely prevented the development of tolerance to this morphine pretreatment regimen, and in this pretreatment group the effect of acute morphine was similar to the effect observed in the saline-pretreated group. In these rats, the effect of acute morphine was still dose-dependent and the overall effect of morphine was enhanced in the nerve-injured paw compared with the contralateral paw. The effect of the highest dose of acute morphine was completely antagonized by naloxone (0.1 mg/kg i.v.), which indicates that the effect of i.v. morphine in these rats is still mediated by opioid receptors.

It has been claimed that, although animals tolerant to opioids may metabolize them somewhat more rapidly, most of the tolerance is pharmacodynamic and results from adaptation of cells in the nervous system to the action of the drug (Jaffe, 1990). The mechanisms of pharmacodynamic tolerance may relate to a reduction in the number of receptors or uncoupling of the receptor from its second messengers (e.g., adenylate cyclase) and/or changes in parallel antagonistic (e.g., cholecystokinin, neuropeptide FF) or facilitatory systems (Portenoy, 1994). Indeed, down-regulation of mu opioid receptors has been demonstrated during chronic morphine exposure (Werling et al., 1989), but other studies have demonstrated that changes in receptor number are not necessary for tolerance to occur (Cox, 1991). The mechanism of the prevention of morphine tolerance by CCK antagonists has not been studied in detail. However, the antiopioid effects of CCK are well documented (Itoh et al., 1982; Faris et al., 1983; O’Neill et al., 1989). Further, both systemically (Zhou et al., 1993) and spinally (Benoilie et al., 1994) administered morphine have been shown to increase the release of CCK from the spinal cord. Therefore, it is highly plausible that chronic administration of the opiate would induce changes in the antagonistic CCK system that would lead to the observed tolerance. Chronic coadministration of CCK<sub>P</sub>-receptor antagonists might prevent such adaptive changes, thus preventing the development of morphine tolerance.

It might be argued that the prevention of opioid tolerance by the CCK<sub>P</sub>-receptor antagonist is only apparent, i.e., this effect is attributable to residual amounts of L-365,260 which enhanced morphine antinociceptive effect on day 5. However, in the animals pretreated chronically with L-365,260 alone, the effect of acute morphine was not modified. This suggests that even though, in the same test, a single acute injection of the CCK<sub>P</sub>-receptor antagonist is able to enhance the antinociceptive effect of an acute low dose of morphine (Idänpään-Heikkilä et al., 1997), the effect of acute morphine is not modified after chronic interaction of L-365,260 with the CCK<sub>P</sub>-receptors, if the lag-time between the last injection of the antagonist and acute morphine is long enough.

Injury to peripheral nerves has been shown to induce a significant increase in the levels of CCK-like immunoreactivity and CCK mRNA (Verge et al., 1993) as well as CCK<sub>β</sub>-receptor mRNA (Zhang et al., 1993) in rat dorsal root ganglion (DRG) cells and it has been proposed that this up-regulation of CCK might explain the mechanism for the development and maintenance of neuropathic pain (Wiesenfeld-Hallin and Xu, 1996). The results on rats with a severe, but incomplete, ischemic spinal cord injury seem to favor this view, because systemic administration of CI-988 alone, a selective CCK<sub>P</sub>-receptor antagonist, was very effective in reducing this allodynia-like state, whereas morphine was ineffective (Xu et al., 1994). However, i.t. administration of L-365,260 has repeatedly been shown to be ineffective in relieving mechanical allodynia in rats with a sciatic nerve root ligation (Nichols et al., 1995, 1996). Similarly, in rats with a constriction injury of the sciatic nerve, the base-line mechanical thresholds were not modified, neither after a single s.c. injection of L-365,260 (Idänpään-Heikkilä et al., 1997) nor, in the present study, after chronic s.c. pretreatment with the CCK<sub>P</sub>-receptor antagonist. These data indicate that, in contrast to spinally injured rats, a tonic activity of the endogenous cholecystokininergic system resulting in persistent pain behavior is unlikely to exist in this rat model of peripheral neuropathic injury.

However, a tonic activity of the endogenous opioidergic system, revealed by administration of opioid antagonists, has been shown to exist in the rats with a chronic constriction injury of the sciatic nerve (Attal et al., 1991; Jazat and Guillaud, 1991; Kayser, 1994). The present findings are in accordance with these previous findings, as in the contralateral paw, the combination of a relatively low dose (0.1 mg/kg i.v.) of naloxone with morphine not only reversed the antinociceptive action of morphine, but also induced a further decrease in the VTPPs. It has been suggested that an acute interaction of CCK with CCK<sub>P</sub>-receptors may result in diminished activity of endogenous opioids (Roques et al., 1993). Accordingly, chronic interaction of L-365,260 with the CCK<sub>P</sub>-receptors might result in an enhanced activity of endogenous opioids, which would be revealed by administration of this relatively low dose of naloxone, which usually does not reinforce allodynia-like behavior in this model (Attal et al.,
1989, 1990). In the nerve-injured paw, no such decrease could be found, which may be partly caused by the already low thresholds of the nerve-injured side.

The systemic administration of drugs represents a common route of administration; it is widely used also in the clinic and delivers drugs to tissues naturally via the circulation. This is a major advantage over other routes of administration. 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 supra-spinal as well as spinal mechanisms should be considered. In the present study, the dose-response curves, after increasing doses of i.v. morphine, were not superimposed. The occurrence of the same phenomenon has been described, as measured by the VTPP test, not only on neuropathic rats (Attal et al., 1991), but also on normal and arthritic rats (Kayser and Guilbaud, 1983). The VTPP test is a supra-spinal integrated test, whereas the paw-withdrawal test, widely used to assess mechanical nociception, is mainly spiral controlled (Kayser and Guilbaud, 1990). Low i.v. doses of morphine have been shown to act mainly on supra-spinal structures to inhibit nociceptive responses (Benoist et al., 1983). With increasing doses spinal structures are also activated. In addition, it has been shown that with higher doses of morphine, an important peripheral component can be detected with the VTPP test on the neuropathic rats (Kayser et al., 1995b). The displaced peaks with increasing doses of morphine could be related to these multiple sites of action of morphine. After i.v. administration of gradually increasing doses of morphine, the importance of supraspinal, spinal and peripheral sites in the inhibition of neural responses to noxious stimuli is suggested to increase in the VTPP test.

In conclusion, the present finding that systemic co-administration of CCK₉-receptor antagonist with morphine prevents the development of tolerance provides evidence that CCK mediates this phenomenon also in neuropathic rats. The present results suggest that CCK₉-receptor antagonists might provide a therapeutic option and be used in conjunction with opioid drugs to enhance analgesia and reduce or prevent tolerance in patients with neuropathic pain.

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Mononeuropathy, CCK and Tolerance

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Send reprint requests to: Dr. Juhana J. Idänpää-Heikkilä, M.D., Ph.D.; Unité de Recherches de Physiopharmacologie du Système Nerveux, INSERM U 161, 2 Rue d’Alésia, 75014 Paris, France.