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Repeated low doses of morphine do not induce tolerance but increase the opioid antinociceptive effect in rats with a peripheral neuropathy

A. Neil, V. Kayser, Y.L. Chen and G. Guilbaud

Unité de Recherche de Physiopharmacologie du Système Nerveux, U161, INSERM, Paris (France)

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In rats with a mononeuropathy, repeated low doses of morphine slightly enhanced its own effect in a paw pressure test of the lesioned limb. While the very effectiveness of morphine in neuropathic rats suggests that at least some nociceptive components of neuropathic pain might be sensitive to opioid receptor mechanisms, the absence of a rapid tolerance in this model indicates that tachyphylactic phenomena do not contribute to the reputed clinical ineffectiveness of opioids in neuropathic pain.

Opioids are generally reputed to be ineffective in the clinical management of pain due to peripheral nerve injury[1,20,25]. However, during recent studies using a novel animal model for neuropathic pain[4] we have shown that an increased sensitivity to morphine develops in animals with a peripheral mononeuropathy[6]. We have earlier shown that a similar increase in the effectiveness of opioids occurs in another model of persistent pain, the arthritic rat[13,19]. The increased sensitivity to opioids in the arthritic rat was accompanied by the unusually rapid development of tolerance to morphine, where after a couple of low doses of the drug (2 × 3 mg/kg s.c.) was almost halved[14]. Since such tachyphylactic mechanisms might, by analogy, contribute to the reputed clinical ineffectiveness of opioids, we decided to investigate if a rapid opioid tolerance does develop upon repeated morphine administration in the mononeuropathic rat.

A unilateral peripheral mononeuropathy was induced in male Sprague–Dawley rats (Charles Rivers, France), weighing 260–280 g, according to the method described in detal by Bennet and Xie[4] through 4 restraining ligatures placed around the common sciatic nerve on the right side only, the left side receiving only a sham wound. Drug pretreatment, morphine hydrochloride (3 mg/kg) diluted in 0.9% NaCl or saline alone was administered twice daily for 4 days, commencing 12 days after the lesion. Sensitivity to an equally effective dose of morphine by the intravenous route (1 mg/kg) was assessed 24 h after the last s.c. injection by a modification of the Randall–Selitto method as described[13]: increasing pressure was applied to the hindpaws until audible squeaks were elicited. After stable base-line readings from both the lesioned and the sham side, morphine was injected i.v. and the thresholds on both sides were redetermined every 10 min up to 1 h after treatment. Postdrug thresholds were calculated as a percentage of mean control threshold, the baseline (100%) was subtracted and an area (% × min) was calculated for the response from 0 to 60 min after drug. The area thus obtained for different paws and treatments in lesioned rats were compared to those obtained earlier for one paw in normal, non-operated animals, receiving the same pharmacological treatments[14] using the two-way analysis of variance.

In preliminary studies, rats receiving repeated injections of morphine seemed, subjectively, to be more sensitive to touch than animals receiving saline. We therefore noted all occurrences of squeaks at the times of drug treatment in the study and found a systematic tendency towards the development of increased sensitivity in morphine-treated lesioned animals (not shown). However, since the neuropathic rats allotted to the morphine group were initially more sensitive than those in the saline group, and lacking the corresponding data from normal rats tested earlier, we did not pursue this issue further, but the increased sensitivity is also reflected in the baseline nociceptive thresholds to mechanical stimuli (Table I).

In saline-pretreated rats (Fig. 1A) morphine induced a more profound effect on the mechanical nociceptive thresholds of the lesioned paw than on either the contralateral, sham-operated paw or normal rats which did not differ from each other. The difference between lesioned and sham sides was even more pronounced in morphine-pretreated animals (Fig. 1B). Here both the

Correspondence: A. Neil. Present address: Department of Pharmacology, Uppsala University, BMC, Box 591, S-751 24 Uppsala, Sweden.

TABLE I

Initial nociceptive thresholds to mechanical stimulation for the rats used

Pretreatment	*Group*	*Paw*	*n*	*Thresholds (g ± S.D.)*
Saline	normal rats		10	500 ± 38
	lesioned rats	sham	8	412 ± 23
		lesion	8	297 ± 28
Morphine	normal rats		9	331 ± 71
	lesioned rats	sham	8	402 ± 11
		lesion	8	244 ± 24

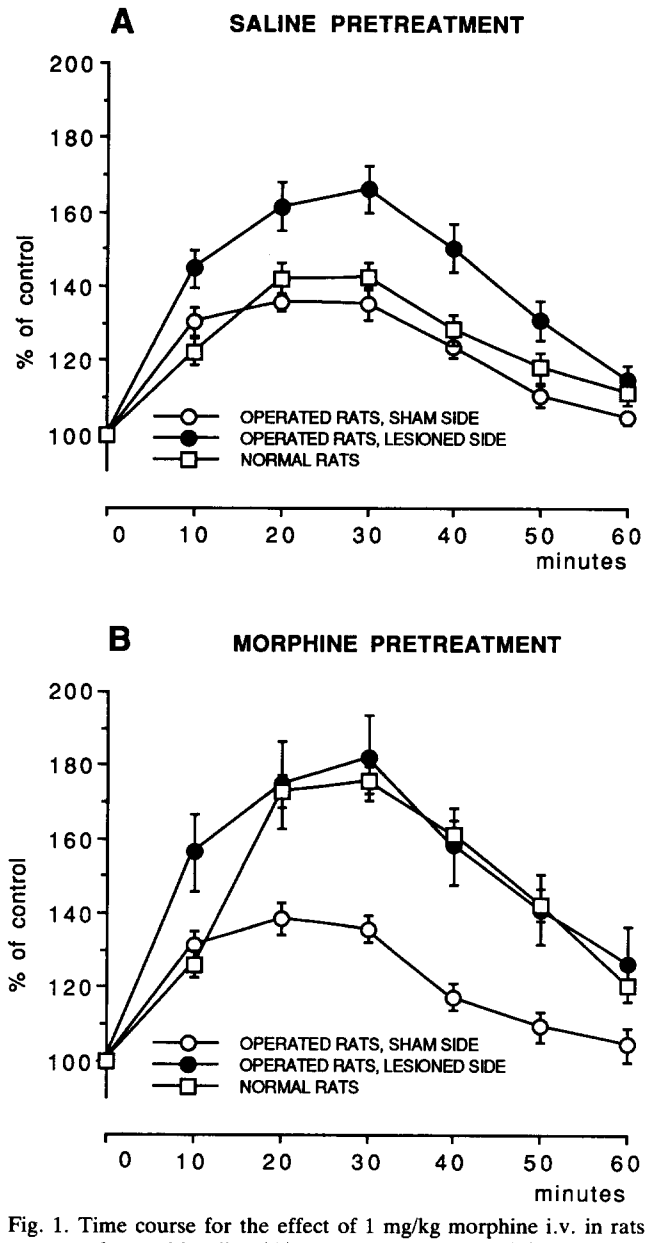

Fig. 1. Time course for the effect of 1 mg/kg morphine i.v. in rats pretreated s.c. with saline (A) or morphine 3 mg/kg (B) twice daily for 4 days. Points are mean values as a percentage of baseline thresholds ± S.E.M.

lesioned paw and normal rats were found to be more reactive to morphine than was the case in saline-pretreated animals, while the opioid effect on the mechanical nociceptive thresholds on the sham-operated paws was unchanged by morphine pretreatment.

Morphine pretreatment with the low doses used here changes the antinociceptive effectiveness of subsequent opioid administration in a complex way that depends on the initial state of the limb tested (Fig. 2). When comparing normal rats to the lesioned side of operated rats, two-way analysis of variance (Table II) revealed that the effect of morphine was dependent on both the previous lesion and previous morphine exposure, but since the interaction term was found insignificant, lesioned rats were not affected more (or less) by previous exposure to morphine than normal animals. In contrast, the comparison between normal animals and the sham side of operated rats showed an alteration (differential increase) in the effect of morphine. Thus, while (as shown earlier in ref. 14) morphine increases its own effectiveness in normal rats, this effect could still be detected as an increase in the already higher effect of morphine on the lesioned side of operated rats, but on the contralateral (sham-operated) side the antinociceptive effect of morphine is altered neither by lesion in saline-pretreated rats nor by previous opioid exposure.

These results differ from those obtained previously in arthritic rats where the acute antinociceptive effect of the same low morphine dosage was slightly greater than in the neuropathic rats. However, after the same morphine pretreatment, the effect of morphine was almost halved after the first day of treatment[14]. It should be mentioned

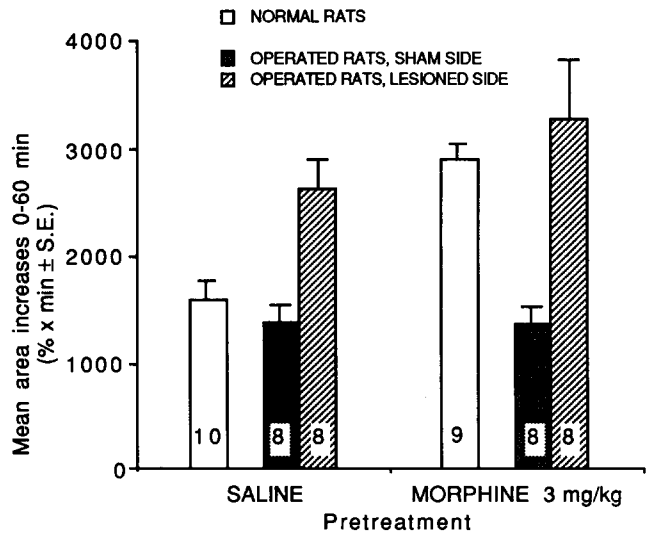

Fig. 2. Total antinociceptive effects of morphine in Fig. 1A and B. Total response area (percent increase from baseline × min) was calculated for each animal and mean values for each group plotted as the mean of % × min ± S.E.M. The number of animals in each group is given at the base of each bar.

TABLE II

Two-way analysis of variance of the increases in the area under the curves induced by i.v. morphine (1 mg/kg)

Comparison	*Source*	*DF*	*MeanSq.*	*F ratio*	*P*
Normal rats vs. operated rats, lesioned side	lesion	1	4151169	5.032	0.03
	pretreatment	1	8296964	10.058	<0.003
	lesion × pretreatment	1	926332	1.123	>0.05
	error	31	824927		
Normal rats vs. operated rats, sham side	lesion	1	6736732	30.769	<0.001
	pretreatment	1	3562737	16.272	<0.001
	lesion × pretreatment	1	3823529	17.464	<0.001
	error	31	218944		

that the resistance to the development of tolerance in neuropathic rats is by no means absolute. In preliminary studies with a higher pretreatment dosage, 10 mg/kg s.c. twice daily for 4 days, we found that while the opioid sensitivity remained stable on the sham side there was a tendency towards a diminished morphine effect on the lesioned side. This pretreatment induced some tolerance in normal rats as well (not shown). However, definite signs of withdrawal can be demonstrated in neuropathic rats using the present treatment (Chen et al., submitted). In other respects, the opioid pharmacology of neuropathic rats is fairly regular. That repeated morphine exposure could increase its own effectiveness has earlier been demonstrated in normal rats and mice (see ref. 14) and might be a species-specific phenomenon. We are not aware of any systematic study on sensitization to analgesic doses of opioids in man. As in the cases of arthritic rats[12] and rats with carrageenin-induced paw inflammation[16], low doses of naloxone act antinociceptively in neuropathic rats on both the sham-operated and the lesioned side[3]. Since such remote sensory changes are reported to develop in some causalgia patients (reviewed in ref. 7) and occur in other experimental models as a consequence of repeated acute trauma[18] or localized edema[14], we were intrigued by the observation that the opioid effectiveness in the limb contralateral to the lesion was unaffected by either lesion or drug pretreatment. This might be due to the lesion involved. As a function of discrete deafferentation, the tissue levels of substance P in the dorsal spinal cord that derives from primary afferent terminals are decreased in neuropathic rats[5], but they were found to be unaltered in arthritic inflammation[17] which comprises more general tissue damage but spares the sensory fibers. It is therefore possible that the observed variations in opioid pharmacology among various experimental pain models result from such differences in innervation and subsequent neurochemical changes.

While the present results do not support the initial hypothesis that rapid development of tolerance in the neuropathic state could contribute to the reputed clinical ineffectiveness of opioids in nerve lesions, they show that opioids could be very effective in this experimental model of neuropathic pain, and that there need not be a direct relationship between opioid antinociceptive efficacy, the rate of tolerance development and other aspects of opioid pharmacology among different models of protracted pain states. The present animal model closely resembles the sympathetically maintained pain states in man: limb guarding, abnormal claw growth, changes in the skin and its temperature[4], hyperalgesic responses to heat, mechanical and chemical stimuli, abnormal responses to innocuous cold stimuli[4,10] (Attal et al., submitted, manuscript in preparation), and the definite beneficial effect of chemical sympathectomy with guanethidine (manuscript in preparation). In this animal model for partial deafferentation pain we were unable to reproduce the reputed clinical ineffectiveness of opioids in nerve lesions, both in tests for phasic mechanical nociception (present study and ref. 6) and in tests for spontaneous, tonic, nocifensive behavior[11]. This might be due to species differences, but the ineffectiveness of opioids in neuropathic pain states has also been contested[2,9,21]. There are reports that patients with neuropathies and phantom limb pain can benefit from long-term opioid treatment[22,26]. Also, a number of neurogenic pain patients benefit from 'acupuncture-like' low frequency transcutaneous stimulation[7]. Since such treatments may be blocked by the opioid antagonist naloxone[23] and levels of opioid active material in the CSF increases[24], it is likely that the treatment acts through opioid receptor mechanisms. It is therefore tempting to speculate that, at least in phasic periods of neuropathic pain states, the levels of nociceptive input which occur are so high that patients on an ordinary dosage regimen are underdosed and that systemic administration of opioids becomes limited by side effects before sufficient concentrations at the antinociceptive receptors are reached. Since experimental animals are generally much less sensitive to adverse side effects as respiratory depression and confusion, this hypothesis remains to be evaluated clinically.

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