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κ-Opioid agonists produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat

[1] G.E. Leighton, [2] R.E. Rodriguez, R.G. Hill & J. Hughes

Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB

1 Nociceptive thresholds to noxious mechanical (paw pressure) and thermal (tail flick) stimuli were recorded in conscious rats. The effects of three selective κ-opioid receptor agonists on the responses to these stimuli were determined following intravenous, intracerebroventricular or intrathecal administration. Results were compared with those obtained with morphine.

2 Following intravenous administration PD117302, U50488, U69593 and morphine produced steep parallel dose-response curves indicating antinociceptive activity when evaluated in the paw pressure test. When U50488 and U69593 were tested at a single dose of 3.3 mg kg^{-1} no effect was seen in the tail flick test.

3 When given by the intrathecal route only morphine was effective at increasing the nociceptive threshold. PD117302, U50488 and U69593 were without effect in either the paw pressure or tail flick tests when tested at doses up to 100 μg per rat. PD117302 caused flaccid paralysis of the hindlimbs following intrathecal administration at the top dose tested. This effect was not reversible by naloxone.

4 All three κ-opioid receptor agonists produced naloxone-reversible antinociception in the paw pressure test, and to a lesser extent in the tail flick test, when injected directly into the third cerebral ventricle with the maximum effect occurring between 5 and 10 min after administration and declining back to control levels by 60 min. Morphine had a much slower onset of action with the peak effect being observed 30 min after dosing.

5 It is concluded that, under our experimental conditions in the rat, the antinociceptive effects of κ-agonists are likely to be operated via an action at a supraspinal rather than a spinal site.

Introduction

There is considerable evidence pointing to the existence of multiple opioid receptors. The original classification of opioid receptor types was derived from *in vivo* studies in the spinal dog (Martin *et al.*, 1976). On the basis of this study receptors were subdivided into μ-, κ- or σ-, with the μ and κ-types being associated with the production of antinociception. Following the discovery of the enkephalins (Hughes *et al.*, 1976), Lord *et al.* (1977) observed a ligand specificity pattern in the mouse vas deferens which was sufficiently different from that of the guinea-pig ileum to propose the existence of a new class of receptor, named δ. There is increasing evidence that activation of the δ-receptor can also produce antinociception (Schmauss & Yaksh, 1984; Rodriguez *et al.*, 1986). Autoradiographic studies have shown that opioid receptor types are differentially distributed in the brain (Goodman *et al.*, 1980; Goodman & Snyder, 1982) and spinal cord (Lamotte *et al.*, 1976; Atweh & Kuhar, 1977) and many studies have been performed in an attempt to determine the level of the neuraxis at which μ-, κ- and δ-opioids act to produce their antinociceptive effects.

There is considerable evidence that morphine can produce antinociception following direct microinjection into discrete brain regions (Herz *et al.*, 1970; Pert & Yaksh, 1974) or following intrathecal administration into the subarachnoid space of the spinal cord (Yaksh & Rudy, 1977; Schmauss & Yaksh, 1984). δ-Receptor agonists have also been shown to be effective following either spinal (Rodriguez *et al.*, 1986) or supraspinal administration (Cowan *et al.*, 1985). A spinal site of action for μ- and δ-selective opioids is supported by the localization of these two types of receptor to primary afferent terminals in the spinal cord (Fields *et al.*, 1980).

The level of the neuraxis at which κ-opioids

[1] Author for correspondence.
[2] Present address: Department of Biochemistry, Faculty of Medicine, University of Salamanca, Spain.

produce their antinociceptive effects is currently under investigation. It has been reported that κ-receptors are found predominantly in the spinal cord (Hiller & Simon, 1980; Traynor et al., 1982) although the pattern of distribution appears to be highly species-dependent (Quirion et al., 1983). Dynorphin A, a potent opioid peptide first discovered in porcine pituitary (Goldstein et al., 1981) and since found to be a selective κ-ligand in smooth muscle bioassays (Corbett et al., 1982; Chavkin et al., 1982) and guinea-pig brain homogenates (James et al., 1982) has been suggested to be the endogenous ligand for the κ-receptor (Huidobro-Toro et al., 1981). Demonstration of dynorphin-like immunoreactivity in the dorsal horn of the spinal cord (Botticelli et al., 1981) together with κ-binding sites has led to the proposal that κ-receptor agonists may exert their antinociceptive effects via an action at this level of the CNS (Gilbert & Martin, 1976; Wood et al., 1981). Many studies have been performed in which the effects of dynorphin have been investigated following intrathecal administration. The results of these studies are far from conclusive however since dynorphin produces flaccid paralysis following intrathecal injection (Faden & Jacobs, 1984; Herman & Goldstein, 1986; Stevens & Yaksh, 1986) at doses that have been reported by some authors to produce antinociception (Hermann, 1982; Przewlocki et al., 1983; Spampinato & Candelleti, 1985). Results are difficult to interpret because different types of noxious stimuli have been used in these studies and κ-receptor agonists are known to be more effective against some forms of noxious stimuli than others (Tyers, 1981).

In an attempt to determine whether κ-agonists exert their antinociceptive effect at a spinal or a supraspinal site we have evaluated three selective κ-opioids, PD117302 (Clark et al., 1988; Leighton et al., 1987), U50488 (VonVoigtlander et al., 1983) and U69593 (Lahti et al., 1985) for their effects against mechanical and thermal noxious stimuli following intravenous, intracerebroventricular or intrathecal administration of drugs. Because of the rapid degradation of dynorphin that occurs *in vivo* and the non-opioid effect of flaccid paralysis that has been reported following intrathecal injection of this peptide, it was decided not to include it in this study.

Methods

Animals

Male Wistar rats (280–310 g Charles River, Margate, or Interfauna, Huntingdon) were implanted with intrathecal or intracerebroventricular cannulae. Male Wistar rats (70–100 g, Interfauna, Huntingdon) were used for the intravenous study. All cannulated animals were housed individually for the duration of the study, other animals being housed in groups of six per cage. Food (Labsure) and water were available *ad libitum* in the home cages. The colony room and the behavioural testing room were maintained on a 12 h light, 12 h dark cycle (lights on 06 h 00 min). All experiments were performed under conditions of constant temperature, humidity and light intensity.

Surgical procedures

Intrathecal cannulae were implanted under tribromoethanol anaesthesia according to the method of Yaksh & Rudy (1976). Prior to implantation the cannulae were cleaned by immersion in 70% v/v ethanol/water and then flushed with sterile saline (0.9% w/v NaCl/water). A period of at least seven days was allowed for animals to recover from surgery before analgesia testing. During this time cannulae were flushed with 20 μl sterile saline on alternate days to ensure that they remained patent. Any animals showing signs of motor deficit following cannula implantation were excluded from the study. Cannula placement was verified at the end of the experiment by anaesthetizing the animals with pentobarbitone then perfusing intracardially with 4% w/v paraformaldehyde solution. Following this procedure a 10 μl injection of pontamine sky blue dye was made through the intrathecal cannula, the spinal column was removed and the tip of the cannula located. Results from any animals with cannulae located on the ventral side of the spinal cord were rejected.

Intracerebroventricular cannulae were implanted in the third cerebral ventricle under chloral hydrate anaesthesia. A stainless steel cannula (23 gauge hypodermic tubing) was inserted using the following stereotaxic co-ordinates: A = −1.3 mm, L = 1.0 mm, V = −4.7 mm (Paxinos & Watson, 1982). The cannula was fixed in place with stainless steel screws (10BA) and acrylic dental cement (Simplex). Animals were allowed a recovery period of at least seven days before behavioural testing. Correct cannula placement was checked by postmortem dye injection (20 μl).

All cannulated animals received 0.2 ml Streptopen injection i.m. (procaine penicillin and dihydrostreptomycin, Glaxovet) and 5 ml dextrose saline i.p. (0.9% sodium chloride and 5% dextrose, Steriflex) immediately after surgery.

Procedure for testing for antinociception

Before behavioural tests, animals were randomly allocated to groups of six animals per drug treat-

ment such that the observers were blind to the treatment schedule. Each animal served as its own control in this series of experiments, with selected groups of animals receiving saline to ensure that the responses to noxious stimuli were not altered by repeated testing. Five minutes before intrathecal or i.c.v. drug administration baseline (predrug) responses were determined in the paw pressure and tail flick tests. Following treatment, responses were reassessed after 5, 10, 30 and 60 min (for i.c.v. dosing) or 15 and 30 min (for intrathecal dosing). These testing intervals were decided upon following preliminary experiments (results not shown). In the intravenous experiment animals were not pretested; instead, a separate group of saline-treated animals was included as control and all animals were tested only once, 5 min after dosing. All behavioural tests were performed between 13 h 00 min and 17 h 00 min.

Paw pressure test

The paw pressure test was performed using an Analgesymeter (Ugo Basile, Milan) fitted with a weight such that a pressure was applied of between 0–1000 g increasing at a rate of 64 g s^{-1}. The nociceptive threshold was taken as the point at which the rat vocalized or made a vigorous attempt to remove the paw. The weight used on the Analgesymeter was reduced for the testing of the smaller rats used in the intravenous test such that the maximum pressure applied to the paw was 250 g. Use of this weight produced a comparable response to that seen in the larger rats when the heavier weight was used.

Tail flick test

Tail flick latency was determined as the time taken for the rat to withdraw its tail from a radiant light source, the method of D'Amour & Smith (1941) being used. The light from a 300 W quartz projection bulb was focused through a narrow aperture adjusted such that the intensity of the beam produced a withdrawal latency of approximately 2.5–4.0 s. A cut off time of 8 s was used in order to prevent tissue damage.

Drugs

Injections through the intrathecal cannulae were made in a volume of 10 μl over a period of approximately 30 s followed by 5 μl saline to flush the cannula (dead space of the cannula = 5 μl). Intracerebroventricular (i.c.v.) injections were made by use of a similar technique but with an injection volume of 20 μl plus 5 μl saline. Intravenous injections were made in a dose volume of 1 ml kg^{-1} and were given via a lateral tail vein. Morphine sulphate (McCarthy), PD117302 hydrochloride ((±)-*trans*-N-methyl- N-[2-(1-pyrrolidinyl)- cyclohexyl]benzo[*b*]-thiophene-4-acetamide, Parke-Davis), and U50488 hydrochloride (*trans*-3,4-dichloro-N-methyl- N-(2-[1-pyrrolidinyl- cyclohexyl]-benzeneacetamide, synthesized at Parke-Davis Research Unit)) were dissolved in sterile saline. It was necessary first to dissolve U69593 ((5,7,8)-(−)-N-methyl- N-(7-(1-pyrrolidinyl-1-oxaspiro(4,5) dec-8-yl)benzeneacetamide, Upjohn) in a few microlitres of 1M HCl and then dilute this with saline before adjusting the pH of the solution to 7 with NaOH. All drug doses quoted refer to the base.

Statistical analysis

Statistical significance of data was determined by the Wilcoxon matched pairs signed rank test when comparing results obtained before and after drug treatment in the same group of animals; or the Kruskal Wallis one way analysis of variance when comparing two different treatment groups (Siegel, 1956).

Results

Intravenous administration of κ-opioids

The effects of morphine, PD117302, U50488 and U69593 in the rat paw pressure test are shown in Figure 1. All compounds produced steep parallel dose-response curves with the highest doses producing maximum antinociceptive effects. U50488 and U69593 were tested at a single dose (3.3 mg kg^{-1}) in the tail flick test and were shown to produce no significant change (Kruskal Wallis ANOVA) in the response latency (controls = 2.3 ± 0.2 s, $n = 5$; U50488 = 3.1 ± 0.2 s, $n = 5$; U69593 = 2.7 ± 0.3 s, $n = 5$).

Intrathecal administration of κ-opioids

PD117302, U50488 and U69593 were administered via the intrathecal route at doses up to 100 μg per

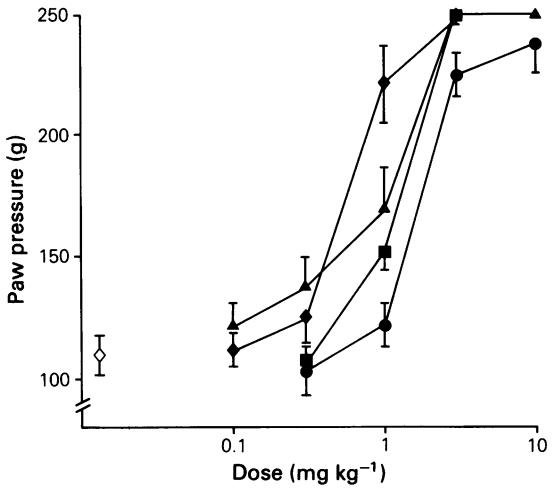

Figure 1 The effects of morphine (◆), PD117302 (■), U50488 (●) and U69593 (▲) in the rat paw pressure test following intravenous administration. Data are shown as mean values with s.e. indicated by vertical lines; $n = 6$ per dose level. (◇) represents the control response.

rat. For PD117302 and U50488 these doses represented the maximum level of solubility. As shown in Figure 2, none of the doses of U50488 or U69593 tested produced any change in the nociceptive threshold in either the paw pressure or the tail flick test. The only results shown for PD117302 are those obtained with 33 μg since higher doses produced flaccid paralysis of the hind limbs that was not reversed by naloxone. PD117302, 33 μg, did not alter the response to either a thermal or a mechanical stimulus. U50488 and U69593 did not produce any impairment of locomotor function as measured by the ability of the animals to maintain their position on an accelerating rotarod. Morphine 5 μg always produced a clear elevation of the nociceptive threshold in both the tail flick and paw pressure tests.

Table 1 A comparison of the lowest doses of morphine, U50488, U69593 and PD117302 to produce a statistically significant effect ($P < 0.05$, Wilcoxon matched pairs signed rank test) in the paw pressure and tail flick tests following i.c.v. administration

	Minimum effective dose (μg per rat)	
Compound	*Paw pressure test*	*Tail flick*
Morphine	0.4	3.7
U50488	33	>100
U69593	11	100
PD117302	33	>100

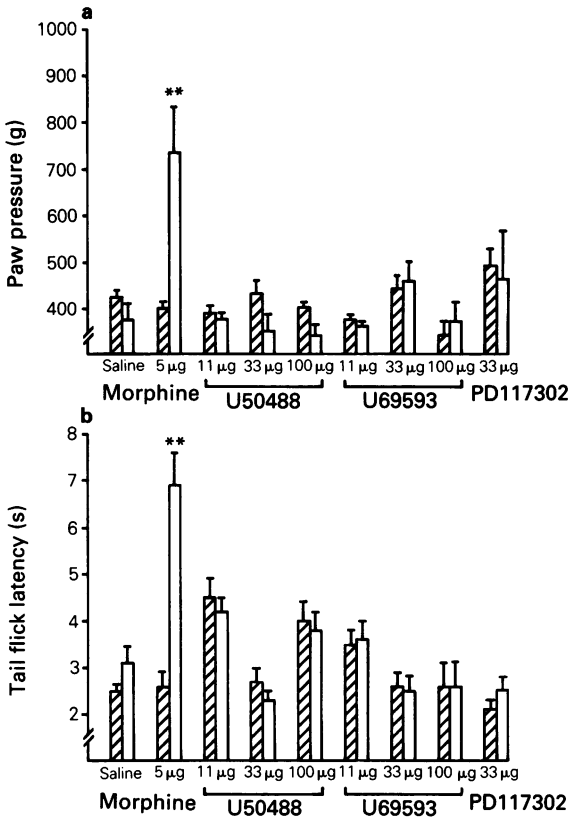

Figure 2 The effects of morphine (5 μg), PD117302, U50488 and U69593 on the paw pressure response (a) and tail flick latency (b) following intrathecal administration. Data are expressed as mean values with s.e. mean shown by vertical lines; $n = 5$ or 6 per dose level. $^{**}P < 0.01$ compared to pretest values (Wilcoxon matched pairs signed rank test). Hatched columns represent pretest values, open columns represent values obtained after treatment.

Intracerebroventricular administration of κ-opioids

Since no antinociception was observed following intrathecal administration of the κ-opioids, it was decided to investigate their effects following direct injection into the third cerebral ventricle. The effects of PD117302, U50488 and U69593 in the paw pressure and tail flick tests are shown in Figure 3 in comparison with the results obtained with morphine. Morphine was the most potent of the compounds tested with an elevation of the nociceptive threshold in the paw pressure test occurring with doses as low as 0.4 μg and an elevation of the threshold in the tail flick test occurring with doses of 3.7 μg and above

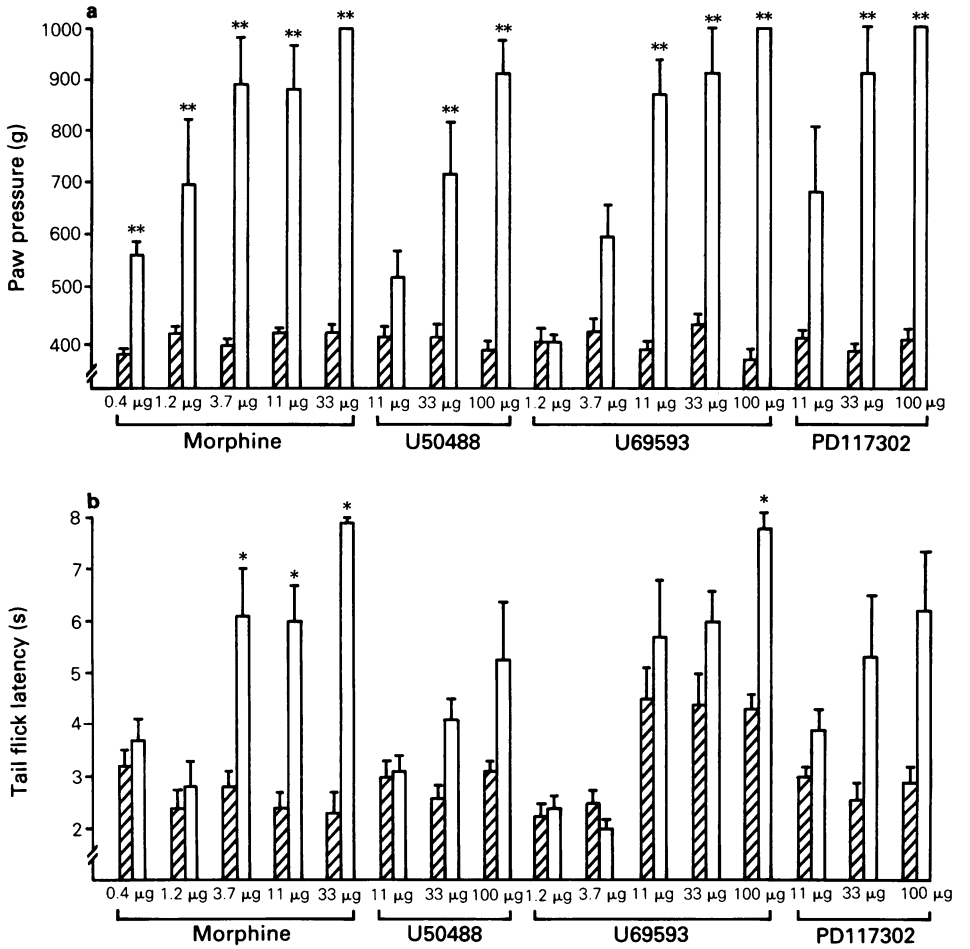

Figure 3 The effects of morphine (30 min after dosing), PD 117302, U50488 and U69593 (all 5 min after dosing) on the paw pressure response (a) and tail flick latency (b) following intracerebroventricular administration. Data are expressed as mean values with s.e. shown by vertical lines; $n = 5$ or 6 per dose level. $^{*}P < 0.01$, $^{**}P < 0.005$ compared to pretest values (Wilcoxon matched pairs signed rank test). Hatched columns represent pretest values, open columns represent values obtained after treatment.

(Table 1). PD117302, U50488 and U69593 all produced statistically significant antinociceptive effects in the paw pressure test.

We also evaluated these compounds in the tail flick test since κ-receptor agonists are reported to be much less effective against thermal than mechanical stimuli when administered peripherally (Tyers, 1981). Some antinociception was observed with all three compounds in this test although the effect reached the level of statistical significance only in the group treated with 100 μg U69593, a dose approximately ten times that required to produce antinociception in the paw pressure test.

Figure 4 shows the typical time course of the effect of morphine (3.7 μg) and U50488 (100 μg) in the paw pressure test following i.c.v. administration. The peak effect produced by morphine does not occur until 30 min after injection whereas the peak effect of U50488 occurs within the first 5 min. These antinociceptive effects were attenuated in animals pretreated with naloxone (1 mg kg^{-1}, s.c.) given 15 min before the i.c.v. administration of the κ-opioid, thus confirming that this antinociceptive effect is mediated by an action at opioid receptors. Results obtained with U69593 are shown in Figure 5.

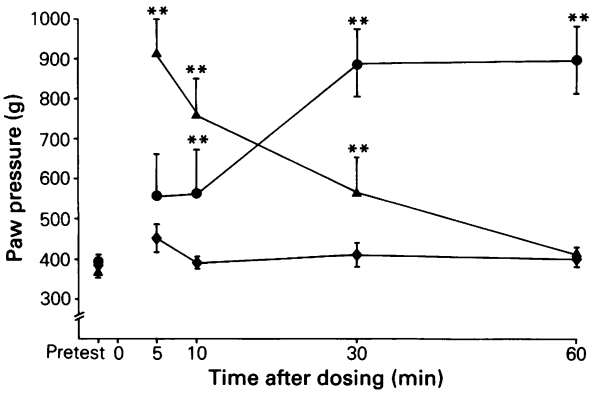

Figure 4 Time course of the antinociceptive effect produced by morphine (●, 3.7 μg) and U50488 (▲, 100 μg) following i.c.v. administration. Data are shown for the paw pressure test in comparison with the responses obtained in animals receiving saline (◆). Data are expressed as mean values with s.e. mean shown by vertical lines; $n = 6$ per group. ** $P < 0.01$ compared to pretest levels, Wilcoxon matched pairs signed rank test.

Discussion

The aim of this study was to produce evidence supporting a spinal and/or a supraspinal site of action for κ-receptor agonists in the production of antinociception. We used three specific κ-opioids, PD117302, U50488 and U69593, in order to avoid the complications associated with working with relatively nonspecific agents such as ethylketocyclazocine or with unstable peptides such as dynorphin. The tests used to detect antinociceptive effects were the paw pressure and tail flick tests. In the paw pressure test all three agents were shown to be effective at increasing the nociceptive threshold following intravenous administration. However, following intrathecal administration no increase in the response threshold was seen after administration of PD117302, U50488 or U69593. These three compounds were also without effect in the tail flick test, in agreement with results reported for U50488 by Stevens & Yaksh (1986). At the highest dose of PD117302 tested in these animals (100 μg per rat) we observed flaccid hindlimb paralysis of rapid onset following intrathecal injection. This paralysis was not prevented by pretreating animals with naloxone. Non-naloxone reversible paralysis has also been reported by several authors following intrathecal injection of dynorphin (Faden & Jacobs, 1983; Herman & Goldstein, 1985). The paralysis following intrathecal injection of dynorphin is seen at doses that are within the range that have been reported to produce antinociception (Przewlocki *et al.*, 1983; Kaneko *et al.*, 1983; Spampinato & Candeletti, 1985). However, since the behavioural tests used by these authors depend upon a motor withdrawal in response to the noxious stimuli, the apparent antinociception reported may reflect motor impairment produced by dynorphin rather than a true antinociceptive effect. The apparent antinociceptive effect of dynorphin has been reported to be resistant to antagonism by naloxone suggesting that it may be a non-opioid effect (Herman & Goldstein, 1985). Relatively few studies have been performed in which the recently described selective κ-opioids U50488 and U69593 have been administered directly into the subarachnoid space of the spinal column. Schmauss & Yaksh (1984) reported that intrathecal U50488 was effective only in suppressing the response to a noxious visceral chemical stimulus but was without effect in hotplate or tail flick tests. Jhamandas *et al.* (1986) described an analgesic effect in the tail flick test following intrathecal administration of U50488; however the maximum effect did not occur until 60 min after injection which suggests that U50488 may not have been acting locally in the spinal cord to produce this effect but rather was diffusing to some distant site. It is noteworthy that we see maximum analgesia following intravenous or i.c.v. administration of U50488 within 5 min of injection.

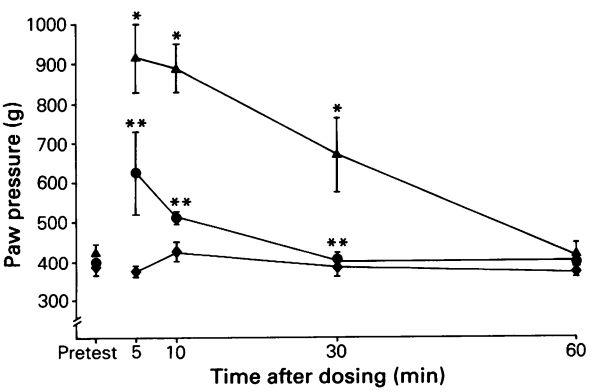

Figure 5 The effect of i.c.v. U69593 (33 μg) following pretreatment (s.c.) with saline (▲, $n = 6$) or 1 mg kg^{-1} naloxone (●, $n = 5$). A third group received saline pretreatment followed by saline i.c.v. (◆, $n = 6$). Data are expressed as mean values with s.e. mean shown by vertical lines; * $P < 0.01$ compared to pretest values, Wilcoxon matched pairs signed rank test. ** $P < 0.01$ compared to the saline + U69593 group, Kruskal Wallis analysis of variance.

Since we were unable to show any analgesic activity following intrathecal administration of κ-agonists yet they were clearly active when given intravenously, we decided to investigate possible supraspinal effects. Following direct injection into the

third cerebral ventricle clear dose-related increases in the nociceptive threshold in the paw pressure test were seen after treatment with PD117302, U50488 and U69593. An antinociceptive effect was also observed in the tail flick test although the doses required were greater than those needed to produce an effect against the mechanical stimulus. Morphine was also found to be active in the paw pressure test at a dose approximately one tenth of that effective in the tail flick test suggesting that the paw pressure test is a more sensitive measure of antinociceptive activity. The observation that the ratio of doses of U69593 required to produce antinociception in the mechanical and thermal tests is the same as that seen with morphine, is in agreement with the observations of Calthrop & Hill (1983) that neither κ- nor μ-selective opioids discriminate between the responses of medullary dorsal horn neurones evoked by mechanical and thermal noxious stimuli. Since the injection volume used for the i.c.v. administration of substances was quite large (20 μl), it was necessary to check that the behavioural effects observed were not due to redistribution of the κ-agonists into the spinal cord (via the central canal). A series of experiments were performed in which [^{3}H]-PD117302 (in a volume of 20 μl) was injected into the third ventricle. Animals were then killed 5, 10 or 30 min after injection and levels of radioactivity in different brain regions determined. Levels of radioactivity in the cervical spinal cord were very low in animals killed 5 or 10 min after dosing and by 30 min after dosing the levels of radioactivity remaining in any of the brain regions was negligible suggesting that PD117302 distributes rapidly from the brain into the blood rather than being redistributed into the spinal cord (unpublished observations).

Hayes *et al.* (1983) and Kaneko *et al.* (1983) report antinociception following i.c.v. administration of dynorphin in the rat using a mechanical noxious stimulus and Unterwald *et al.* (1987) have reported a raised escape threshold to an aversive electrical brain stimulation of the median forebrain bundle, a model of supraspinal analgesia, following treatment with ethylketocyclazocine. These reports support our suggestion that the antinociceptive effects of κ-agonists are mediated primarily via an action at a supraspinal site. However, Schmauss & Yaksh (1984) and Stevens & Yaksh (1986) report that κ-agonists administered intrathecally are potent antinociceptive agents when tested against a visceral chemical stimulus and it is therefore possible that κ-agonists act at two different levels within the central nervous system to produce antinociception, depending upon the precise nature of the noxious stimulus used.

It is interesting to note that the presence of κ-receptors in the spinal cord does not necessarily imply that these receptors have an obligatory role in antinociception. Intrathecally administered κ-agonists have been found to inhibit suckling-evoked oxytocin release (Wright & Clarke, 1986), and κ-receptors may have functional importance in a number of such non-nociceptive sensory reflexes.

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