Antinociceptive actions of intrathecal xylazine: interactions with spinal cord opioid pathways

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Summary
We have studied rats with chronically implanted subarachnoid catheters. Xylazine, an \(\alpha_2\) adrenoceptor agonist, was injected intrathecally and nociceptive thresholds measured at two skin sites: the tail and the neck. Intrathecal xylazine (dose range 24.3–389 nmol) produced increases in electrical thresholds for nociception in the tail without any change in the neck; this observation suggested that the antinociceptive action of this drug was confined to the caudal part of the spinal cord responsible for tail innervation. The magnitude of this effect was dose-dependent. Tail flick latency also increased in these rats and the antinociceptive effects were antagonized in a dose-dependent manner by the selective \(\alpha_2\) adrenoceptor antagonist idazoxan (dose range 6.7–540 nmol). Intrathecal idazoxan also suppressed the increase in tail flick latency caused by the mu opioid agonist fentanyl (0.74 nmol) given intrathecally. This effect was also dose-dependent. The idazoxan dose–response curve for this suppression of fentanyl antinociception assessed with tail flick latency was the same as that for suppression of xylazine. In contrast, the antinociceptive effects of intrathecal xylazine were not affected by concurrent administration of opioid or GABA\(_A\) antagonists. We conclude that intrathecal xylazine produced spinally mediated antinociceptive effects by combination with spinal cord \(\alpha_2\) adrenoceptors and that neither opioid nor GABA-containing propriospinal neurones were involved in the mediation of this effect. However, \(\alpha_2\) adrenoceptors in the spinal cord appear to be involved with antinociception produced by intrathecal fentanyl. (Br. J. Anaesth. 1996; 76: 544–551)

Key words

Drugs selective for \(\alpha_2\) adrenoceptors and even subtypes of \(\alpha_2\) receptors have been shown to produce antinociceptive effects assessed with a variety of tests, such as hot plate, tail flick, paw pressure and visceral nociception [1–4]. Antinociceptive effects are readily demonstrated after intrathecal injection of these drugs. However, rarely is evidence provided that would indicate that the drug was confined in its action to the spinal cord and that it did not produce the antinociceptive effects by spreading to the brain.

It has been shown recently that differential or segmental antinociceptive effects occur after intrathecal injection of a variety of opioid and benzodiazepine agonists [5–9]. In experiments where segmental antinociceptive effects were clearly demonstrated with the electrical current threshold (ECT) test, increases in tail flick latency after some intrathecal drugs have also been shown [9–11]. In such experiments some of the antinociceptive effects of intrathecal 5-HT (increases in tail ECT but not tail flick latency) resulted from activation of \(\mu\) opioid and GABA\(_A\) systems within the spinal cord [11, 12]. A similar interaction between opioid and adrenoceptor spinal cord systems has been suggested, although proof of restriction of drug effects to the spinal cord, as described above, was not provided [13–15]. In this study, we report the segmental antinociceptive effects of an intrathecal \(\alpha_2\) adrenoceptor agonist and characterize these antinociceptive effects using ECT and tail flick latency tests. The combination of the drug with \(\alpha_2\) adrenoceptors was responsible for these effects and the possible involvement of spinal cord opioid or GABA\(_A\) receptor systems was investigated.

Materials and methods
This work was carried out with permission from the licensing authorities in the UK (Home Office Licence No. PPL50-00131). In all experiments attention was paid to ethical guidelines for investigation of experimental pain in conscious animals [16]. Experiments were performed on male Wistar rats (weights 150–200 g) with chronically implanted lumbar subarachnoid catheters. Two tests, measurements of electrical current threshold for nociception (ECT) and tail flick latency, were used to demonstrate the antinociceptive effects of intrathecally administered \(\alpha_2\) adrenoceptor agonist (xylazine) and opioid agonists fentanyl (\(\mu\) selective) and U50488H (\(\kappa\) selective). Dose-response relationships of xylazine...
given alone were assessed and then in a separate series of experiments, we investigated the effects of various antagonists on spinally mediated antinociception caused by a standard dose of intrathecal agonist. Dose–response curves for the opioids were determined in previous experiments [9]. Idazoxan (Research Biochemicals International; RBI) was used as the $\alpha_2$ adrenoceptor antagonist, naloxone (Du Pont) as the opioid antagonist and bicuculline (Sigma, London) as the GABA$\alpha$ antagonist. All drugs were given intrathecially in a volume of 5 µl made up in physiological saline or 6% glucose solution.

**CATHETER IMPLANTATION**

Rats were anaesthetized with halothane in oxygen-enriched air ($F_{O_2} = 0.5$) and a mid-lumbar laminectomy performed under aseptic surgical conditions using a 1.5-mm dental drill. The dura mater was exposed and incised. A catheter was manufactured from a 5-cm length of Portex tubing (i.d. 0.25 mm, o.d. 0.75 mm) stretched to a length of 10 cm in hot water (i.d. 0.1 mm approximately). It was introduced 1.0 cm into the subarachnoid space through an incision in the dura to lie next to the most caudal segments of the spinal cord, as described previously [9]. The catheter deadspace volume was measured before implantation; this varied between 4.5 and 7.5 µl. After closure of the surgical wounds with nylon and silk sutures (Ethilon and Mersilk; Ethicon Ltd) the animals were allowed to recover from general anaesthesia; this usually took 5–10 min.

After full recovery from general anaesthesia the animals were observed carefully for normal movement and behaviour. Any animal exhibiting signs of neurological damage was killed immediately (typically 5% of rats). The rest of the animals were subjected to a lignocaine test; we have previously found this to be a reliable test for correct intrathecal positioning of the catheter [8]. This test was performed after each cannulation and after each experiment in which intrathecal drug was administered. Thus intrathecal injection of drugs was confirmed. Any animal exhibiting a negative lignocaine test after cannulation (typically 10%) was excluded.

**NOCTIVE TESTS**

A series of nociceptive tests was performed on each animal, the first on the day after cannulation. Although this is early after surgery, this preparation has been shown to produce stable baseline readings for the whole of the first week after cannulation [6, 9, 11, 17]. One test was performed each day on each animal up to a maximum of six. The rats were kept in groups of five before intrathecal cannulation and then in single boxes in rooms with a 12-h light-dark cycle. They were allowed free access to food and water throughout. They were placed individually in a proprietary restrainer and experiments were carried out in a quiet, darkened environment to minimize distraction of the animal by extraneous noise and light. Nociceptive thresholds were measured every 5 min using the ECT. In some experiments tail flick latency was also measured.

**ELECTRICAL CURRENT THRESHOLD**

Needle electrodes were placed 1 cm apart in the skin at the base of the neck. Wire electrodes were applied to the surface of the skin 1 cm and 5 cm from the base of the tail. The electrical current threshold (measured in mA at each skin site) was assessed, as described previously [9], every 5 min by the “up-down” method until three consecutive stable control readings had been obtained. The intrathecal drug or combination of drugs (see below) was then injected down the intrathecal catheter using the catheter swellings (used as markers to determine the catheter deadspace volume before implantation) to determine precisely the injection volume and dose. The electrical current threshold was measured at both skin sites every 5 min for at least another 20 min.

Individual responses were measured in mA. It has been found previously that these values for nociceptive thresholds vary between individuals and in the same individual at different times because of differences in electrode placement. They were therefore standardized by dividing the mean of the three threshold readings obtained 5, 10 and 15 min after intrathecal injection of agonist by the mean of the three corresponding readings taken before injection. This standardized response (denoted $r$) was a multiple of the control value.

**TAIL FICK LATENCY TEST**

Tail flick latency was measured before assessment of ECT in the tail. The heat from a 150-W projector bulb was focused on to the tip of the tail painted black. Tail flick latency (measured in seconds) was assessed every 5 min until three stable control (pre-drug administration) measurements had been achieved (typically four trials), and then every 5 min after intrathecal drug administration. The increase in tail flick latency was standardized as a percentage of maximum possible effect (% MPE) calculated as follows:

$$\% MPE = \frac{\text{tail flick latency (predrug)} - \text{tail flick latency (predrug)}}{\text{cut-off time} - \text{tail flick latency (pre-drug)}} \times 100$$

The cut-off time was the maximum time allowed for the animal to flick its tail away from the heat source before the lamp was automatically switched off. This was set at 10 s to avoid burning the tail.

**ANTINOCICEPTIVE EFFECTS OF XYLAZINE**

ECT was measured in the tail and neck in 48 experiments on 24 rats. In 30 of these experiments (11 rats) tail flick latency was also measured. Xylazine was injected intrathecally (24.3–389 nmol dissolved...
in 6% glucose 5μl over a period of 30 s with the animal tilted 45° head up to restrict distribution of the drug to the lumbar spinal cord. The standardized responses in both the neck and the tail were grouped for each dose of xylazine and the mean (SEM) calculated.

In nine of the above experiments xylazine 195 nmol was given intrathecally. The responses (ECT and tail flick latency) were standardized differently from those above to produce average time–response curves. The three control readings taken before intrathecal injection were averaged for ECT in the tail and neck. All values of current threshold (mA) at each 5-min period (whether before or after drug administration) were then divided by the corresponding average pre-injection value for that pair of electrodes. For tail flick latency each reading was standardized as %MPE using the equation above. The values thus obtained in different experiments for tail flick latency and ECT (tail and neck) were combined for each time of testing to produce means that were plotted on time–response curves. Thus the timing of onset of segmental antinociceptive effects (increases in tail ECT without any change in the neck threshold) could be compared with the onset of antinociceptive effect demonstrated by the tail flick latency test.

EXPERIMENTS USING IDAZOXAN

Xylazine

We performed 42 experiments in nine rats using tail flick latency and ECT tests. Intrathecal xylazine was given alone (195 nmol dissolved in 6% glucose 5 μl) at the beginning and after a series of once daily experiments with idazoxan in each rat. In intervening experiments (maximum of four) the same dose of xylazine was given intrathecally 5 min after a range of doses of idazoxan (6.7–540 nmol). The response of idazoxan was calculated as percentage suppression of the response to agonist given alone for both the ECT and tail flick latency tests. This was done by calculating the mean (R) of all tail responses to xylazine 195 nmol given alone for each test. Percentage suppression of control agonist response was calculated from the following equation:

\[ \text{% suppression} = \frac{R - r}{R - 1} \times 100\% \]

where R = response (either ECT (tail x control) or increase in tail flick latency, %MPE) in each individual experiment with antagonist. For the results of tail flick latency a value of 0, not 1, was used in the denominator. The results for percentage suppression by each dose of antagonist in individual experiments were grouped and the mean (SEM) calculated. These results were used to plot idazoxan dose–response curves.

Fentanyl

The experimental design in these experiments was the same as that for xylazine. Both noxious tests were used to assess the effect of intrathecal fentanyl 0.74 nmol dissolved in 6% glucose 5 μl. This dose was chosen from the dose–response relationship determined in previous experiments [9]. This dose caused segmental antinociceptive effects similar to those of xylazine. Sixty-five experiments were performed in 18 rats in which responses to agonist alone were assessed before and after an intervening series of experiments in which fentanyl 0.74 nmol was injected intrathecally 5 min after idazoxan (dose range 6.7–540 nmol), also injected intrathecally. We plotted percentage suppression of the antinociceptive effects of fentanyl by idazoxan, derived as above, and idazoxan dose–response curves.

Controls

Tail flick latency and ECT (tail) were measured every 5 min for 15 min before and 20 min after intrathecal injections of either idazoxan 180 or 540 nmol dissolved in 6% glucose 5 μl, or 6% glucose 5 μl alone in 13 rats. We searched for evidence of residual drug effects or progressive neurological damage at each time of testing by comparing the absolute values of ECT in the tail obtained at each time of testing with the first measurements obtained in that animal.

EXPERIMENTS USING NALOXONE

Xylazine

Sixteen experiments were performed in 11 rats. Both noxious tests were used to assess the effect of naloxone 2 mg kg⁻¹ dissolved in saline 1 ml given i.p. on the antinociceptive effects of intrathecal xylazine 195 nmol dissolved in 6% glucose 5 μl. One experiment was performed on each animal per day. First the animal received intrathecal xylazine alone. On the following day, after three stable pre-injection readings had been obtained with both tests, naloxone was given i.p. 5 min before intrathecal injection of xylazine. Both noxious tests were carried out every 5 min for another 20 min. The results from each experiment were combined to produce mean (SEM) values for the responses in both tests to agonist alone and to agonist after i.p. injection of naloxone.

U-50488H

Larger doses of naloxone are required to suppress the effects of κ-selective compared with μ-selective opioids. U-50488H, a selective κ opioid agonist, was used to assess the effectiveness of the dose of naloxone to suppress all opioid responses in the xylazine experiments. The same design was used in these experiments as in those described above for fentanyl, xylazine and naloxone. We performed four experiments in four rats. Measurement of ECT (tail and neck) was used to assess the effects of intrathecal U-50488H on its own (210 nmol dissolved in saline 10 μl) and after i.p. naloxone 2 mg kg⁻¹. Tail flick latency was not used because the drug does not alter this measure of antinociception [7, 18, 19]. Results were expressed as mean (SEM) for U-50488H given alone and in the presence of naloxone.
Comparisons of responses to xylazine, fentanyl and U-50488H were made with an unpaired t test to confirm that the doses used were equipotent. The effect of naloxone on the antinociceptive responses of xylazine and U-50488H was assessed using paired t tests.

EXPERIMENTS WITH BICUCULLINE

Eight experiments were performed on four rats in which measurements of both ECT and tail flick latency were used to assess the effects of the GABA_A antagonist bicuculline on the antinociceptive action of intrathecal xylazine 195 nmol dissolved in 6% glucose 5 μl. A dose of 50 pmol of bicuculline was chosen from previous studies [6, 12]. In addition, intrathecal bicuculline 50 pmol given alone had no effect on the ECT in the tail [6]; these control experiments were therefore not repeated.

In another four rats the antinociceptive effects of intrathecal xylazine given alone and injected 5 min after administration of intrathecal bicuculline 50 pmol were assessed using both nociceptive tests. The responses in each group were combined to produce mean (SEM) values and compared using a paired t test.

Results

ANTINOCICEPTIVE EFFECTS OF INTRATHECAL XYLAZINE

Figure 1 shows the dose-response curve for the antinociceptive effects of intrathecal xylazine assessed with both nociceptive tests. Doses up to 389 nmol produced highly significant increases in the electrical current threshold in the tail (mean 2.67 (SEM 0.16) × control; n = 8; P < 0.01 paired Student’s t test) with no significant increase in the electrical current threshold measured in the neck (1.1 (0.07); n = 8). At the same time intrathecal xylazine caused significant increases in tail flick latency (76 (10) %MPE; n = 8; P < 0.01 paired Student’s t test).

Figure 2 shows time–response curves for tail flick latency and ECT responses in nine rats that received intrathecal xylazine 195 nmol. Intrathecal xylazine caused simultaneous significant increases in tail flick latency and ECT in the tail. These effects lasted for the duration of the experiment and were accompanied by no significant changes in electrical threshold in the neck. The time to offset of the responses could not be measured in this preparation because licensing restrictions only allowed restraint for this time. However, examination of control (pre-drug injection) and subsequent electrical current thresholds in the tail revealed no evidence for drug effects lasting until the next day or the occurrence of neurological damage; thresholds did not increase in any of the experimental series’ as they progressed.

ANTAGONISM WITH INTRATHECAL IDAZOXAN

Xylazine

Doses of intrathecal idazoxan (6.7–540 nmol) given intrathecally 5 min before intrathecal xylazine 195 nmol caused dose-dependent suppression of the antinociceptive effects of xylazine assessed by both tests (fig. 3). There were no significant differences (paired Student’s t test; P < 0.5) between the responses to xylazine given alone at the beginning of the series of experiments (ECT 2.05 (0.06) × control; tail flick latency 90.2 (5.5) %MPE) and those
obtained after the series of experiments (ECT 2.2 (0.11) × control; tail flick latency 94.8 (4.9) %MPE).

Fentanyl

The antinociceptive effects of fentanyl assessed with the tail flick latency test were suppressed by idazoxan, with the same dose–response relationship as that for suppression of the antinociceptive effects of xylazine assessed by both nociceptive tests (fig. 3). However, the effects of fentanyl assessed by the ECT test were not suppressed by idazoxan. There were no significant differences between the antinociceptive responses to intrathecal fentanyl given alone at the beginning of the series of experiments (ECT 1.93 (0.1) × control; tail flick latency 73.2 (5.9) %MPE) or after the series of experiments (ECT 2.18 (0.12) × control; tail flick latency 79.9 (4.7) %MPE). There were no significant increases in neck ECT (1.08 (0.11 × control). These responses also did not differ significantly from the corresponding responses to intrathecal xylazine given alone. The doses of the two drugs were therefore equieffective in the production of antinociceptive effects assessed by both tests, and the actions of both and interactions with idazoxan were restricted to the spinal cord. Control (pre-injection) and subsequent responses indicated that there was no drug accumulation or progressive neurological deficit as the experimental series progressed.

Both doses of idazoxan and 6 % glucose did not cause significant changes in thresholds assessed by either nociceptive test (table 1).

EXPERIMENTS WITH NALOXONE

The dose of naloxone used (2 mg kg\(^{-1}\) i.p.) was sufficient to suppress totally the segmental antinociceptive effects produced by U-50488H, confirming previous studies with \(\kappa\) selective opioids [7, 17]. This high dose of naloxone did not produce any significant suppression of the antinociceptive effects caused by intrathecal xylazine (table 2). There were no significant differences between the responses to intrathecal xylazine and U-50488H given alone. All the responses to intrathecal U-50488H were segmental; no significant increases in neck ECT occurred (0.95 (0.11) × control).

EXPERIMENTS WITH BICUCULLINE

Intrathecal xylazine alone (195 nmol) produced significant segmental antinociceptive effects in the ECT test (1.96 (0.1) in the tail and 0.99 (0.01) in the neck; \(n = 4\)) and significant increases in tail flick latency (83 (17) %MPE; \(n = 4\)). Concurrent intrathecal administration of bicuculline 50 pmol did not significantly suppress either the ECT response (2.17 (0.1) in the tail and 1.0 (0.02) in the neck; \(n = 4\)) or tail flick latency (82.5 (13) %MPE; \(n = 4\)).
Antinociception by intrathecal xylazine

Table 1 Antinociceptive effects of idazoxan and 6 % glucose (mean (SEM) (n)), as assessed by the electrical current threshold for nociception (ECT) and tail flick latency (TFL). %MPE = percentage of maximum possible effect

<table>
<thead>
<tr>
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<th>ECT (× control)</th>
<th>TFL (%MPE)</th>
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<tr>
<td></td>
<td>Tail electrodes</td>
<td>Neck electrodes</td>
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<tr>
<td>Controls</td>
<td></td>
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<tr>
<td>6 % Glucose 10 µl</td>
<td>0.99 (0.03) (10)</td>
<td>1.01 (0.02) (10)</td>
</tr>
<tr>
<td>Idazoxan 180 nmol</td>
<td>1.02 (0.03) (7)</td>
<td>0.98 (0.02) (7)</td>
</tr>
<tr>
<td>Idazoxan 540 nmol</td>
<td>0.95 (0.09) (7)</td>
<td>0.97 (0.04) (7)</td>
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Table 2 Effects of naloxone on the antinoceptive effects of U-50488H and xylazine, as assessed by the tail Electrical current threshold for nociception (ECT) and tail flick latency (TFL)

<table>
<thead>
<tr>
<th></th>
<th>ECT (× control)</th>
<th>TFL</th>
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<tbody>
<tr>
<td>U-50488H alone</td>
<td>2.04</td>
<td>86.5</td>
</tr>
<tr>
<td>U-50488H+ naloxone</td>
<td>1.01</td>
<td>85.3</td>
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<tr>
<td>Xylazine alone</td>
<td>2.25</td>
<td>8.84</td>
</tr>
<tr>
<td>Xylazine+ Naloxone</td>
<td>0.20</td>
<td>9.26</td>
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Discussion

Intrathecal injections of xylazine produced segmental antinociceptive effects in the ECT test and simultaneously caused increases in tail flick latency. These effects occurred without any signs of sedation. The antinociceptive effects of xylazine assessed by both tests exhibited the same pattern of onset and were maintained for the entire experiment. Xylazine antinociception was maintained for much longer periods than any of the opioids or benzodiazepines in this preparation [7, 9]. The drug effects had disappeared 24 h later because there was no increase in control (pre-drug) tail electrical thresholds in subsequent experiments. These experiments have confirmed the antinociceptive effects of spinal α2 adrenoceptor agonists reported by many others [1–4, 20–23]. Although most of those studies did not perform any of the manoeuvres mentioned above to prove a spinal cord action of the drug administered, the weight of evidence indicates a spinal site is responsible for the antinociception in a wide variety of tests. Waterman, Livingston and Bouchenafa showed segmental antinociceptive effects (five dermatomes around the catheter site) after spinal administration of xylazine in conscious sheep [22]; this effect was reversed completely by idazoxan. Our experiments allowed precise measurements of electrical thresholds and comparisons of dose–response curves to analyse drug interactions at the level of the spinal cord.

The dose–response curve for the tail flick latency response lies significantly to the left of that for the electrical current threshold response. Similar observations were made by Skingle, Hayes and Tyers [24] using clonidine, although they did not show differential responses in skin areas innervated by caudal compared with rostral spinal cord segments (a segmental effect). We have reported such differences in the dose–response curves for the two tests after intrathecal 5-HT [11].

Differences in the mechanisms responsible for the antinociception caused by 5-HT in tail flick latency compared with the ECT have also been demonstrated. The antinociceptive effects of 5-HT assessed by ECT could be suppressed by naloxone and bicuculline. However, the antinociceptive effects of 5-HT assessed by tail flick latency were not affected by either antagonist [11, 12]. Therefore, these results indicated involvement of propriospinal systems using GABA A and μ opioid receptors in the pathways excited by ECT.

In the present study we have demonstrated that the antinociceptive effects of intrathecal xylazine are mediated by a combination of the agonist with spinal cord α2 adrenoceptors because both the tail flick latency response and ECT were suppressed selectively by intrathecal idazoxan. This confirms the findings of Waterman, Livingston and Bouchenafa, although they did not perform a detailed dose–response analysis of the antagonism [22]. The same dose of idazoxan produced similar suppression of both responses. In contrast with the results obtained previously for intrathecal 5-HT [11, 12] it was not possible to demonstrate involvement of either an opioid or GABA A receptor-mediated intermediate stage in the antinociception produced by xylazine; neither naloxone nor bicuculline suppressed increases in tail flick latency or ECT caused by intrathecal xylazine. The dose of naloxone used was sufficient to block all spinal opioid receptors as it was capable of completely suppressing the antinociceptive effects of the κ selective opioid against U-50488H in this series of experiments and has previously been shown to be capable of suppressing spinal antinociception caused by a variety of opioids and benzodiazepines [7, 17]. The dose of bicuculline used was also sufficient to block spinal GABA A receptors as it has been shown previously to be effective in the suppression of spinally mediated antinociceptive effects of midazolam and 5-HT [12].

As segmental effects were demonstrated in all of our experiments for all intrathecal agonists it must be concluded that the actions of the agonists and their interactions with the antagonists must have occurred at the spinal cord level. We conclude therefore that the antinociceptive effects of xylazine result from an action on spinal α2 adrenoceptors;
neither opioid nor GABA–benzodiazepine systems are involved as neither naloxone nor bicuculline suppressed these effects of xylazine.

Moreover, some of the spinally mediated antinoceptive effects of intrathecal fentanyl (ECT effects) result from actions at native \( \mu \) opioid receptors and do not involve any other propriospinal system. In contrast, for increases in tail flick latency caused by intrathecal fentanyl there seems to be an interaction between the native \( \mu \) opioid pathway and those pathways activated by \( \alpha_2 \) adrenoceptor agonists, as the selective \( \alpha_2 \) adrenoceptor antagonist idazoxan suppressed the effects of intrathecal fentanyl on tail flick latency. The results of these experiments with fentanyl and idazoxan indicate that the \( \mu \) selective opioid binds with opioid receptors leading to activation of a spinal cord pathway that involves a native \( \alpha_2 \) adrenoceptor; this is an essential part in the production of an increase in tail flick latency.

Interactions between \( \mu \) opioid and \( \alpha_2 \) adrenoceptor systems have been reported by several authors. For example, potentiation of the antinoceptive effects of intrathecal morphine by oxymetazoline has been reported [14]. Paw pressure and tail flick latency measurements were used but no proof of confinement of the drugs to the spinal cord was given. The potentiation (not simply the addition of effects) might therefore have been because of the actions of either or both drugs at supraspinal and spinal sites. The results presented in this study indicate that some potentiation is possible because the pathways activated by fentanyl and xylazine were not totally convergent. A similar relationship between the adrenoceptor and opioid systems has been reported from cross tolerance studies [13, 15, 25]. In one of these studies [25] it was shown that rats tolerant to ST-91 (an \( \alpha_2 \) adrenoceptor agonist) showed no cross tolerance to morphine but morphine-tolerant rats demonstrated cross tolerance to ST-91. This might be predicted by the results of this study; induction of tolerance at the \( \alpha_2 \) adrenoceptor by ST-91 also reduces the effect of morphine which acts via the adrenoceptor after binding with the opioid receptor. The nociceptive test used in this latter study was the tail flick latency test, the test which revealed convergence of the two spinal cord systems in the experiments reported here. Both of the other studies also used tail flick latency [13, 15] although one [13] also used paw pressure; neither study provided proof of confinement of the drugs to the spinal cord. Both of these latter studies seem to be at variance with the results reported here. The first [15] reported that chronic administration of clonidine (\( \alpha_2 \) adrenoceptor agonist) decreased the antinoceptive effect of intrathecal morphine as tolerance to morphine developed, a result that would be predicted by the present study. However, they [15], in common with the second group of investigators [13], showed that chronic administration of morphine to cause morphine tolerance also decreased the effect of intrathecal \( \alpha_2 \) adrenoceptor agonist. It is likely that morphine acted at sites in the brain leading to continuous activity in descending systems releasing noradrenaline onto spinal cord \( \alpha_2 \) adrenoceptors [21, 26, 27]. This might have induced down-regulation of these receptors thereby inducing cross tolerance with the \( \alpha_2 \) adrenoceptor agonists.

Acknowledgement

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References


18. Schmauss C. Spinal kappa-opioid receptor-mediated antino-
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