INTRATHECAL MIDAZOLAM IN THE RAT: EVIDENCE FOR SPINALLY-MEDIATED ANALGESIA

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It has been suggested that benzodiazepines might have analgesic properties via an action in the spinal cord. Rigoli (1983) reported that the extradural administration of midazolam to post-operative patients and individuals with chronic pain afforded significant analgesia. However, relatively large doses were used and significant sedation was produced, thus making the measurements of pain by analogue scores unreliable.

Benzodiazepine stereospecific binding sites have been demonstrated in the spinal cord (Mohler and Okada, 1977) and endogenous benzodiazepine-like substances have been discovered in human cerebrospinal fluid (CSF) (Deckert, Kuhn and Przuntek, 1984); however their role has not been identified as yet. Elsewhere in the central nervous system membrane preparations of benzodiazepine receptors have been linked with the receptors for gamma amino butyric acid (GABA) in the control of chloride channels (Schoch et al., 1985); opening of these channels on the surface membrane of a neurone produces hyperpolarization and, therefore, inhibition of firing. GABA has been implicated in spinal cord anti-nociceptive mechanisms (Game and Lodge, 1975), but interaction between benzodiazepine stereospecific binding sites in the spinal cord and GABA in antinociception has not been proven.

Whitwam and colleagues (1982) showed that lumbar intrathecal application of the water soluble imidazo benzodiazepine, midazolam, interrupted reflexes in renal sympathetic nerves evoked by electrical stimulation of mixed peripheral nerves at intensities sufficient to recruit group III and IV afferent fibres (some of which carry pain information); resting sympathetic nerve activity was not decreased and only the reflex from the hind leg (input to lumbar dorsal horn—the site of application of the drug) and not the foreleg (input to cervical dorsal horn) was attenuated. The observed effects were, therefore, likely to result from interruption of pathways in the lumbar dorsal horn, although a mild local anaesthetic effect affecting very fine nerves only could not be ruled out. The purpose of our investigation was to determine if the interruption of the afferent pathways taken by nociceptive afferents could afford spinally-mediated analgesia, and to define whether this effect was the result of a stereospecific interaction with benzodiazepine receptors, rather than a local anaesthetic effect.

SUMMARY

This study investigated the possible analgesic effect of midazolam as a result of interruption of those spinal cord pathways taken by pain afferents. Experiments were performed on 15 male Wistar rats with chronically implanted lumbar subarachnoid catheters. The threshold for pain induced by brief passage of electric current between pairs of electrodes placed on the tail and the skin of the neck was measured before and after subarachnoid injections of midazolam. Intrathecal midazolam caused a significant (P < 0.02) increase in the threshold for pain in the tail, but not in the neck; this response was not produced by intrathecal injections of vehicle and was blocked by prior intraperitoneal injections of the benzodiazepine antagonist RO 15–1788. We also performed experiments on frog sciatic nerves which showed that midazolam did not have a local anaesthetic action. We conclude that intrathecal midazolam causes spinally-mediated analgesia by binding to benzodiazepine receptors in the spinal cord.
MATERIALS AND METHODS

Investigations in the rat

**Catheter implantation.** Fifteen male Wistar rats (150–250 g) were anaesthetized with 5% halothane in oxygen-enriched air. Anaesthesia was maintained with 1.75% halothane in oxygen-enriched air given by face mask, the animals being placed in the prone position. A lower lumbar laminectomy was performed and a Portex catheter (i.d. 0.25 mm, o.d. 0.75 mm) was introduced to the lumbar subarachnoid space as previously described (Bahar, Rosen and Vickers, 1984); the catheter was advanced in the subarachnoid space until the tip lay in the upper lumbar region. The catheter was tailor-made for each rat and the deadspace was measured using a Hamilton micro syringe after subcutaneous tunnelling to an exit wound at the back of the neck before the subarachnoid insertion of the catheter. The wound at the back of the neck, before the nylon (Ethilon) sutures and the animal allowed to recover from anaesthesia.

Soon after recovery (10–15 min) the catheter position was verified by the intrathecal injection of 2% plain lignocaine solution 10 μl followed by a volume of normal saline (the previously measured deadspace volume) to flush the catheter. The catheter was judged to be in the correct position if the hind limbs of the rat became paralysed and were dragged behind the animal within 20–30 s of the injection. The animals were then allowed a further period of 2–4 h to recover fully from the effects of the local and general anaesthetics, although they were moving around their cage, eating and drinking normally within 1 h of the surgical preparation.

Animals with a negative lignocaine test or with obvious neurological damage following laminectomy were excluded from the study at this stage and were killed under anaesthesia. Five animals were used for a study of possible effects of intrathecal injection of vehicle and 10 were used for a study of differential spinal analgesia. The subarachnoid position of catheters was confirmed histologically 1 month after implantation (fig. 2).

**Intrathecal vehicle.** Each rat was placed in a restrainer (fig. 1) and two stimulating electrodes were placed on the skin of the tail. These electrodes consisted of two wires wrapped around the tail which was moistened with electrode jelly; the negative wire was placed 2 cm from the base of the tail and the positive wire 5 cm distal to the negative. These were connected to a peripheral nerve stimulator (Digitimer DS 10) which delivered rectangular 1-ms pulses at a frequency of 50 Hz, 1 ms pulses, 0–5 mA Stimulator.

FIG. 1. Schematic representation of equipment used to measure current threshold for pain in the tail, and in the neck.
50 Hz at a voltage up to 100 V. The current delivered through the electrodes was measured as a voltage decrease across a 1-kΩ resistor in series with the electrodes using a Gould digital storage oscilloscope. The minimum current necessary to produce an obvious reaction to pain (movement and vocalization) was measured every 10 min.

After 30 min, 25 µl of vehicle (normal saline) balanced to pH 3.5 with sodium hydroxide and hydrochloric acid injected through the intrathecal catheter and the deadspace (the volume of which was measured at the time of catheter insertion) was flushed with physiological saline. The minimum current necessary to produce pain was again measured in the skin of the tail every 10 min for 30 min. This was followed by an intrathecal injection of midazolam 25 µl (125 µg, flushed in with physiological saline) and the current threshold for pain measured for a further 30 min, as before. If the animals were released at this stage they behaved normally; that is, they were able to move around their cages, stand on their rear limbs, and feed and drink normally with no obvious signs of central nervous system depression. Finally, the catheter position was confirmed by another intrathecal injection of 2% plain lignocaine solution, as above.

Differential analgesia. Each animal was placed in the restrainer as above. In addition to the pair of electrodes on the tail, two needle electrodes were also placed 1 cm apart in the loose skin of the neck. The minimum electrical current (50 Hz, 1 ms, 0–5 mA) necessary to produce signs of discomfort was measured for each of these pairs of electrodes every 5 min. After six control readings a 2-ml intraperitoneal (i.p.) injection of either physiological saline (five rats) or RO 15-1788 5 mg (five rats) was given through a hole in the side of the restrainer without removal of the animal; the operator performing the measurements of current thresholds of pain did not know whether the rat under investigation had received saline or benzodiazepine antagonist.

The current threshold for pain was measured as before every 5 min for a further 30 min before and after the intrathecal injection of midazolam 10 µl. Care was taken to inject only 10 µl of midazolam (50 µg) to the subarachnoid space, by injecting a precise volume down the catheter (10 µl plus catheter deadspace) at a rate of 1 µl s⁻¹, using a Hamilton microsyringe; thus knowing the precise volume of the catheter deadspace (measured at the time of the catheter insertion) allowed us to be certain that only 10 µl of midazolam solution emerged from the catheter tip into the subarachnoid space.

Finally, the catheter position was confirmed by another intrathecal injection of 2% plain lignocaine solution, as above.

The mean current thresholds for pain in each 30-min period obtained with intrathecal vehicle and with differential analgesia were calculated and the results obtained after i.p. and intrathecal injections were normalized for each animal by dividing these values by the control values in each case. Student's t test for paired data was then applied to these data to compare thresholds before and after i.p. injections, and before and after intrathecal injections, of either vehicle or midazolam.

Investigations in the frog

Two adult frogs were stunned by a blow to the back of the head, and pithed. The sciatic nerves of each frog were dissected out from the spine to the knee. The distal portion of each nerve was placed on bipolar silver stimulating electrodes with the negative terminal proximal. The proximal portion of each nerve was placed on bipolar recording electrodes and the evoked potentials amplified (Gould Universal Amplifier) and displayed on a Digital storage oscilloscope; the captured waveforms were subsequently printed out on an ink jet recorder (Mingograf).

Supramaximal electrical stimuli were delivered to the nerve and, after control recordings had been obtained, the following solutions were placed in sequence in a pool on the nerve between the stimulating and recording electrodes: (a) vehicle: physiological saline with HCl and NaOH (pH of 3.5); (b) midazolam solution 0.5 ml (5 mg ml⁻¹); (c) 2% plain lignocaine solution 0.5 ml.

RESULTS

Investigations in the rat

Histological. Histological examination of the spinal cords of some of the rats 4–6 weeks after the study confirmed the subarachnoid position of the catheter. Figure 2 shows a photomicrograph of a transverse section of rat spinal cord stained with haematoxylin and eosin. Among the nerve roots in the subarachnoid space is a circular object which is arachnoid infiltration around a Portex catheter.
which has been removed during sectioning; this is probably a reaction to the catheter, but the possibility remains that it may be the result of the administration of drug or vehicle.

The effect of vehicle. Figure 3 shows the current threshold for pain in the skin of the tail in one rat before and after the intrathecal injection of vehicle and midazolam 25 μl (125 μg) intrathecally. The current threshold for pain in the tail was not altered after the intrathecal injection of vehicle, but increased markedly after the intrathecal injection of midazolam. This single result was confirmed by statistical analysis of the grouped data (table I). The mean current threshold for pain has been calculated before and after the intrathecal injection of vehicle, and after the intrathecal injection of midazolam 125 μg for each of the five rats. These results were normalized by dividing the figures obtained before and after the intrathecal injections of vehicle and of midazolam by the control figures to allow grouping of the results for statistical analysis. The mean thresholds varied between animals because of differences in electrode and tail resistances and
Differential analgesia. Figure 4 shows the current threshold for pain in the neck and the tail for one rat before and after the intrathecal injection of saline, and the intrathecal injection of midazolam 50 μg (10 μl).

Figure 5 shows the results from another rat which received the benzodiazepine antagonist RO 15-1788 5 mg i.p. 30 min before intrathecal midazolam. The features shown on figures 4 and 5 are supported by the grouped data (table II) when analysed statistically as above, viz.:

(a) intrathecal injection of midazolam caused an increase in the current threshold for pain in the skin of the tail but not in the neck \((P < 0.02);\) paired \(t\) test, \(M'\) v. \(S'\) in tables II (i) and II (ii));

(b) the increase in current threshold for pain in the skin of the tail was prevented by the prior i.p. injection of RO 15-1788 (paired \(t\) test \(M'\) v. \(RO'\) in table II (iv));

(c) there was no change in the current threshold for pain in the skin of the tail or neck after i.p. normal saline or RO 15-1788 (paired \(t\) test \(S'\) v. \(C'\) in tables II (i) and II (ii)) and \(RO'\) v. \(C'\) in table I (iii) and II (iv)).

The speed of injection and volume of injectate were found by experience to be crucial in obtaining this differential blockade. Figure 6 shows the result of a study performed before the investigation of differential blockade; the rat received midazolam 25 μl intrathecally without control of the speed of injection. The current

### Table I. The mean current thresholds for pain in the tail of five rats before \((C)\) and after the intrathecal injection of vehicle \((V)\) and after the intrathecal injection of midazolam \((M)\).

<table>
<thead>
<tr>
<th>Rat</th>
<th>(C)</th>
<th>(V)</th>
<th>(M)</th>
<th>(C')</th>
<th>(V')</th>
<th>(M'^{*})</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.90</td>
<td>1.03</td>
<td>0.83</td>
<td>1.00</td>
<td>1.15</td>
<td>10.93</td>
</tr>
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<td>0.22</td>
<td>2.24</td>
<td>1.00</td>
<td>0.28</td>
<td>2.93</td>
</tr>
<tr>
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<td>1.04</td>
<td>1.25</td>
<td>8.00</td>
<td>1.00</td>
<td>1.20</td>
<td>7.66</td>
</tr>
<tr>
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<td>0.19</td>
<td>0.75</td>
<td>1.00</td>
<td>0.31</td>
<td>3.46</td>
</tr>
<tr>
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<td>0.62</td>
<td>1.64</td>
<td>1.00</td>
<td>1.70</td>
<td>4.46</td>
</tr>
</tbody>
</table>

also differences between the animals themselves. These changes also occurred to a smaller extent within the same animal as the study progressed. It is therefore more appropriate to use comparisons of the figures in columns \(M'\) with \(S'\) and \(M'\) with \(RO'\) in table II, rather than \(M'\) with \(C'\). The application of Student's \(t\) test for paired data to \(C'\) v. \(V'\) and \(V'\) v. \(M'\) shows that there is a statistically significant \((P < 0.025)\) increase in the current threshold for pain in the skin of the tail after intrathecal midazolam, but no significant change after intrathecal injection of vehicle.
threshold for pain in the neck and the tail both increased after this injection and the subsequent injection of 2% plain lignocaine solution 25 \mu l to verify the position of the catheter led to a total spinal block, with extreme pallor and paralysis of all four limbs.

**Investigations in the frog**

The results from three experiments on frog sciatic nerve are shown in figure 7. Compound action potentials evoked by supramaximal stimulation of the distal ends of each sciatic nerve are shown as single sweeps of the oscilloscope: A during the control period before any application of substances to the nerve; B after the application of vehicle to the nerve trunk between the stimulating and recording electrodes; C after the application of midazolam 5 mg ml\(^{-1}\), and D after the application of 2% plain lignocaine solution to the nerve trunk. Neither midazolam nor the vehicle caused a diminution in the size of the compound action potential, unlike lignocaine which caused a typical local anaesthetic block.

**DISCUSSION**

The results from the first series of rats in which tail electrodes only were used, indicate that the intrathecal injection of midazolam causes an increase in the current threshold for pain in the tail, while the intrathecal injection of vehicle had no effect. This may be the result of spinally-mediated analgesia or general central nervous system depression from rostral spread of the drug. No obvious depression of the central nervous system occurred after the intrathecal injections of midazolam (the animals were not asleep and moved around the cage normally when released), but this possibility led us to design the second series of experiments with two sets of stimulating electrodes—one set on the tail and the other pair on the skin of the neck. It can be seen from the results of the second series of rats that intrathecal midazolam caused a differential effect when care was taken to control the speed and volume of injection; the increase in the current threshold for pain only occurred in the skin of the tail and not in the neck and this, therefore, implies a spinal mechanism for the analgesia.
It may be said that the performance of these experiments only 2–4 h after the surgical preparation and intrathecal injection of lignocaine would mean that the measurements had been made in the presence of an altered baseline. The animals were all moving around their cages and eating and drinking normally within 1 h of this preparation and the sudden change in the current threshold for pain in the tail and not in the neck following intrathecal midazolam must have been attributable to the midazolam and not just to the residual effects of the surgical preparation and intrathecal lignocaine. The volume of 2% lignocaine used (10 μl) to confirm the intrathecal position of the catheter (which was subsequently verified 1 month later by histopathological examination) is much smaller than Bahar, Rosen and Vickers (1984) found necessary to cause paralysis of the rear limbs (32 ± 3 μl). Since confirmation of the intrathecal position of all catheters was not reported in that study, one possible explanation for these differences may be that a proportion of their catheters were extradural and not intrathecal.

Two possibilities exist for the mechanism of spinally-mediated analgesia: a local anaesthetic effect on afferent nerves going into the spinal cord; and interference with neurotransmission of nociceptive afferent information, possibly mediated by combination with benzodiazepine receptors. Frog sciatic nerve was chosen as a convenient preparation for the study of possible local anaesthetic effects and the possibility of a local anaesthetic action was ruled out by the results of these studies. We were unable to demonstrate any local anaesthetic effect of midazolam which was applied at high concentration (5 mg ml⁻¹) to the sciatic nerve trunk, in contrast to the local anaesthetic blocking action of lignocaine. The second possibility is much more likely, since we were able to block the analgesic effect of intrathecal midazolam by prior administration of the benzodiazepine antagonist RO 15–1788 (figs 4, 5; table II).

Therefore we conclude that intrathecal midazolam causes spinally-mediated analgesia by binding to benzodiazepine receptors in the spinal cord. It may be that this combination with benzodiazepine receptors potentiates the antinociceptive effects of gamma amino butyric acid (Buckett, 1980); more work needs to be performed to elucidate the precise mechanism involved.
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REFERENCES


