Anesthesiology
70:780–786, 1989

Intrathecal Midazolam and Fentanyl in the Rat: Evidence for Different Spinal Antinociceptive Effects

J. M. Serrao, M.B., B.S., F.F.A.R.C.S., S. C. Stubbs, B.Sc.,†*
C. S. Goodchild, M.A., M.B., B.Chir., Ph.D., F.F.A.R.C.S., J. P. Gent, M.A., Ph.D.†*

The effects of intrathecal midazolam and fentanyl on electrical current threshold for pain were measured using stimulating electrodes in the neck and tail of rats with chronically implanted lumbar subarachnoid catheters. This involved the measurement of the minimum current (50 Hz 2 ms pulses 0–5 mA), which made the rat squeak when applied alternately to electrodes at each skin site. The responses measured in milliamperes were expressed as a number of times control readings. Equieffective doses of both midazolam and fentanyl produced a significant increase in electrical threshold for pain in the tail (mean ± SEM 3.14 ± 0.51 and 2.89 ± 0.35: $P < 0.05$; Wilcoxon sum rank test) in the absence of any change in the neck (mean ± SEM 1.28 ± 0.13 and 0.96 ± 0.12, NS), thus demonstrating a spinal effect. Fentanyl caused a significant simultaneous increase in tail flick latency (mean ± SEM 67.8 ± 20.1%, $P < 0.05$), but midazolam did not (mean ± SEM 4.22 ± 2.76%, NS). Intraperitoneal injections of naloxone (0.25 mg/kg) blocked the response to fentanyl in both tests and did not affect the response to midazolam. Intraperitoneal flumazenil (5 mg/kg) blocked the midazolam antinociceptive effect but did not affect the response to fentanyl in either test. Tail withdrawal in response to non-noxious stimulation was preserved in all animals with spinal analgesia, indicating that myelinated afferent and efferent pathways were still functioning. Righting reflex, coordination, motor power, and alertness were also preserved in the presence of both drugs. Both drugs caused spinally mediated antinociceptive effects that were qualitatively different. (Key words: Analgesics, intrathecal; fentanyl; midazolam. Anesthetic techniques: spinal. Pain: antinociception.)

HOT PLATE (HP) tests and measurements of tail flick latency (TFL) are established methods of assessing analgesia.[1–3] Using the HP and TFL tests, Yaksh and Henry[4] showed that intrathecal injections of small amounts of opiates in the intact rat produced powerful effects upon nociceptive thresholds that could be antagonized with naloxone.

The demonstration of dermatomal effects is crucial to the assessment of spinal analgesia. A behavioral test that distinguishes between spinally and supraspinally mediated antinociception is needed to evaluate the effects of epidural and intrathecal drugs. The HP test has a number of end points (*e.g.*, paw licking and jumping) that involve a strong supraspinal component.[1,2,5] This makes it difficult to discriminate between the spinal and supraspinal effects of the analgesic drug. TFL, however, is consistently increased by mu opioid agonists and has a well-defined quantitative end point. As a result of its sensitivity to the analgesic effect of the opioids and because early interest in the study of selective spinal analgesia was largely centered around the opioids, measurements of TFL became the mainstay of behavioral nociceptive tests in the rat. Subsequently, its use was extended to demonstrate the analgesic effects of other intrathecally and epidurally administered drugs. TFL measurement cannot be considered an ideal test of spinal analgesia because it does not lend itself to the demonstration of dermatomal effects. Although Irwin *et al.*[6] have shown that tail flick is preserved in animals with spinal cord transections, tail flick latency may be increased by a supraspinal action of a drug in the intact animal. It is difficult, using intact animals, to demonstrate convincingly that a spinally administered drug produces its analgesic effect by an action confined to the spinal cord.

The electrical current threshold for pain (ECTP) has been shown to be elevated by intraperitoneal and intrathecal opiates when stimuli were applied to dermatomes corresponding to caudal cord levels.[1,7] This test is reproducible, has a quantitative end point, and would lend itself to demonstration of segmental analgesia if another more rostral set of electrodes were used as well. Using ECTP measurements at lumbar and cervical levels we have demonstrated segmental antinociceptive effects following intrathecal injections of the imidazobenzodiazepine, midazolam (Hypnovel,‡ Roche).[8]

In this study we determined the equianalgesic intrathecal doses of midazolam and fentanyl (Sublimaze, Janssen) using ECTP and compared the effects of these on TFL. ECTP measurements in the neck and tail demonstrated that analgesia produced by intrathecal injection of both drugs was solely due to an action on the spinal cord. We also compared the effect of benzodiazepine and opioid antagonists on rises of ECTP and TFL produced by equieffective doses of midazolam and fentanyl.

Received from the *Departments of Anaesthesia and †Pharmacology, University of Leeds, Leeds, United Kingdom. Accepted for publication December 16, 1988. Presented in part to the 5th World Congress of Pain, Hamburg, FRG, 1987.

Address reprint requests to Dr. Serrao: Department of Anaesthesia, University of Leeds, 24 Hyde Terrace, Leeds LS2 9LN, United Kingdom.

‡ Hypnovel is a preparation of midazolam dissolved in 0.9% sodium chloride balanced to *p*H 3.5 with sodium hydroxide and hydrochloric acid. It contains no benzyl alcohol or other preservatives.

Materials and Methods

Catheter Implantation

Male Wistar rats weighing 150–200 g had intrathecal catheters implanted *via* lower lumbar laminectomies performed while anesthetized with halothane. The method of implantation was similar to that previously described by Bahar *et al.*[9] with some changes: a number of swellings, 1.5 cm apart from each other, were placed at both ends of the catheter. Only 1.5 cm of catheter was passed through the dura. The catheter was fixed to the vertebral bone by means of bone cement, the last swelling on the catheter being situated in the laminectomy crater and embedded in the bone cement. The catheter was subcutaneously tunnelled to an exit wound at the base of the neck. The volume of each catheter was measured between the first swelling on the exteriorized end of the catheter and the catheter tip prior to intrathecal insertion, using a Hamilton microsyringe; this allowed accurate injection of known volumes of drug into the cerebrospinal fluid (CSF).

Correct catheter placement was confirmed by injection of 10 μl of 2% lidocaine into the subarachnoid space 10 min after recovery. The catheter was judged to be intrathecal if paralysis and dragging of the hind legs occurred within 30 s of this injection. Animals with negative lidocaine tests and those with obvious neurologic damage after catheter implantation were excluded from the study.

Intrathecal Midazolam and Fentanyl Tests

Four hours after recovery from local and general anesthesia the animals were placed in a plexiglass restrainer (Broome, Harvard Apparatus) and ECTP and TFL were measured. In addition a nonnociceptive test, the tail withdrawal test, was used as an indicator of intact afferent and efferent myelinated pathways. The animals were released from the restrainer when these tests had demonstrated that spinally mediated analgesia had been established and were observed for drowsiness, motor incoordination, righting reflexes, ability to navigate an incline, and ability to feed.

ECTP. Pairs of stimulating electrodes were moistened with electrode jelly and placed on the surface of the tail and in the skin of the neck, the cathode of each pair being placed rostral to the anode. The two electrodes were separated by 1 cm on the neck and 3 cm on the tail. Current was delivered for 0.5 s to each pair from an electrical nerve stimulator (Digitimer-DS 10®) delivering rectangular pulses of current (2 ms, 50 Hz, 0–10 mA, 0–100 V). Stimulation of the neck electrodes always followed 10–15 s after stimulation of the tail. The current delivered was measured on a storage oscilloscope as a voltage drop across a 1 k ohm resistor in series with the electrodes. The current threshold for pain was defined as the minimum current necessary to produce an obvious aversive movement and/or strong vocalization. Segmental analgesia was defined as an increase in ECTP in the skin of the tail, with no significant change in the neck threshold.

TFL. This was the time taken for the rat to flick its tail away from noxious heat provided by a 150-W lamp focussed on a blackened spot on the tail from a distance of 10 cm.

Tail withdrawal test. A positive response was defined as reflex tail withdrawal in response to stroking the fine hairs at the base of the tail. Tail movement was recorded by a linear displacement transducer and displayed on an oscilloscope.

All three measurements were made on animals placed in a darkened restrainer in a quiet environment. Measurements were made every 10 min for a 30-min control period and again for 30 min after the intrathecal injection of test drug.

The results were standardized for each rat in two ways. First, we calculated an analgesic response or "R" value by dividing the mean of three current threshold readings after the intrathecal injection by the mean of three control readings; this was the method used for plotting the dose–response curves and the histograms in the figures. Second, each 10-min reading of ECTP and TFL was divided by the mean of all three control readings for each animal to produce time–response curves. Such standardization of the responses was undertaken to remove "between animals" variation in the absolute current values, which inevitably resulted from differences in electrode placement. The mean predrug and postdrug TFL values were recorded for each animal. In the absence of a response, the cutoff time was set at 20 s to prevent permanent damage to the tail. The scores were converted for each rat to percentage maximum possible effect (%MPE).

$$\%\ \text{MPE} = \frac{(\text{TFL postdrug} - \text{TFL predrug}) \times 100}{(\text{TFL cutoff time} - \text{TFL predrug})}$$

Analgesic Dose–Response Relationships for Midazolam and Fentanyl

ECTP was measured as above before and after 10 μl intrathecal injections of fentanyl (nine rats; dose range 0.25–6.85 nmol) or midazolam (six rats; dose range 5.1–414 nmol). Each rat received a number of different doses (1–5) on separate occasions at least 4 h apart. R values for each response were calculated as above and the means ± SEM of these values were plotted on a log dose–response curve.

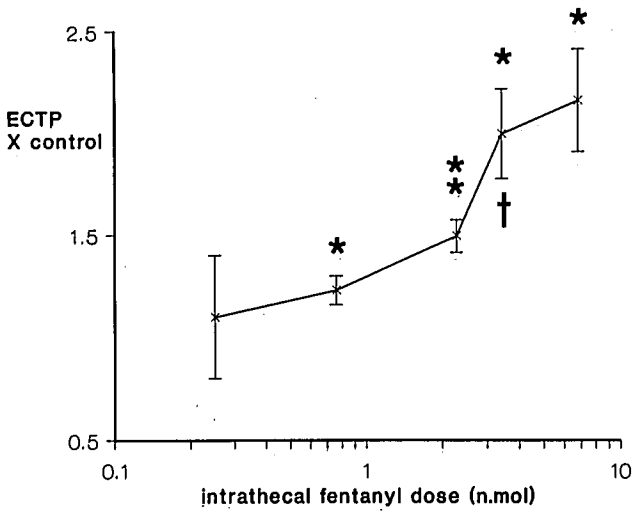

FIG. 1. The mean ± SEM of the R values obtained following a range of doses of intrathecal fentanyl (volume 10 μl) are shown. All animals included in this study showed segmental effects, *i.e.*, rises in ECTP in the tail with no significant changes in the neck. The antinociceptive effect on the tail is a dose-dependent effect that saturates, *i.e.*, additional increases in dose do not produce an additional increase in the antinociceptive response that remained segmental for the doses used in this study. *$P < 0.05$, ‡$P < 0.01$, compared with control. †$P < 0.05$ compared with responses following 0.25 nmol.

Comparison of the Analgesic Effects of Midazolam and Fentanyl Using ECTP and TFL

Four animals (group 1) received an intrathecal injection of 6.85 nmol fentanyl dissolved in 10 μl of normal saline. Six rats in groups 2 and 3 (consisting of three rats each) received 138 nmol of intrathecal midazolam dissolved in 10 μl of normal saline. The fentanyl group and the three animals from group 2 receiving midazolam had all three tests (ECTP, TFL, and the tail withdrawal test) performed in rapid succession in that order at each test period, whereas group 3 had ECTP and TFL measurements alternating at intervals of 5 min. This was done to determine if any bias was introduced by the test protocol. The tail withdrawal test was performed immediately after TFL measurement in group 3.

Comparison of the Reversal of Analgesic Effects of Midazolam and Fentanyl by a Benzodiazepine and an Opioid Antagonist

Equianalgesic doses of midazolam (46 nmol) and fentanyl (3.43 nmol) were chosen from the previously calculated analgesic dose–response curves for ECTP. These doses produced a just maximal spinal analgesic effect, *i.e.*, just at the top of the log dose–response curve. ECTP and TFL were measured every 10 min for the 30 min before and after intrathecal injection of agonist. Five animals received fentanyl and five received midazolam as the intrathecal agonist. A control R value was obtained after intrathecal agonist and intraperitoneal antagonist; either flumazenil (R015-1788; Anexate, Roche) 5 mg/kg or naloxone (Narcan, Du Pont) 0.25 mg/kg. These tests were performed on 3 successive days, and the order of testing the antagonists was randomized.

Confirmation of Drug Placement

Catheter position was confirmed at the end of all experiments by an additional intrathecal injection of 10 μl of 2% lidocaine solution.

Effect of Vehicle

The effect of vehicle alone was not investigated in these studies because this had been done previously by members of our group.[8] These results showed that intrathecal vehicle had no effect on ECTP.

Statistical Methods

The measurement of ECTP and TFL were analyzed for differences between groups by application of the Wilcoxon sum-rank test to avoid assumptions about the data distribution. Results were considered significant if $P < 0.05$.

Results

Control values of ECTP in the tail varied from 0.1 to 1.0 mA between animals. This was dependent upon electrode positioning, contact area and resistance, and tail size. All results of ECTP were therefore standardized.

Analgesic Dose–Responses for Midazolam and Fentanyl

Midazolam and fentanyl caused dose-related increases in ECTP in the tail (figs. 1 and 2). Intrathecal doses of midazolam of 46 nmol and fentanyl of 3.43 nmol produced maximal analgesia while retaining a segmental analgesic effect. In addition, these doses of the two drugs were equieffective in producing spinally mediated analgesia measured by ECTP, *i.e.*, the R values produced by midazolam and fentanyl at these doses were not significantly different from each other.

Comparison of Effects of Midazolam and Fentanyl on ECTP and TFL

Both drugs caused similar increases of ECTP in the tail (fentanyl, fig. 3; midazolam, fig. 4) with no change in the neck. Fentanyl caused an increase in TFL, whereas midazolam did not. The results shown in figures 3 and 4 are time–response curves for each drug group. There were no significant differences between groups 2 and 3

in ECTP or TFL results. The two groups have therefore been combined in figure 4. The responses in figures 3 and 4 consist of data standardized by dividing each individual measurement by the mean of the control readings. Both drugs produced an increase in ECTP for the tail within the first 10 min after intrathecal injection. However, only fentanyl caused an increase in the TFL, which followed the same time course as the segmental analgesia revealed by the ECTP; this was significantly different from the midazolam response ($P < 0.05$) at each of the three readings after drug administration.

To make a comparison between drugs, the data have been further transformed. The responses in the neck and tail were standardized by dividing the mean of the three 10-min readings after the inthrathecal drug by the mean of the three control readings. Such calculations show the following:

1. Both midazolam and fentanyl caused a significant increase in ECTP in the tail (mean ± SEM, 3.24 ± 0.51 and 2.89 ± 0.35; $P < 0.05$). This increase occurred in the absence of any change in ECTP in the neck for the two groups (mean ± SEM, 1.28 ± 0.13 and 0.96 ± 0.12), indicating that both drugs caused a spinal segmental block.
2. The magnitude of increase in ECTP in the tail was similar in both groups, confirming that the doses chosen from the dose–response curves were equieffective.
3. The %MPE was calculated for each animal and the results grouped for each drug. There was a significant increase in TFL after intrathecal fentanyl (mean ± SEM, 67.8 + 20.1%; $P < 0.05$). Intrathecal midazolam did not produce any significant effect upon TFL (mean ± SEM, 4.2 ± 2.76%).

Reversal of the Analgesic Response to Midazolam (46 nmol) and Fentanyl (3.43 nmol)

Flumazenil. Flumazenil (5 mg/kg ip) suppressed the rise in ECTP after intrathecal midazolam (mean ± SEM = 0.91 ± 0.13), whereas it did not significantly affect ECTP (mean ± SEM = 52.7 ± 13.4%) after intrathecal fentanyl (figs. 5 and 6).

Naloxone. Naloxone (0.25 mg/kg ip) did not significantly affect ECTP (mean ± SEM = 1.92 ± 0.18) after intrathecal midazolam. Naloxone significantly suppressed the rise in ECTP (mean ± SEM = 1.02 ± 0.08) and TFL (mean ± SEM = 31.2 ± 5.9) after intrathecal fentanyl (figs. 5 and 6).

A positive result in the tail withdrawal test was obtained in all animals with segmental analgesia; these animals remained alert with normal motor coordination and righting reflexes. Subsequent intrathecal injection of lidocaine caused paralysis and dragging of the hind limbs within 30 s in all animals included in the study.

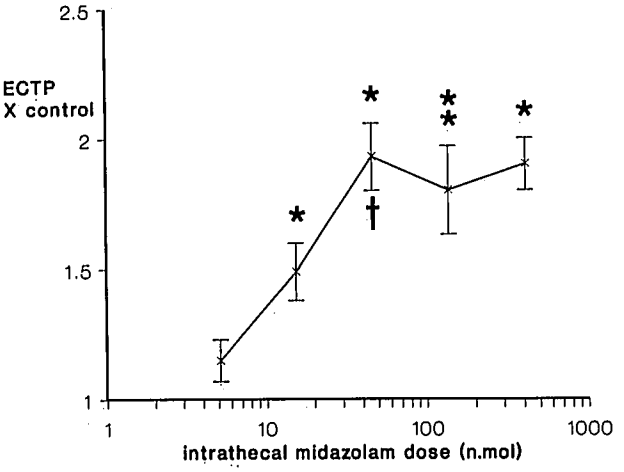

FIG. 2. The mean ± SEM of the R values obtained following a range of doses of intrathecal midazolam (volume 10 μl) are shown. All animals included in this study showed segmental effects, *i.e.*, rises in ECTP in the tail with no significant changes in the neck. The antinociceptive effect on the tail is a dose-dependent effect that saturates, *i.e.*, additional increases in dose do not produce an additional increase in the segmental antinociceptive response. *$P < 0.05$, ‡$P < 0.01$, compared with control. †$P < 0.05$ compared with responses following 5.1 nmol.

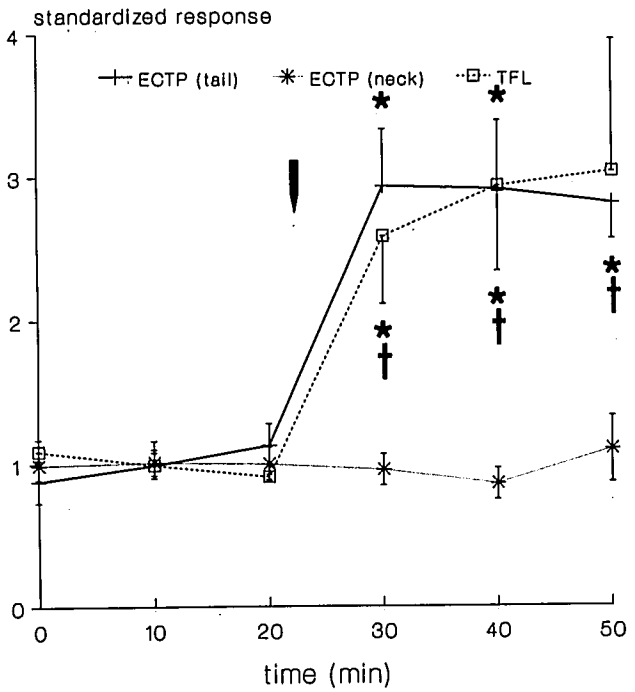

FIG. 3. Time response curves for four rats that received 6.85 nmol fentanyl intrathecally in 10 μl saline given at the arrow. Each individual reading of ECTP (mA in neck or tail) and TFL has been standardized by dividing it by the mean of the three corresponding control readings for that animal. Data for the four rats have then been grouped for each 10-min testing period. Fentanyl caused segmental analgesia and coincident rises in both ECTP (tail) and tail flick latency. *$P < 0.05$, compared with control. †$P < 0.05$ compared with responses following midazolam (fig. 4).

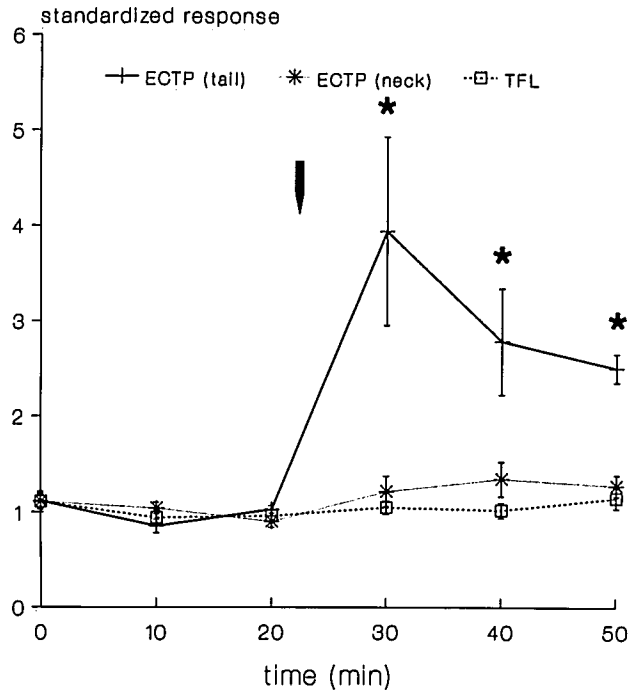

FIG. 4. Time response curves for six rats that received 138 nmol midazolam intrathecally in 10 μl given at the arrow. Midazolam caused segmental analgesia with a rise in ECTP in the tail and not in the neck. Midazolam did not affect TFL. *$P < 0.05$ compared with control readings.

Discussion

A number of methods have been used in the past to determine the relative contribution of spinal and supraspinal actions of intrathecal drugs:

1. Equivalent doses of a drug given intrathecally and subcutaneously have been compared for analgesic effect.[10]

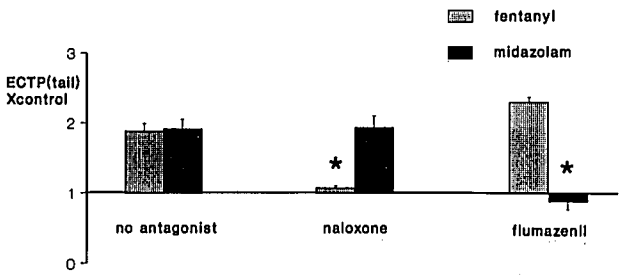

FIG. 5. Histogram showing grouped R values for ECTP (tail) in ten rats. Five rats received fentanyl (3.43 nmol in 10 μl intrathecally) and five rats received midazolam (46 nmol in 10 μl intrathecally). Responses to agonist alone and agonist given with each of two antagonists are shown: naloxone 0.25 mg/kg ip and flumazenil 5.0 mg/kg ip. The opioid antagonist (naloxone) significantly reduced the response to fentanyl (*$P < 0.05$) but did not affect the midazolam response. The benzodiazepine antagonist (flumazenil) only blocked the midazolam response (*$P < 0.05$).

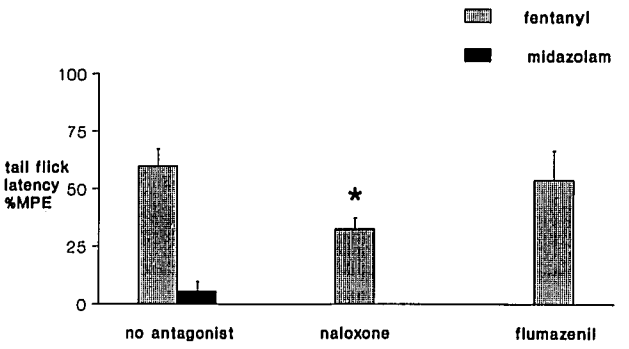

FIG. 6. Histograms showing grouped %MPE for TFL in the same rats as figure 5. Midazolam did not increase TFL. The fentanyl response was significantly reduced by naloxone (*$P < 0.05$) but not by flumazenil.

Intrathecal administration has been shown to be more effective, but this method does not take into account the rostral spread of a drug injected intrathecally. Drug movement through CSF following a spinal injection may lead to higher concentrations at supraspinal centers than when the drug is given by other routes. This would lead to a greater analgesic effect following the spinal administration of a drug, but it would not necessarily be the result of an action confined to the spinal cord.

2. Tests using aversive responses to pinching and squeezing at different points on the body involve tactile and visual stimuli as well as pain, thus making the cause of the aversive response difficult to ascertain.[5]

3. The application of drugs to the spinal cord in animals with spinal cord transections has been used to demonstrate a spinal antinociceptive effect,[6] but it may not account for all the effects of the spinally administered drug in the intact animal.

4. The spectrum of effects of drugs given spinally and by other routes has been compared, but the placement of drugs intrathecally does not rule out systemic absorption.[11] The preservation of normal alertness, respiratory rates, muscle tone and mobility, righting, and placing reflexes has often been used as evidence that a drug is acting spinally, rather than by causing a more general depression of the CNS.[5,11] However, these observations are not objective and have qualitative, weak end points.

Opioids and other drugs acting on the spinal cord do not abolish all modalities of sensation. It would not be surprising, therefore, to find that different drugs produce different results with different nociceptive tests.

Recently, Schmauss and Yaksh[12] showed that drugs acting upon different subpopulations of opiate receptors had different pharmacologic profiles when studied with a battery of nociceptive tests using visceral chemical stimuli and cutaneous thermal stimuli. Although they gave the drugs intrathecally, they provided no evidence that the actions of these drugs were confined to the spinal cord.

Our results show that intrathecal injection of either midazolam or fentanyl provides analgesia that is clearly segmental and is therefore spinally mediated. This demonstration of a drug effect confined to the spinal cord is frequently omitted in studies of this type; we believe it is an essential prerequisite for comparing the analgesic efficacies of different spinal drugs. We have shown previously that analgesia following intrathecal midazolam is not produced by injection of vehicle (normal saline buffered to *p*H 3.5) alone.[8] We observed no effects of either drug on motor coordination, on the ability to navigate inclines, or on normal movement and feeding. These observations confirmed the evidence provided by the preserved tail withdrawal reflex; the two drugs had selective antinociceptive effects. Furthermore, our results indicate that when fentanyl and midazolam were administered intrathecally at doses that were equipotent and produced maximal spinally mediated analgesia in the ECTP test, only fentanyl caused an increase in TFL. This confirmed the results of Yaksh *et al.*[11] who used HP and TFL tests to demonstrate a rapid and profound antinociceptive effect of intrathecal fentanyl. Their study used intact animals, but no direct evidence, such as the demonstration of segmental effects, was provided for a wholly spinal action of the drug.

Benzodiazepines are not normally considered to be analgesics. When these drugs are given by any route that causes high blood levels of drug, it is impossible to demonstrate analgesic effects over and above their effects on consciousness and anxiety. However, one may confine the action of midazolam to the spinal cord by giving it intrathecally, thus allowing access to receptors that mediate analgesia, the measurement of which is not confused by changes in the level of consciousness. As such, this is analogous to the antihypertensive drug clonidine, which is an effective analgesic in some conditions when given epidurally.[13]

Additional evidence for the analgesic effects of spinal benzodiazepines in the unanesthetized animal arises from work done by Pieri and Moreau.[14,15] In Pieri's first study,[14] no effect upon TFL was shown following epidural injection of midazolam in rats with spinal transections. Low dural permeability may have prevented sufficient drug from crossing to the spinal cord. Also, sole confirmation of catheter position and therefore of correct placement of midazolam was provided by injection of a large dose of morphine, the effects of which may have been produced initially by vascular absorption rather than by spread from the epidural space to the spinal cord. In the second study, Moreau and Pieri[15] injected midazolam intrathecally and observed potentiation of the effects of intrathecal morphine on TFL by midazolam but no effect of midazolam alone. Although these observations are in accord with our results for TFL with intrathecal midazolam, Pieri and Moreau[15] provided no evidence of dermatomal effects of either drug; therefore, it is impossible to be sure that the site of drug interaction was confined to the spinal cord in their experiments.

When ECTP was used as the nociceptive test, this study confirmed our previous findings that intrathecal midazolam causes segmental analgesia, an effect that is abolished by the prior intraperitoneal administration of the benzodiazepine antagonist flumazenil.[8] Members of our group have also demonstrated segmental analgesic effects in humans following intrathecal midazolam; the analgesic effects were specific for somatic pain, and no effects were observed on sympathetic tone or reflexes or on muscle power and coordination.[16]

We have shown in the present study that a dose of naloxone (0.25 mg/kg), which suppressed the response of the fentanyl group to both ECTP and TFL, did not affect the analgesic response to ECTP produced by midazolam. Conversely, the benzodiazepine antagonist flumazenil (5 mg/kg) suppressed the analgesia produced by midazolam while leaving the fentanyl response unaffected. The results from this small series of experiments with the antagonists indicate that fentanyl and midazolam produce their spinal antinociceptive effects by binding to different receptors.

When drugs are assessed for spinal analgesic effects, the results of a single nociceptive test, such as the TFL, may be misleading as shown by this study. This notion is not new; disparity between the results from different nociceptive tests has been reported for three classes of opioids.[12] Although the ECTP test is not usually employed in screening for analgesic efficacy, it has been used to demonstrate analgesic effects of opiates that are also active in elevating pain thresholds in other tests, such as the TFL.[1,7] All previous reports have shown that ECTP is increased by known analgesics. These observations, combined with those indicating that intrathecal midazolam is analgesic in humans,[15] indicate that this is a good general screening test for spinally administered analgesics. It seems likely that each nociceptive test may activate a different pathway or combination of pathways within the spinal cord. The ECTP test is likely to activate all nociceptive afferent pathways, whereas the applications of heat, pressure, and chemicals are likely to be more specific. Each spinal drug may affect one or more of these pathways, which may account for such disparity between results obtained with different tests and results obtained with different antagonists. The results of this study suggest that midazolam produces spinally mediated analgesia that is different in quality from that produced by the mu opioid agonist fentanyl. The results of a variety of nociceptive tests and dose–response curves for a variety of antagonists may shed more light on the analgesic effect of intrathecal midazolam.

The authors thank S. Barlow and M. Bruce for secretarial assistance, D. Myers for technical assistance, and Hoffman La Roche for supplying flumazenil.

This work was carried out with permission from the licensing authorities in Great Britain (Home Office Licence PPL 50-00131) and in all experiments attention was paid to ethical guidelines for investigation of experimental pain in conscious animals.[17]

References

1. Yaksh TL, Rudy TA: Analgesia mediated by a direct spinal action of narcotics. Science 192:1357–1358, 1976
2. Woolf G, MacDonald AD: The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther 80:300–307, 1944
3. D'Amour EF, Smith DL: A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79, 1941
4. Yaksh TL, Henry JL: Antinociceptive effects of intrathecally administered human beta endorphin in the rat and cat. Can J Physiol Pharmacol 56:754–759, 1978
5. Yaksh TL, Rudy TA: Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J Pharmacol Exp Ther 202:422–428, 1977
6. Irwin S, Houde RW, Bennett DR, Hendershot LC, Seevers MH: The effects of morphine, methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. J Pharmacol Exp Ther 101:132–143, 1950
7. Carroll MN, Lim RKS: Observations on the neuropharmacology of morphine and morphine-like analgesia. Arch Int Pharmacodyn Ther 125:383, 1960
8. Goodchild CS, Serrao JM: Intrathecal midazolam in the rat—evidence for spinally mediated analgesia. Br J Anaesth 57:1563–1570, 1987
9. Bahar M, Rosen M, Vickers MD: Chronic cannulation of the intradural or extradural space in the rat. Br J Anaesth 56:405–410, 1984
10. Tang AH, Schoenfeld MJ: Comparison of subcutaneous and spinal subarachnoid injections of morphine and naloxone on analgesic tests in the rat. Eur J Pharmacol 52:215–223, 1978
11. Yaksh TL, Noueihed RY, Durant PAC: Studies of the pharmacology and pathology of intrathecally administered 4-anilinopiperidine analogues and morphine in the rat and cat. ANESTHESIOLOGY 64:54–66, 1986
12. Schmauss C, Yaksh TL: In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther 228:1–12, 1984
13. Glynn CJ, Jamous MA, Teddy PJ, Moore RA, Lloyd JW: Role of spinal noradrenergic system in transmission of pain in patients with spinal cord injury. Lancet 2:1249–1250, 1986
14. Pieri L: Neuropharmacology of benzodiazepines, Pharmacological Basis of Anesthesiology—Clinical Pharmacology of new Analgesics and Anesthetics. Edited by Tiengo M, Cousins MJ. New York, Raven Press, 1983, pp 37–48
15. Moreau JL, Pieri L: Modification of morphine antinociception by intrathecally (IT) administered benzodiazepine receptor ligands. Br J Pharmacol 92:652P, 1987
16. Goodchild CS, Noble J: The effects of intrathecal midazolam on sympathetic nervous system reflexes in man—a pilot study. Br J Clin Pharmacol 23:279–285, 1987
17. Zimmerman M: Ethical guidelines for investigations of experimental pain in conscious animals (Guest editorial). Pain 16:109–110, 1983