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DIRECT SPINAL EFFECT OF INTRATHECAL AND EXTRADURAL MIDAZOLAM ON VISCERAL NOXIOUS STIMULATION IN RABBITS

M. E. CRAWFORD, F. MOLKE JENSEN, D. B. TOFTDAHL AND J. B. MADSEN

SUMMARY

We measured alterations in a noxious visceromotor reflex in rabbits subjected to intestinal distension, after i.m., extradural or intrathecal injection of midazolam or saline. Spinal catheters were inserted and tunnelled surgically and the animals allowed to recover for 2 weeks. A balloon catheter was placed in the distal part of the descending colon, in the awake rabbit. Intraluminal pressures were increased continuously by water instillation until a sudden withdrawal of the pelvis was observed. Pressure values at withdrawal threshold were recorded immediately before the injection and after 5, 15 and 30 min. Pain thresholds were unaltered after saline. Extradural midazolam 12.5–250 μg kg^{-1} produced a dose-dependent increase in the percent maximum possible effect ranging from 7% after the smallest dose to 80%. Similar dose-dependent effects were observed after intrathecal injection of midazolam 25–62.5 μg kg^{-1}. Extradural and intrathecal, but not i.v. injection of flumazenil 25 μg kg^{-1} (a benzodiazepine receptor antagonist) reduced the antinociceptive effect of extradural and intrathecal midazolam to pretreatment levels. A segmental effect of intrathecal midazolam was demonstrated using transcutaneous electrical stimulation in the areas of the neck and the lower back. The effect of intrathecal midazolam 62.5 μg kg^{-1} was restricted to the lumbar region, demonstrating a selective action on the spinal cord. Thus extradural and intrathecal midazolam produced a dose-dependent effect on the reflex response to visceral distension in rabbits. This effect is caused by a direct spinal action on benzodiazepine receptors in the spinal cord. (Br. J. Anaesth. 1993; **70**: 642–646)

KEY WORDS

Anaesthetic techniques: intrathecal, extradural. Pain: visceral, intestinal distension. Pharmacology: midazolam, flumazenil.

Benzodiazepine binding sites are located in the spinal cord [1], with the greatest density of binding sites found within lamina II of the dorsal horn [2]. This region plays an important role in the processing of noxious information. Based on radioligand binding assays and electrophysiological studies, the benzodiazepine binding site appears linked to the $GABA_A$ receptor complex [3].

Benzodiazepines are known to enhance GABA-induced responses of central nervous system neurones *in vitro* [4–6]. Bicuculline, a $GABA_A$ receptor antagonist, has been shown to attenuate the antinociceptive effect of intrathecal midazolam, a water soluble benzodiazepine [7,8]. GABA may play an important role in the modulation of noxious stimuli, as administration of the $GABA_A$ agonist tetrahydro-isoxazolo-pyridinol (THIP) produces antinociception [9]. In addition, midazolam possesses analgesic properties when administered extradurally in humans [10] and intrathecally in rats [8,11–13]. The antinociceptive properties of midazolam appear to be dependent on the type of noxious stimulus used, as it is inactive in the tail flick test [12] and elicits only a weak effect when assessed in the hot-plate tests, in a dose range which lacks additional motor impairment [14].

We evaluated the effect of midazolam, a water soluble benzodiazepine, against visceral noxious stimuli in rabbits and determined if it produced a direct effect on the spinal cord, evaluated by the visceromotor response elicited by intestinal distension.

MATERIALS AND METHODS

The study was approved by the Danish Committee for Animal Research under the Department of Justice and was carried out in accordance with the ethical guidelines for investigation of experimental pain in conscious animals.

Eighteen female New Zealand albino rabbits, body weight 3.5–4.0 kg, were studied.

Catheter implantation

The animals were anaesthetized with a combination of i.m. ketamine 25 mg and xylazine 10 mg, supplemented with 1 % lignocaine 5 ml administered s.c. into the area of the skin incision. The skin between L5 and L7 had been shaved previously and disinfected with alcohol. After skin incision the muscular fascia was opened, muscles bluntly detached from the spinous processes and haemostasis obtained by compression. After visualization of the

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ligamentum flavum between the spinous process of L7 and the caudal articular process of L6, a superficial incision was performed in the ligament, and a Portex extradural catheter, o.d. 0.9 mm, inserted and advanced 5 cm cranially. Correct extradural placement produced a rubber-like resistance during insertion. The catheter was secured with a drop of histacrylic glue. The muscular fascia was closed around the catheter and the free end mounted with a detachable Luer lock connector to which a rubber injection membrane had been attached. This end was positioned s.c. and dorsally between the forelegs. The skin was sutured and the animal left for 1–2 weeks; each was in a spacious wooden cage and with free access to food and water.

Intrathecal catheters were inserted using the same procedure except for the incision in the ligamentum flavum. Using a 21-gauge needle, an incision was made through the ligament and dura, resulting in a free flow of cerebrospinal fluid.

Animals presenting any signs of motor dysfunction after catheter implantation were excluded from the study. Cathether position was tested with 1% lignocaine. Injection of 1% lignocaine 0.5 ml extradurally produced weakness of the hindlimbs, whereas 1.5 ml resulted in total paralysis of the same limbs of the extradural batch. Total paralysis was obtained with 0.5 ml of the same solution in all animals of the intrathecal group. Six rabbits had extradural catheters implanted, six had intrathecal catheters and six rabbits were allocated to the group for i.m. injection. One animal in the last group suffered intestinal rupture because of a sudden increase in balloon pressure in the early phase of the study and was excluded and killed immediately.

Tests

The experimental method has been described earlier [15]. In brief, it consisted of a closed circuit containing water. A latex rubber balloon-tipped PVC catheter was used (William Cook, Europe), with an outer diameter of 0.2 cm and a double lumen providing the possibility of simultaneous water infusion and continuous pressure recording inside the balloon. All pressures were measured by a transducer with concomitant paper recording of the pressure curve. A stop-cock and a 20-ml syringe attached allowed a rapid reduction of pressure to 0 after pelvic withdrawal. The balloon catheter was introduced approximately 8 cm into the colon from the anus. Cut-off pressure was set at 100 mm Hg to avoid intestinal rupture.

With a water infusion rate of 15 ml min^{-1}, balloon pressure was increased until a sudden pelvic withdrawal by the rabbit was seen. The maximum intraluminal pressure at the time of this reaction was used to calculate test and control values. This procedure was repeated four times, 30 s apart.

Two investigators performed these measurements: one made the injection, restrained the rabbits loosely during introduction of the balloon, made a note on the recorder of the pressure at which pelvic withdrawal occurred, and emptied the balloon; the other investigator introduced the balloon catheter and observed the rabbit during distension of the balloon. At the time of the visceromotor reflex, this information was passed to the first investigator, who marked it on the recorder. In this way, the experiments were performed blinded to the observer of the visceromotor reflex. Furthermore, this observer was unaware of the drug, dose and route of administration used.

The test and control values were calculated as the mean of the last three measurements, because they had been found in a previous study to be more consistent [15]. In the dose–response study, thresholds were converted for each rabbit to percentage maximum possible effect (%MPE):

$$\%\text{MPE} = \frac{\text{test value} - \text{control value}}{100 - \text{control value}} \times 100$$

where control value = threshold obtained before injection; test value = threshold obtained after drug administration. The cut-off pressure was 100 mm Hg.

Pressure values were recorded before and at 5, 15 and 30 min after intrathecal injections, and at 15 and 30 min for extradural and i.m. injections. The 5-min measurement was avoided in the extradural and i.m. groups in order to allow diffusion of the drugs to the central nervous system. Transcutaneous electrical stimulation was performed via two metal skin electrodes, 2 cm apart. The rabbits were tested in the neck and lower back regions. Electrical energy was applied with a TENS stimulator which provided a constant current over a wide range of skin resistance (Elpha 500, Biometer, DK). Threshold values were defined as the current necessary to produce escape behaviour, expressed in mA, and calculated as the mean of three consecutive measurements.

Drug regimen

The dose–response relationship was assessed utilizing extradural doses of midazolam 250, 125, 25 and 12.5 μg/kg body weight and intrathecal doses of midazolam 62.5 and 25 μg kg^{-1}. I.m. doses were 250 and 62.5 μg kg^{-1}. The extradural doses of midazolam were chosen from pilot experiments preceding this study. The initial intrathecal dose was 25 μg kg^{-1} and increased to 62.5 μg kg^{-1} in order to obtain near maximal effect. The i.m. doses were chosen to correspond to the largest doses of intrathecal and extradural injections.

Drug injections were performed in volumes of 1.5 ml for extradural, 0.5 for intrathecal and 1.0 ml for i.m. injections. Previous studies in our laboratory have shown these volumes to give a distribution of injectate to T4 [16]. All placebo injections consisted of isotonic saline. The benzodiazepine antagonist, flumazenil (Ro 15-1788), was injected into animals in the extradural and intrathecal groups after administration of midazolam in order to evaluate if the effects observed were receptor mediated. Flumazenil injections were either parenteral or spinal (extradural or intrathecal). The dose was 25 μg/kg body weight for both routes of administration. This dose was the largest obtainable with the commercial solution of flumazenil given in a volume of 1 ml and was chosen in order to ensure antagonism of the effects after intrathecal and extradural midazolam.

Statistical methods

Data were analysed using the Wilcoxon rank sum

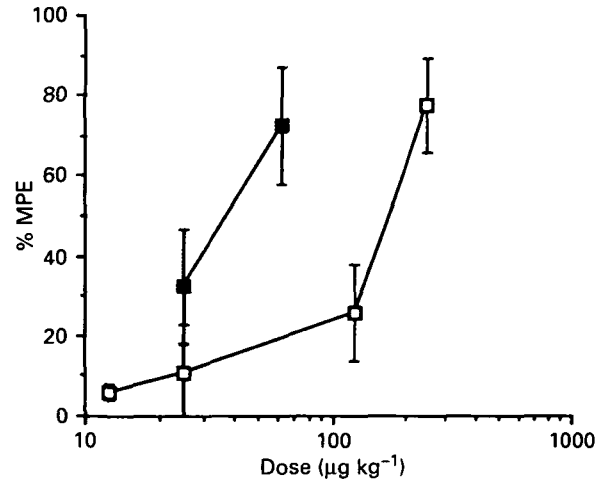

FIG. 1. Dose response 15 min after extradural (□) and intrathecal (■) midazolam: visceromotor reflex (percent of maximal possible effect (% MPE)) elicited by intestinal distension in rabbits. Each point represents mean, SEM; $n = 6$ in both groups.

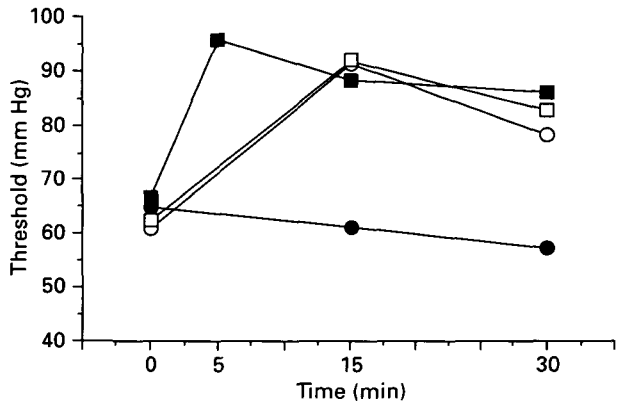

FIG. 2. Increase in intestinal distension thresholds after intrathecal (■) ($n = 6$), extradural (□) ($n = 6$) and i.m. (● = 62.5 μg kg^{-1}; ○ = 250 μg kg^{-1}) ($n = 5$) midazolam. Each point represents mean values. SEM values are omitted for clarity.

test for paired data and Mann–Whitney U test for unpaired data. When more than two groups were compared, the Friedmann two-way ANOVA was used, allowing for multiple testing within the group, when $P < 0.05$. This level of significance was chosen throughout the study.

RESULTS

None of the animals had signs of localized or generalized infection, or signs of motor dysfunction produced by catheter implantation. Body weights were comparable in the three groups.

Extradural or intrathecal injection of lignocaine showed that all catheters were placed correctly.

Saline injections

Injection of isotonic saline 1.5 ml in the extradural and 0.5 ml in the intrathecal group did not produce significant change in threshold to intestinal distension (4.9 (SEM 8.2)% and 0.2 (5.2)% change after extradural and intrathecal saline, respectively).

Extradural administration of midazolam

Extradural midazolam produced a dose-dependent response in the visceral response threshold (fig. 1). The effect of the applied doses was 250 ⩾ 125 > 25 = 12.5 = saline ($P < 0.05$, Wilcoxon rank-sum test, Friedmann test). After the maximal dose of midazolam, there were signs of slight motor impairment in one rabbit, judged by the ability of the animal to walk.

The ability to jump evoked by provocation remained intact. This effect on the motor system lasted for approximately 10 min, thus at the first test 15 min after injection no signs of motor impairment were present. Muscular rigidity was not observed.

Intrathecal administration

The dose–response relationship after midazolam 25 and 62.5 μg kg^{-1} is shown in figure 1. The effect after the large dose was significantly different from placebo at all times (fig. 2), whereas the effect after 25 μg kg^{-1} reached significance only at 5 min after administration (not illustrated). The increase produced by midazolam 62.5 μg kg^{-1} was prolonged and remained stable during the study (fig. 2).

The extradural dose was approximately five times larger than the intrathecal dose required for a 50% increase in MPE (calculated from figure 1).

I.m. administration

Midazolam 250 μg kg^{-1} produced mean increases in distension thresholds comparable to those observed after extradural administration of the same dose (fig. 2). The smaller dose, 62.5 μg kg^{-1}, produced an insignificant decrease in the distension threshold at 15 and 30 min.

Segmental effect

Using the electrical stimulation test, intrathecal midazolam 62.5 μg kg^{-1} elicited a significant increase in escape threshold in the lumbar region (before: 17.0 (1.3) mA; after 15 min: 21.6 (1.6) mA; 30 min: 21.2 (2.3) mA) ($P < 0.05$), whereas in the cervical region thresholds remained unaltered (9.5, 9.1 and 9.7 mA, before, after 15 and 30 min, respectively) (fig. 3).

Duration of response

The effect was significantly increased throughout the 30-min study period after intrathecal, extradural and the large i.m. dose of midazolam (fig. 2).

Antagonist

After i.v. administration of flumazenil 25 μg kg^{-1}, distension thresholds after extradural and intrathecal midazolam remained virtually unaltered (fig. 4). The same dose of antagonist extradurally and intrathecally not only abolished the response after midazolam administered by the same route (fig. 4), but reduced the distension threshold to less than control values (fig. 4).

Behaviourial results

Before each retraction of the pelvis, the rabbit usually showed signs of awareness to the distending stimulus—sitting still, movement of the tail and redness of the ears. These reactions were also observed after administration of midazolam; no other signs of sedation were observed. There were no signs of scratching or biting the lower extremities.

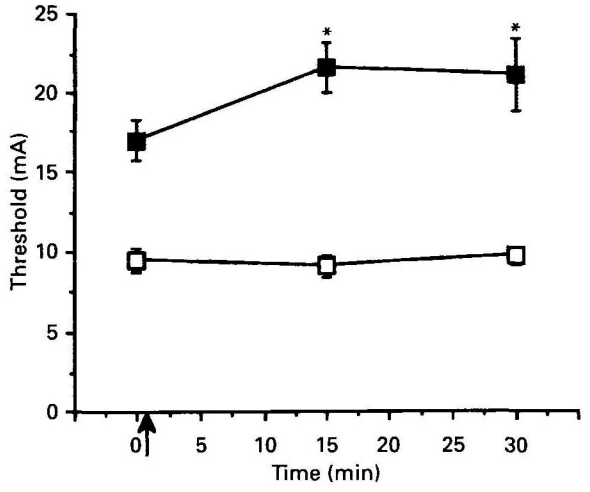

FIG. 3. Changes in electrical stimulation thresholds (mean, SEM) after intrathecal midazolam 62.5 μg kg^{-1}. ■ = Lumbar region; □ = cervical region. Arrow indicates injection time. *$P < 0.05$ compared with control values.

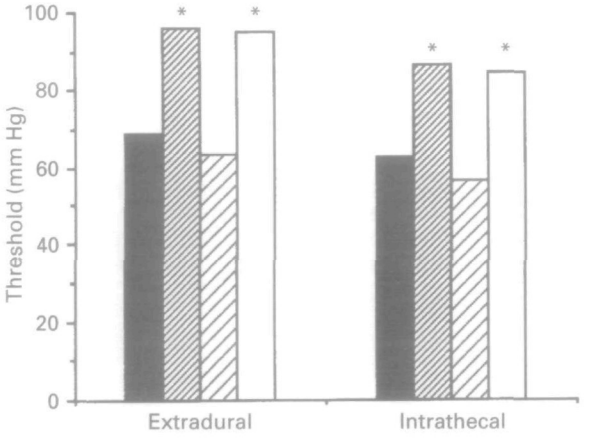

FIG. 4. Effect of extradural or intrathecal (▨) and i.v. (□) flumazenil 25 μg kg^{-1} on extradural midazolam 250 μg kg^{-1} (left) and intrathecal midazolam 62.5 μg kg^{-1} (right) antinociception against intestinal distension. (In columns ▨, spinal flumazenil was administered by the same route as the midazolam.) ▨ = Midazolam alone. ■ = Values before drug administration (control). *$P < 0.05$ compared with control.

DISCUSSION

Distension of the colon of the rabbit has been shown to be a reliable and reproducible method of eliciting visceral noxious stimuli in rabbits [15]. This method has been used in several other species, including man, and similar reproducible results were obtained (for review see [17]). The end-point of the test, pelvic withdrawal, depends on normal function of the motor system. In the present study, signs of motor impairment were observed in one rabbit for 10 min (slight dysco-ordination of the hindlimbs during walking, but with an intact ability to jump). At the 15-min time of testing there were no signs of motor impairment. Thus the possible effects on motor function did not occur to any extent after the doses of midazolam used. In a pilot study performed to determine the dose range for the present study, extradural midazolam 1000 μg kg^{-1} produced a flaccid paralysis of the hindlimbs for approximately 1 h in all rabbits. This dose is four times the maximum dose used in this study and these results are in accordance with the results obtained by Yanez and co-workers [14], who also observed paralysis in rats given approximately six times the antinociceptive dose of midazolam.

A receptor link between the $GABA_A$ site and the benzodiazepine site in the spinal cord has been described earlier [3]. Recent work by Edwards and co-workers [8] confirmed this linkage. They found that bicuculline, a $GABA_A$ receptor antagonist, was able to attenuate the effect of spinally administered midazolam.

This fact, combined with the antinociceptive effect after intrathecal administration of THIP, a specific $GABA_A$ agonist [9], supports a physiological and an anatomical link between the two receptors. This link may also be responsible for the motor effects observed after larger doses of intrathecal and extradural midazolam. The existence of GABA receptors on motor neurone membranes in combination with a link with the benzodiazepine receptor explains the antispastic effect of benzodiazepines.

In the present study, extradurally and intrathecally administered midazolam produced a dose-dependent, pronounced effect against visceral noxious stimulation. The apparently increased potency after intrathecal compared with extradural midazolam is in accordance with experience from human studies, in which morphine 0.5 mg intrathecally appeared equianalgesic with extradural morphine 2–4 mg.

The effects of midazolam were antagonized by the benzodiazepine antagonist flumazenil, but only after concomitant extradural or intrathecal administration and not by i.v. administration. These observations, in combination with the greater efficacy following intrathecal administration of midazolam and the segmental analgesia obtained in the electrical stimulation test, strongly suggest a selective spinal action of extradural and intrathecal midazolam. Furthermore, if the antagonist action of intrathecal and extradural flumazenil were caused by a central distribution to the brain, one would expect parenteral administration of flumazenil to exert at least some degree of antagonism against both intrathecal and extradural midazolam. The direct spinal action of midazolam has been confirmed in previous studies [8,12,13].

The antinociceptive effect after the largest i.m. midazolam dose, 0.25 mg kg^{-1}, could be mediated via a local action on the spinal cord, as midazolam penetrates the blood–brain barrier easily. The reduction in thresholds after parenteral injection of a small dose of midazolam (62.5 μg kg^{-1}) supports this hypothesis and is in accordance with the work by Daghero, Bradley and Kissin [18]. They found that small doses of midazolam in the spinal cord produced analgesia, whereas small doses in the brain produced hyperalgesia. The hypothesis by Fields, Heinricher and Mason [19] concerning on- and off-cells in the rostral ventromedial medulla (RVM) exerting a descending control of noxious stimuli at the level of the spinal cord and the inhibition of off-cells by GABA resulting in hyperalgesia, is in accordance with present and previous observations of various

effects after benzodiazepines: they are able to attenuate opioid-induced antinociception [20] and produce hyperalgesia when administered parenterally or in the brain, but elicit antinociception when they are administered locally at the spinal cord.

The reduction in distension thresholds to less than control values obtained by intrathecal administration of flumazenil 25 $\mu g\ kg^{-1}$ could implicate a tonic active GABA–benzodiazepine system in the modulation af visceral pain. This hypothesis is supported by the work of Edwards and co-workers [8], who found that bicuculline, a $GABA_A$ antagonist, produced hyperalgesia together with allodynia, when given intrathecally in rats. Ness and Gebhart [21] have recently discovered the existence of a descending inhibitory system of neurones in the spinal cord, which are excited by colorectal distension. The inhibition originated from areas in both the periaqueductal grey matter and the RVM.

The effect after intrathecal midazolam appears to be dependent on both the nociceptive test used and the choice of species. Yanez and co-workers [14] found morphine to be approximately 15 times as effective as midazolam on a molar basis, when using the hot-plate test in rats. Serrao and co-workers [13] found fentanyl was only five times as effective as midazolam against electrical stimulation in rats, whereas they found no effect of midazolam on the tail-flick response. The present results compared with unpublished data from our laboratory reveal midazolam to be approximately 20 times as effective as morphine against intestinal distension in rabbits. These differences in effect after morphine and midazolam on different noxious stimuli are physiological evidence of the anatomical separation of intraspinal pathways of visceral and somatic afferent fibres [22,23]. The results obtained in this study have not confirmed those of a preliminary study in humans [24], in which different doses of intrathecal midazolam were found to be effective against per- and postoperative somatic pain and ineffective against sympathetic reflexes produced by manipulation of abdominal contents and against postoperative pain of assumed visceral origin. The preliminary nature of this study, the differences in methods of visceral stimulation and the different species studied make comparison impossible, but point to the need for further studies in humans to determine the effects of spinally applied midazolam on different types of visceral pain.

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