DEPRESSION OF NOCICEPTIVE SYMPATHETIC REFLEXES BY THE INTRATHECAL ADMINISTRATION OF MIDAZOLAM

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The effect of the intrathecal administration of midazolam 0.5-1.0 mg in 1-2 ml of physiological saline solution, has been observed on responses evoked in renal sympathetic nerves by supramaximal electrical stimulation of radial and tibial nerves. In artificially ventilated dogs anaesthetized with α-chloralose, the intrathecal administration of midazolam caused a marked depression of reflexes evoked from the tibial nerve but had no effect on either spontaneous sympathetic activity or reflexes evoked by radial nerve stimulation.

Neither the small amount (1-2 μl) of benzyl alcohol, present as a preservative (administered intrathecally), nor midazolam 1 mg kg⁻¹ i.v. caused any significant depression of the evoked somato-sympathetic reflexes. The effects of intrathecal midazolam were reversed by the benzodiazepine antagonists Ro 15-1788 1 mg kg⁻¹ i.v. and Ro 15-3505 1-2 mg kg⁻¹ i.v. but not by naloxone 2 mg i.v. It is suggested that the antinociceptive effect of locally applied midazolam could be the result of a non-opioid GABA-mediated system which may have implications in the management of pain.

Specific benzodiazepine receptors have been described in the central nervous system (Möller and Okada, 1977; Squires and Braestrup, 1977) and, in addition, it has been suggested that benzodiazepines may modulate the affinity of γ-amino butyric acid (GABA) for its receptors, and so enhance its activity (Guidotti, Toffano and Costa, 1978).

Recently, a new water soluble benzodiazepine, midazolam, has been introduced (Fragen, Gahl and Cadwell, 1978; Reeves, Corssen and Holcomb, 1978) which permits, for the first time, the topical application of a benzodiazepine to nervous tissue.

The present study was undertaken to investigate the potential effect of midazolam, when administered in the intrathecal space, on reflexes evoked by nociceptive stimulation.

It has been shown in the dog, that both group III (small myelinated) and group IV† (unmyelinated) nerve fibres in afferent somatic nerves, evoke responses in efferent sympathetic nerves (Whitwam, Kidd and Fussey, 1979). Thus, the observation of evoked sympathetic reflexes in the anaesthetized dog, can be used to assess the effectiveness of drugs, administered intrathecally, on nociceptive stimulation.

MATERIALS AND METHODS

Experiments were performed on 13 mongrel dogs weighing between 10 and 20 kg. Anaesthesia was induced with methohexitone 12.5 mg kg⁻¹ ± 0.5 (mean ± SEM - used for all data), the trachea intubated and the lungs ventilated artificially with oxygen-enriched air. Cannulae were inserted to the right femoral artery and, via the right femoral vein, to the inferior vena cava. Anaesthesia was maintained using a 1% solution of α-chloralose in an initial dose of 32 ± 1 mg kg⁻¹, followed by a continuous infusion of 14 ± 1 mg kg⁻¹ h⁻¹. Suxamethonium 1 mg kg⁻¹ every 30 min was administered to provide neuromuscular blockade. Oesophageal temperatures were maintained between 36.5 and 38.5 °C and PaO₂, PaCO₂ and arterial pH (Radiometer ABL 1) within the ranges 14.2-15.4 kPa; 5.3-5.7 kPa and 7.25-7.31 unit respectively, by alterations in tidal volume and inspired oxygen concentration and, when necessary, by the administration of bicarbonate. The haematocrit was maintained in the range 39-43% by infusion of physiological saline approximately 8-10 ml kg⁻¹ h⁻¹.

The mean arterial and airway pressures were measured using calibrated Statham strain gauges and displayed with the ECG and the beat-by-beat heart rate (Devices 4522) on an ultraviolet light

†At the Nobel Symposium I (1966) "Muscle Afferent! and Motor Control" in Stockholm, it was agreed that the α-δ and C terminology (Erlanger and Gasser, 1937) should be used for efferent nerve fibres while I - IV (Lloyd, 1943) should be used to describe afferent fibres. The subject has been reviewed by Whitwam (1976).
Drugs were injected to the intrathecal space following the removal of the laminae of the 1st and 2nd lumbar vertebrae and exposure of the dura mater.

The lateral branch of the superficial (cutaneous) branch of the left radial nerve was exposed in the foreleg and the tibial nerve in the left hind leg. Both nerves were desheathed, cut distally and mounted on silver–silver chloride electrodes in a mineral oil pool. The renal sympathetic nerves were exposed retroperitoneally, close to the renal artery, and recordings of efferent activity made from desheathed fascicles of the nerves, which were immersed in mineral oil, using bipolar silver–silver chloride electrodes. The signals were processed through a preamplifier (Tektonix type 122) and displayed on either an ultraviolet recorder (SE Laboratories, type 2112) using conditioning amplifiers and galvanometers with a frequency response of 3000 Hz, or a dual-beam oscilloscope (Tektronix type 565).

Supramaximal electrical stimuli (intensity 50 V, duration 0.5 ms) were applied to the peripheral nerves using a Grass S88 Stimulator with matching direct-coupled isolation units (Grass type 478 A) at a frequency of 0.33 Hz.

Average transients of the evoked responses in the renal sympathetic nerves were recorded using a Neurolog system (N.L. 750 Digitimer). In addition, these responses were rectified and integrated (N.L. 90).

The operating table was arranged so that the preparation was in a head-up tilt of approximately 15°.

Commercial midazolam hydrochloride contains 5 mg per ml of drug and 10 μlitre per ml of benzyl alcohol as preservative. This was diluted 10 times in 0.9% sodium chloride to give a solution containing 0.5 mg ml⁻¹ of midazolam and hence 1 μlitre ml⁻¹ of benzyl alcohol. After control responses were obtained, 1–2 ml of the midazolam solution (that is, 0.5–1 mg of drug) was administered to the intrathecal space at the level of L1 and the effects on spontaneous, and reflexly evoked, sympathetic activity observed.

In order to exclude the possibility that the observed effects were caused by the preservative, benzyl alcohol, the following experiments were performed (preservative-free midazolam hydrochloride is not available, as yet):

(a) 1–2 ml of a solution of 1 μlitre ml⁻¹ of benzyl alcohol in 0.9% sodium chloride was administered intrathecally, in some of the preparations, before the midazolam.

(b) After the maximum effect of midazolam had occurred, the effects of the specific benzodiazepine antagonists, Ro 15-1788 (dissolved in a solution of gum acacia and Tween 80) and Ro 15-3505 (available commercially in a solution of 1 mg ml⁻¹) in doses of 1–2 mg kg⁻¹, were observed following administration i.v.

To exclude the remote possibility that the observed effects were caused by systemic effects of the drug as a result of absorption from the intrathecal space, the effects of midazolam 0.5 mg, 0.5 mg kg⁻¹ and 1 mg kg⁻¹ i.v., on the evoked sympathetic reflexes, were observed.

In some preparations, the effect of naloxone 2 mg i.v. administered after the intrathecal midazolam, was noted. The schema of the experimental preparation is illustrated in figure 1.

RESULTS

The early and late responses in the renal sympathetic nerves evoked by stimulation of tibial and radial nerves are illustrated in figure 2.

In all preparations the intrathecal administration of midazolam 1 mg produced a marked decrease (approximately 80–90%) in the sympathetic responses evoked by stimulation of the tibial nerve. In some preparations these responses were virtually abolished (fig. 3). Midazolam had no effect on either spontaneous sympathetic activity (fig. 4) nor on the responses evoked by radial nerve stimulation (fig. 3). The effect of the drug on the tibial reflexes was observed within 3–4 min although the maximal depression of the reflex response required 8–10 min.

The effect of the drug persisted unchanged for as long as observations were made, that is, for up to 2 h, and throughout this time there was no evidence of recovery.

The benzodiazepine antagonist Ro 15-1788 1 mg kg⁻¹ i.v. caused partial reversal of the effects of midazolam. In contrast, the newer antagonist Ro 15-3505 1–2 mg kg⁻¹ i.v. produced complete reversal of the depressant effect of the midazolam (fig. 3). Naloxone 2 mg i.v. did not reverse the effects of midazolam. Neither 1–2 μlitré of benzyl alcohol 1 μlitre ml⁻¹ in 0.9% sodium chloride administered intrathecally (fig. 5) nor midazolam, up to 1 mg kg⁻¹ i.v., caused any significant effects on the observed somatosympathetic reflexes.

The intrathecal administration of midazolam did
Stimulating electrodes
Radial nerve


FIG. 2. Individual examples of responses evoked in renal sympathetic nerves by supramaximal stimulation (50 V intensity, 0.5 ms duration) of cutaneous nerves. Left = radial; right = tibial. The tibial response shows two components to the early (short latency, group III) response (Whitwam, Kidd and Fussey, 1979).
Fig. 3. Effect of intrathecal midazolam 1.0 mg in 2 ml of 0.9% sodium chloride injected at L1 on responses in renal sympathetic nerves evoked by stimulation of the radial (R) and tibial (T) nerves. Lower traces = average transients of eight responses. Upper traces = rectified integral of average signals. From left to right: control responses; 10 min after intrathecal midazolam 1.0 mg; 3 min after Ro 15-3505 1.0 mg kg$^{-1}$ i.v.; 3 min after a further dose of this drug (see text).

Fig. 4. Effect of intrathecal midazolam 1.0 mg in 2 ml of 0.9% sodium chloride injected at L1 on spontaneous activity in renal sympathetic nerves. 1 = Control; 2 = after midazolam, showing no effect.
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FIG. 5. Effect of benzyl alcohol 1 μlitre in 1 ml of 0.9% sodium chloride injected intrathecally at L1 on evoked sympathetic responses. Lower traces = average transients; upper traces = rectified integral of averaged signal.

not affect heart rate or arterial pressure in any of the preparations except for small, and transient, decreases in arterial pressure and increases in heart rate immediately after the injection of 1 mg kg⁻¹ i.v.

DISCUSSION

It is accepted widely that nociception is mediated by small myelinated (group III) and unmyelinated (group IV) nerve fibres. The "input – output" relationships, that is, the reflexly evoked responses, in the sympathetic nervous system have been examined in many species of animals (Sato and Schmidt, 1973). In the dog it has been shown that supramaximal stimulation of nerves containing cutaneous fibres evokes responses in thoracic and renal sympathetic nerves which have two components referred to as the early and late responses. These responses are caused by stimulation of afferent nerve fibres in group III and group IV, respectively (Whitwam, Kidd and Fussey, 1979). Thus, the observation of changes in reflexly evoked sympathetic responses provides an experimental model with which to study the effects of drugs on nociception.

In the experiments reported here, the intrathecal injection of midazolam in the upper lumbar region caused a marked decrease in the sympathetic responses evoked by stimulation of the tibial nerve. The drug had no effect on either spontaneous renal sympathetic activity or on responses evoked by stimulation of the radial nerve. This suggests that midazolam depressed the afferent nociceptive pathways from the tibial nerve and did not spread to higher levels to any significant extent.

The facts that 1 μlitre of benzyl alcohol injected to the intrathecal space, at the same level, had no effect on these reflexes, and that the effect of midazolam was totally reversed by Ro 15-3505 i.v., indicate that the observed depression of the tibial reflexes was caused by midazolam and not by the very small amount of preservative in the formulation of this drug. Further evidence for a local topical effect of midazolam was the absence of any significant effect on somatosympathetic reflexes when the drug was administered i.v. in high doses.

In some preparations the sympathetic reflexes evoked by stimulation of the tibial nerve were totally abolished (fig. 3). In this figure the integrator signal, after the intrathecal administration of the drug, merely represents the ongoing integral of the rectified signal from the average transient of spontaneous sympathetic activity without any evidence of evoked responses.

The question may be asked as to how locally applied benzodiazepines can influence a nociceptive system. Haefely and colleagues (1975) and Costa and co-workers (1975) have suggested that the benzodiazepine system interacts with the GABA system. Subsequent studies have confirmed this interaction showing that GABA enhances the binding of benzodiazepines to their receptors (Tallman, Thomas and Gallager, 1978; Braestrup and Nielsen, 1981) and, conversely, by binding to their relative receptors benzodiazepine has an indirect modulating effect on GABA recognition sites which, by decreasing their affinity to GABA (Guidotti, Toffano and Costa, 1978), causes more-free GABA to be available.

It has also been shown that morphine analgesia is enhanced either by increases in the concentration of GABA in the CNS (brought about by decreasing the degradation of GABA by inhibition of the enzyme GABA transaminase) (Contreras, Tamayo and Quijada, 1979; Buckett, 1980a), or by the administration of the GABA receptor agonist, muscimol (Biggo et al., 1977). However, GABA itself has analgesic properties (Buckett, 1980b) and is found in high concentrations in the dorsal root area (Kuriyama and Yoneda, 1978).

As specific benzodiazepine receptors are present throughout the nervous system, including the spinal cord (Möbler and Okada, 1977; Squires and Braestrup, 1977), it might be expected that the introduc-
tion of an exogenous benzodiazepine into the CSF around the spinal cord will reach the benzodiazepine receptors in high concentration and could have a pronounced effect on local GABA activity. Thus, benzodiazepines can gain access to analgesic systems mediated by GABA.

Although Dingledine, Iversen and Breuker (1978) have shown that naloxone can reverse GABA activity, Buckett (1980b) found that this drug did not antagonize the analgesic effect of GABA. In the event that benzodiazepine is acting indirectly through GABA, the findings of the present study agree with those of Buckett (1980b) and suggest that benzodiazepine-induced nociceptive depression is not dependent on an opioid system.

It has been suggested that the benzodiazepine receptor blocking drug Ro 15-1788 is also a partial agonist (Nutt, Cowen and Little, 1982) and this may explain the greater effectiveness of Ro 15-3505 in reversing the effects of midazolam.

In conclusion, supramaximal electrical stimulation of a peripheral nerve provides a massive nociceptive input to the CNS. The findings of the present study and the rapidly increasing evidence in the literature concerning the interaction of benzodiazepine and GABA on nociceptive systems, suggests that benzodiazepine applied locally (intrathecal or extradural) could be of value in the management of pain. This has been made possible with the introduction of midazolam which, being water soluble, does not require a potentially neurotoxic vehicle.

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REFERENCES


DEPRESSION DES REFLEXES SYMPATHIQUES NOCICEPTIFS PAR L'ADMINISTRATION INTRATHECALE DE MIDAZOLAM

RESUME

Nous avons étudié les effets de l'administration intrathécale de midazolam 0,5-1,0 mg dans 1-2 ml de chlorure de sodium à 9% sur les réponses des nerfs sympathiques rénaux évoquées par la stimulation électrique supramaximale des nerfs radial et tibial. Chez des chiens en ventilation contrôlée, anesthésiés avec de l'alpacho-aloral, l'administration intrathécale de midazolam provoquait une dépression marquée des réflexes évoqués à partir du nerf tibial mais n'avait d'effet ni sur l'activité sympathique spontanée ni sur les réflexes évoqués par la stimulation du nerf radial. Ni la faible quantité (1-2 µl) de benzylalcool, présent...
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COMME CONSERVATEUR (ADMINISTRÉ PAR VOIE INTRATHÉCAL), NI L'ADMINISTRATION DE MIDAZOLAM 1 mg·kg⁻¹ I.V., N'ONT ENTRAÎNÉ DE DÉPRESSION SIGNIFICATIVE DES RÉFLEXES SOMATO-SYMPATHIQUES ÉVOCÉS. LES EFFETS DU MIDAZOLAM INTRATHÉCAL ÉTAIENT ANNULÉS PAR LES ANTAGONISTES DES BENZODIAZÉPINES RO 15-1788 1 mg·kg⁻¹ I.V. ET RO 15-3505 1-2 mg·kg⁻¹ I.V. MAIS PAS PAR LA NALOXONE 2 mg·I.V.

Nous suggérons que les effets antinociceptifs du midazolam, appliqué localement, pourraient être dus à un système dont le médiateur est le GABA, qui n'est pas un opiacé, système qui pourrait être impliqué dans le traitement de la douleur.

UNTERDRUCKUNG DER NOZIZEPTIVEN SYMPATHISCHEN REFLEXE DURCH INTRATEKALE VERABREICHUNG VON MIDAZOLAM

ZUSAMMENFASSUNG

An Reaktionen des renalen Sympathikusnerven auf supramaximale elektrische Stimulation der Nn. radialis und tibialis wurde die Wirkung einer intrathekalen Verabreichung von 0,5-1,0 mg Midazolam in 1-2 ml physiologischer Kochsalzlösung beobachtet. Bei künstlich beatmeten, mit a-Chloralose narkotisierten Hunden führte die Verabreichung von Midazolam zu einer ausgeprägten Unterdrückung von Reaktionen auf den Tibialis-Reiz, hatte jedoch keinen Effekt auf die spontane Sympathikus-Aktivität oder auf Reaktionen auf Radialis-Stimulation. Auch die geringe Menge (1-2 ml) Benzylalkohol als Konserverungsmittel (intrathekal verabreicht) oder Midazolam 1 mg·kg⁻¹ I.V. verabreicht führten zu keiner signifikanten Unterdrückung der ausgelösten somato-sympathischen Reflexe. Die Wirkung des intrathekalen Midazolams wurde durch die Benzodiazepin-Antagonisten Ro15-1788 1 mg·kg⁻¹ I.V. und Ro15-3505 1-2 mg·kg⁻¹ i.v. aufgehoben, nicht jedoch durch Naloxon 2 mg·I.V. Wahrscheinlich ist der antinocizeptive Effekt lokal verabreichter Midazolams auf ein nicht-opioides, GABA-vermitteltes System zurückzuführen, das bei der Behandlung von Schmerz von Bedeutung sein könnte.

DISMINUCION DE LOS REFLEJOS NOCICEPTIVOS SIMPATICOS MEDIANTE LA ADMINISTRACION INTRATECAL DE MIDAZOLAM

SUMARIO

Se observaron los efectos de la administración intratecal de 0,5-1,0 mg de midazolam en una solución fisiológica salina de 1-2 ml en las respuestas provocadas en los nervios simpáticos renales, a causa de la estimulación eléctrica supramaximal de los nervios radial y tibial. La administración intratecal de midazolam en perros anestesiados con a-clorasa y sometidos a ventilación artificial, produjeron una notable disminución de los reflejos provocados desde el nervio tibial, pero no tuvo efecto alguno en la actividad simpática espontánea ni en los reflejos provocados por estimulación del nervio radial. Ni la pequeña cantidad de alcohol benéfico (1-2 ml), presente como conservativo y administrado intratecalmente, ni el midazolam intravenoso (1 mg·kg⁻¹), causaron disminución significativa alguna en los reflejos simpáticos comiticos provocados. Los efectos del midazolam intratecal se contrarrestaron mediante los antídotos de la benzodiazepina, Ro 15-1788, 1 mg·kg⁻¹ intravenoso, y Ro 15-3505, 1-2 mg·kg⁻¹ intravenoso, pero no mediante naloxona, 2 mg intravenosa. Se sugiere que el efecto antinociceptivo del midazolam administrado localmente podría ser consecuencia de un sistema no opioide comunicado por GABA, el cual puede tener repercusiones en la gestión del dolor.