Spinal and supraspinal midazolam potentiates antinociceptive effects of isoflurane

Y. Taira¹, K. Nakakimura*, M. Matsumoto and T. Sakabe

Department of Anesthesiology-Resuscitology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan

¹Present address: Department of Anesthesia, Aso-Yamanami Hospital, 115-1 Miyaji, Itinomiya-cho, Aso-gun, Kumamoto 869-2612, Japan

*Corresponding author

The effects of lumbar intrathecal (i.t.) and intracerebroventricular (i.c.v.) midazolam on nociception during isoflurane anaesthesia were studied in rats using the tail-flick test. Rats received i.t. midazolam 2 and 4 µg or i.c.v. midazolam 4 and 8 µg during 1.1, 1.2 and 1.3% isoflurane or without isoflurane. Neither i.t. nor i.c.v. midazolam alone at doses studied influenced nociceptive responses. 1.1% isoflurane showed a minimum antinociceptive effect which was not influenced by i.t. or i.c.v. midazolam. 1.2 and 1.3% isoflurane produced moderate antinociception which was markedly potentiated by both i.t. and i.c.v. midazolam. The effects of midazolam shown in the present study are different from the reported effects of midazolam on opioid-induced antinociception; where spinally administered midazolam potentiates and supraspinal midazolam inhibits the antinociceptive effects of morphine. The present results suggest that midazolam potentiates isoflurane-induced antinociception at doses where no effect is seen alone.

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Midazolam is commonly used as an adjunct to general anaesthesia and has been shown to decrease the anaesthetic requirements of volatile agents after i.v. administration in both animals¹ and humans.²³ However, in respect of a nociceptive action, both hyperalgesic⁴⁻⁵ and antinociceptive⁶ effects of systemically administered midazolam have been reported. Different results may be ascribed to the action of midazolam on the spinal cord and/or brain. Regionally administered midazolam within the central nervous system has been reported to produce different effects on nociception. Lumbar intrathecal (i.t.) administration of midazolam produces antinociception,⁴⁻⁸ while intracerebroventricular (i.c.v.) injection produces a hyperalgesic response.⁵ In combination with opioids, the effects of midazolam on nociception are also different depending on the route of administration; where i.t. injection potentiates the antinociceptive effects of morphine and i.c.v. injection inhibits opioid-induced antinociception.⁹⁻¹¹ These modulating effects of midazolam on opioid-induced antinociception have been suggested to be mediated at least partially through gamma-aminobutyric acid A (GABA⁶) receptors.⁹¹⁰¹² With respect to the interaction of i.t. midazolam with inhalation anaesthetics, there has been one report¹³ which showed that i.t. midazolam decreased isoflurane minimum alveolar concentration (MAC) in rats. No study has examined whether a difference exists between the spinal and supraspinal actions of midazolam on antinociception induced by a volatile anaesthetic as observed in narcotic-induced antinociception. The current study therefore examined the influence of i.t. or i.c.v. midazolam on nociception in rats anaesthetized with various concentrations of isoflurane.

Materials and methods
This study was approved by the Committee of Ethics on Animal Experiments at the Yamaguchi University School of Medicine.

Male Wistar rats weighing 250–300 g (60–70 days after birth) were used. The animals were housed in a cage at 21–25 °C under diurnal light condition and allowed free access to food and water prior to the experiment.

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For implantation of an i.t. catheter, rats were anaesthetized with halothane, and a PE-10 catheter was aseptically inserted through the atlanto-occipital membrane into the intrathecal space. The catheter was advanced 8 cm caudally to position its tip at the level of lumbar enlargement of the intrathecal space. The catheter was advanced 8 cm caudally to position its tip at the level of lumbar enlargement of the spinal cord and secured to the subcutaneous tissue.

For implantation of an i.c.v. cannula, rats were anaesthetized by intraperitoneal administration of pentobarbital (50 mg kg\(^{-1}\)) and a 24-G stainless steel cannula was stereotaxically inserted into the right lateral ventricle under aseptic conditions according to the atlas of Paxinos and Watson (1986) (0.8 mm posterior from bregma, 1.5 mm lateral from the midline and 3.5 mm depth from the skull). The cannula was fixed to the skull bones with methyl methacrylate cement.

A minimum period of 48 h elapsed between catheter implantation and the following experiment which was performed in animals with no neurobehavioural abnormality.

All catheters' positions were verified at the end of experiment; i.t. catheter-implanted rats were given i.t. injection of 10 µl of 2% lidocaine and all showed bilateral paralysis hindlimbs. In addition, for postmortem confirmation, i.t. catheter position and i.c.v. cannula, and for the assessment of the spread of solution, 10 µl and 5 µl of Evans Blue was injected i.t. and i.c.v., respectively.

Rats implanted with either i.t. catheter or i.c.v. cannula were assigned into four groups; Group I (n=24) received no inhalation of isoflurane, Groups II, III and IV (n=30 in each) received isoflurane 1.1%, 1.2% and 1.3%, respectively. Group I was further divided into four midazolam-treated groups (n=6 in each), each of which received 2 µg i.t., 4 µg i.c.v. and 8 µg i.c.v. Each of Groups II, III and IV was further divided into five groups (n=6 in each): control group that received either i.t. (n=3) or i.c.v. (n=3) saline and four midazolam groups (2 µg i.t., 4 µg i.t., 4 µg i.c.v. and 8 µg i.c.v.).

Midazolam (F. Hoffmann-La Roche, Swiss) was dissolved in 0.9% saline, so that desired dose was contained in 10 µl for i.t. injection or 5 µl for i.c.v. injection. In control groups at each isoflurane concentration, rats received 10 µl of i.t. saline or 5 µl of i.c.v. saline. The i.t. and i.c.v. injections were made using a microsyringe over a period of 5 min and 10 min, respectively.

The doses of midazolam and concentrations of isoflurane were selected on the basis of our preliminary results and of reported information.\(^{11-13}\) Preliminary, using rats with femoral artery catheterization, we found that \(P_{\text{aco}_2}\), and mean arterial pressure respectively were maintained at 4.7–6.0 kPa and above 70 mm Hg for more than 120 min under spontaneous respiration at 1.3% isoflurane alone or with i.c.v. administration of 8 µg midazolam. Rats given midazolam 4 µg i.t. or 8 µg i.c.v. behaved normally. When the isoflurane concentration was increased to 1.4%, tail-flick latencies were prolonged to greater than 15 s and severe respiratory depression or sometimes death ensued.

Before administration of midazolam or isoflurane, an awake value of tail-flick latency (Analgesia meter, MK-330, Muromachi Kikai, Tokyo) was measured in each rat. The tail of each rat was placed on a focused projector lamp so that the beam was focused on the proximal third of the tail. The end-point of the test was represented by the number of seconds until the rat removed its tail from the beam. Beam intensity was regulated so that pre-anaesthetic awake latencies were 4–5 s. The cut-off time was set at 15 s.

Rats of Group I were minimally restrained in a specially designed plastic box flushed continuously with total 2 litres min\(^{-1}\) of nitrogen and oxygen (\(F_{\text{IO}_2}=0.25\)). In the other groups, anaesthesia was induced with 3% isoflurane in the same gas mixture as Group I (\(F_{\text{IO}_2}=0.25\)) and, thereafter, maintained with preselected anaesthetic concentrations (1.1%, 1.2% and 1.3%) under spontaneous respiration until the end of experiment. The concentrations of inhalational isoflurane and oxygen were monitored continuously with a Datex Capnomac Anesthetic monitor (Helsinki, Finland). Rectal and skin temperature of the tail base were measured by a digital thermometer (6510 TC, Mallinckrodt Medical Inc., Ireland). Application of thermistor probe neither disturbed spontaneous movement of the tail nor influenced tail-flick latencies. Rectal and tail temperatures were maintained by means of external heating at 36.8–37.2°C and 36.2–36.4°C, respectively.

After a 30-min stabilization period at each concentration of isoflurane (Groups II–IV), baseline tail-flick latencies were measured, and then midazolam or saline was administered i.t. or i.c.v. In order to avoid repeated stimulation without anaesthesia, the baseline measurement was omitted in Group I, and midazolam was administered i.t. or i.c.v. after a 30-min stabilization.

Measurement of tail-flick latencies were repeated at 10, 15, 20, 30, 45, 60, 90 and 120 min after the administration of midazolam or saline.

The antinociceptive effects of isoflurane and midazolam were expressed as percentage of the maximum possible effect (% MPE) as follows:

\[
\% \text{ MPE} = \frac{(\text{postdrug value – awake value})}{(\text{cut-off time – awake value}) \times 100}
\]

Analysis of variance for repeated measures was used to evaluate the time-course effect of treatments. To compare % MPE and maximum antinociceptive effect (peak % MPE) among groups, factorial analysis of variance was used. Multiple comparisons were performed using Fisher’s protected least significant difference test. \(P<0.05\) was considered statistically significant.

**Results**

In Group I (isoflurane 0%), i.t. or i.c.v. midazolam alone studied did not significantly change % MPE over the 120-min observation period.
In the control group at each concentration of isoflurane (Groups II, III and IV), there were no differences in % MPE between i.t. and i.c.v. saline administration and therefore the data of i.t. and i.c.v. saline at each isoflurane concentration were combined and served as control.

In Group II, mean % MPE of the control group was significantly but minimally increased to 6±10% during 1.1% isoflurane when compared with the awake value. Neither i.t. nor i.c.v. midazolam caused further changes in % MPE following administration.

The time courses of % MPE after administration of i.t. and i.c.v. midazolam in Groups III and IV respectively are shown in Figures 1 and 2. In Groups III and IV, mean % MPE of the control group was significantly increased to 31–38% during 1.2% isoflurane and to 45–51% during 1.3% isoflurane when compared with awake values. Both i.t. and i.c.v. midazolam at all doses caused a further increase in % MPE (Figs 1, 2). At each anaesthetic concentration, there were no significant differences in % MPE between midazolam given either i.t. or i.c.v.

Table 1 shows peak % MPE (maximum % MPE during the measurement period in each rat) in each group. In Group I (isoflurane 0%), there were no differences in peak % MPE between the subgroups given i.t. or i.c.v. midazolam. In Group II (1.1% isoflurane), there were no significant differences in peak % MPE between control and any treatment groups given midazolam. In Groups III and IV (1.2 and 1.3% isoflurane), there were significant differences in peak % MPE between respective control and all treatment subgroups with no inter-treatment group differences. Peak % MPEs in control and all treatment subgroups in Groups III and IV are significantly higher than those in the corresponding subgroups of Groups I and II. There were no differences in peak % MPE in any subgroup between Groups III and IV, and no difference between Groups I and II.

Postmortem examination revealed that Evans Blue injected through the i.t. catheter and i.c.v. cannula spread...
in the lumbar spinal cord and in both lateral cerebroventricles and third ventricle, respectively.

**Discussion**

In the present study, isoflurane 1.1–1.3% showed antinociceptive effects in a dose-related fashion, and both spinal and supraspinal administration of midazolam at doses not affecting nociceptive responses alone potentiated the antinociception produced by isoflurane 1.2 and 1.3%. These results are in contrast to the reported effects of midazolam on opioid-induced antinociception which depend on the route of administration of midazolam (i.e., i.t. injection of midazolam potentiates opioid-induced analgesia, while i.c.v. injection inhibits).9–11

Anatomical localization of the spinally or supraspinally administered agents in addition to their effects on motor function deserve comment. We confirmed that the distribution of Evans Blue injected i.t. (10 µl) and i.c.v. (5 µl), was confined in the spinal and supraspinal localization, respectively. Thus, either effect of i.c.v. and i.t. drug is assumed independent, and not resulted from distribution to the remote subarachnoid space. As for the motor function, we did not observe any disturbance induced by midazolam at doses studied. Our i.t. doses of midazolam are far less than the minimum dose (40 mg) of i.t. midazolam required to produce catalepsy.11 Midazolam 8 mg i.c.v. has been shown to cause catalepsy in rats.11 However, in the present study, no animals exhibited catalepsy, and thus the observed results have not been influenced by motor disturbance.

Several studies have demonstrated that intravenous midazolam decreased the requirement for volatile anaesthetic agents.1–3 However, little is known about the exact site or mechanism of action. There has been only one report to our knowledge investigating the interaction of i.t. midazolam with inhalation anaesthetics with respect to nociception or anaesthetic potency.13 Schwieger and co-workers13 reported in rats that i.t. midazolam decreased isoflurane MAC in a dose-dependent manner. A dose of 5 µg of i.t. midazolam decreased isoflurane MAC by 16%13 which is comparable with our observations that i.t. injection of 2 and 4 µg of midazolam potentiated the antinociception produced by 1.2 and 1.3% isoflurane. I.t. injection of midazolam alone has been reported to produce antinociception in humans14 and animals7–9,11,15 which is believed to be mediated by potentiation of the effects of GABA on GABA<sub>A</sub> receptors. However, over the range of the doses studied (2–4 µg), no significant antinociceptive effects of i.t. midazolam have been reported in rats11 as observed in the present experiments. Several studies have indicated that the spinal cord is an important site for antinociceptive action of inhalation anaesthetics16–19 as assessed by a blockade of motor response to noxious stimulation. Mason, Owens and Hammond20 have shown that suppression of pinch-evoked movement by halothane is antagonized by i.t. injection of bicuculline and picrotoxin at doses that have no influence on the response latency when given alone, suggesting that halothane exerts its antinociceptive action through a GABA<sub>A</sub> receptor-related mechanism within the spinal cord.

The potentiation effect of i.t. midazolam on antinociception induced by isoflurane in the current study is likely to be mediated by enhancement of the action of GABA on GABA<sub>A</sub> receptor in the spinal cord. Furthermore, antinociceptive effects of i.t. midazolam is suggested to be mediated via mechanisms related to opioid receptors.7,15,21 Goodchild and co-workers8 have strongly suggested that i.t. midazolam causes antinociception by a mechanism involving the delta opioid receptor. This possibility, though not examined in the present study, may be in part responsible for the observed potentiation.

In contrast to the observation that opioid-induced antinociception was antagonized by i.c.v. midazolam, i.c.v. injection of midazolam potentiated isoflurane-induced antinociception in the present study. The potentiating effects of isoflurane-induced antinociception by i.c.v. midazolam might be attributable to anaesthetic efficacy in contrast to analgesic effects of midazolam. However, such speculation cannot readily explain the current results. I.c.v. midazolam, given with 1.1% isoflurane, produced no potentiation, while the effect of 1.2 and 1.3% isoflurane (baseline % MPE: 35–50%) were increased by i.c.v. midazolam (% MPE to 90–100%). Although the possibility that an undetectable anaesthetic efficacy of midazolam may have contributed to increase the antinociceptive effects induced by isoflurane 1.2 and 1.3% cannot be entirely excluded, the fact that there was no modulatory effect of the

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**Table 1** I.t. and i.c.v. midazolam potentiated antinociceptive effects of isoflurane. Peak % MPE (mean (SEM)) in each group inhaled isoflurane concentration. Peak % MPE is a peak value of % MPE of the rat during the experiment. i.t.=intrathecal administration, i.c.v.=intracerebroventricular administration. *P<0.005 compared with the control group at the corresponding concentration of isoflurane. †P<0.025 compared with the corresponding subgroup of Group I. ‡P<0.005 compared with the corresponding subgroup of Group II.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control 2 µg i.t.</th>
<th>Midazolam 4 µg i.t.</th>
<th>Midazolam 4 µg i.c.v.</th>
<th>Midazolam 8 µg i.c.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 0%</td>
<td>3.9 (1.6)</td>
<td>6.3 (1.4)</td>
<td>6.0 (1.3)</td>
<td>5.3 (2.7)</td>
</tr>
<tr>
<td>Group II 1.1%</td>
<td>13.6 (3.1)</td>
<td>16.3 (2.7)</td>
<td>19.1 (7.8)</td>
<td>16.3 (2.0)</td>
</tr>
<tr>
<td>Group III 1.2%</td>
<td>48.2 (7.0)‡‡</td>
<td>90.3 (9.7)∗‡‡</td>
<td>83.7 (11.9)∗‡‡</td>
<td>90.2 (9.0)∗‡‡</td>
</tr>
<tr>
<td>Group IV 1.3%</td>
<td>63.2 (9.8)‡‡</td>
<td>90.4 (7.1)∗‡‡</td>
<td>97.7 (1.5)∗‡‡</td>
<td>100 (0)∗‡‡</td>
</tr>
</tbody>
</table>
same doses of midazolam given with isoflurane 1.1% suggests this possibility is unlikely.

Several investigators have recently demonstrated that anaesthetic potency measured as MAC is determined by the action of anaesthetics at the spinal cord and/or brain stem. Isoflurane MAC for goats was increased during preferential delivery of isoflurane to the brain and decreased during preferential delivery to the spinal cord, compared to MAC measured by systemic administration.\textsuperscript{16, 17} Rampil\textsuperscript{19} also showed that MAC was not changed after spinal cord transection in rats. The results of these studies suggest that deepening of anaesthetic level by i.c.v. midazolam (even if this occurs) may not obscure the somatic response to the stimulation.

Anaesthetic potency of an agent does not necessarily correlate with analgesic action. Deady and co-workers\textsuperscript{22} found that the ratio of anaesthetic potency measured by MAC or concentration inhibiting righting reflex \textit{versus} analgesic potency measured by tail-flick latency was different among volatile anaesthetics. Niv and co-workers\textsuperscript{4} also reported in rats that intraperitoneal injection of 10 mg kg\textsuperscript{-1} of midazolam produced complete abolition of spontaneous movement but decreased tail-flick latencies compared with the awake values, suggesting a dissociation between anaesthetic depth and analgesic actions.

With respect to i.c.v. administration of midazolam alone, our results are consistent with a previous report which observed only minor influence, if any, on nociceptive responses.\textsuperscript{11} In addition to enhancement of the binding of GABA to GABA\textsubscript{A} receptors, midazolam has been reported to inhibit GABA metabolism in brain synaptosomes, suggesting GABA concentrations may be increased at the synapse.\textsuperscript{23} Volatile anaesthetics also have modulatory actions on the function of GABA\textsubscript{A}-mediated inhibitory systems in the brain. This modulation seems to contribute to anaesthetic and possibly analgesic action, though the modulatory effects of anaesthetics are not simple. Banks and Pearce\textsuperscript{24} reported that volatile anaesthetics produced both reduction of the peak amplitude and prolongation of the duration of miniature inhibitory postsynaptic currents in hippocampal neurons of brain slices, and concluded that net action is via enhanced inhibition. Moreover, in an \textit{in vitro} study it was suggested that isoflurane also increases GABA levels in the synaptic cleft and thus may enhance synaptic inhibition.\textsuperscript{25} When used in conjunction with opioids, i.c.v. injection of midazolam has been shown to inhibit the antinociceptive effects of opioids through a mechanism related with GABA\textsubscript{A} receptor.\textsuperscript{9, 10, 12} In addition, Rady and Fujimoto\textsuperscript{9} have demonstrated an elimination of the effect of i.c.v. midazolam by i.t. pretreatment with dynorphin antiserum, indicating that the inhibitory effect of i.c.v. midazolam on opioid antinociception is mediated by the release of dynorphin A (1–17) in the spinal cord. Dynorphin A (1–17) is a putative endogenous kappa selective opioid and is reported to be a mediator of antianalgesic descending system in the spinal cord.\textsuperscript{26} To our knowledge, the effects of volatile anaesthetics on antianalgesic descending systems have not been investigated. It has been reported that isoflurane dose-dependently blocks spinal cord potentials evoked by a feedback loop through supraspinal structures,\textsuperscript{27} though the relationship relevance to our data is unclear. When considering the effects of volatile anaesthetics on opioid receptor subtypes, various results have been reported. Campbell, Rowbotham and Lambert\textsuperscript{28} reported that clinical concentrations of halothane had little effect on mu and delta opioid receptor binding, whereas in another study halothane inhibited both mu and kappa receptor binding.\textsuperscript{29} These modulating effects of volatile anaesthetics and midazolam on GABA\textsubscript{A} and/or opioid receptors in the brain may contribute the potentiating effects of i.c.v. midazolam on isoflurane-induced antinociception in the current study, though the precise mechanism remains to be elucidated.

In summary, both spinal and supraspinal midazolam doses with no antinociceptive effects alone, potentiated isoflurane-induced antinociception. These results are different from the effects of midazolam on opioid-induced antinociception, which is potentiated by spinal administration but inhibited by supraspinal injection of midazolam. The present study suggests that low doses of midazolam may enhance the antinociceptive effects of isoflurane.

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