Cerebral Effects of High-dose Midazolam and Subsequent Reversal with Ro 15-1788 in Dogs

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The effects of a continuous high-dose infusion of midazolam on cerebral function, metabolism, and hemodynamics were studied in nine dogs receiving a spinal anesthetic and breathing 65% nitrogen/35% oxygen. In five dogs, the effects of 65% nitrogen oxide (N₂O) inspired and the benzodiazepine antagonist Ro 15-1788 were also examined. Midazolam was infused at a rate of 0.66 mg·kg⁻¹·min⁻¹ for 60 min for a total dose of 40 mg·kg⁻¹. Cerebral metabolic rate for oxygen (CMRO₂) and cerebral blood flow (CBF) (measured by venous outflow technique) both decreased until a plateau level was reached at approximately 75% of control values (4.0 ± 0.2 ml·min⁻¹·100 g⁻¹ and 49 ± 3 ml·min⁻¹·100 g⁻¹, respectively, mean ± SEM). This occurred after 6-10 mg·kg⁻¹ of midazolam, corresponding to serum midazolam levels between 18.4 ± 3.8 and 31.2 ± 3.3 µg·ml⁻¹. Serum midazolam levels increased throughout the midazolam infusion, reaching a mean value of 53 ± 5.5 µg·ml⁻¹ by the end of the midazolam infusion. A similar plateau was seen for changes in the electroencephalogram (EEG), which never developed burst suppression. Five dogs inspired 65% nitrogen oxide/35% oxygen during minutes 30-45 of the midazolam infusion, rather than 65% nitrogen/35% oxygen. Nitrous oxide had no effect upon CMRO₂, but significantly increased CBF when compared to dogs receiving nitrogen. Ro 15-1788, 1.0 mg·kg⁻¹ caused a return of CMRO₂ and EEG activity to control levels. CBF and intracranial pressure (ICP) increased markedly, to greater than control levels immediately following Ro 15-1788. After 15 min, both CBF and ICP had decreased to near levels seen immediately prior to Ro 15-1788 administration. Ro 15-1788 administration was associated with a decrease in MAP requiring treatment with intravenous fluids and a low-dose phenylephrine infusion to maintain mean arterial pressure (MAP) above 60 mmHg. Samples for cerebral metabolic determination taken either at the end of the midazolam infusion, or following Ro 15-1788, were essentially normal. The authors conclude that midazolam produces a limited dose-related decrease in CMRO₂ which correlates with decreases in neuronal function. There were no apparent toxic effects of midazolam 40 mg·kg⁻¹. The combination of midazolam and Ro 15-1788 was effective in reversing the cerebral effects of high-dose midazolam, without untoward effects upon cerebral metabolic concentrations. However, Ro 15-1788 administration was associated with a significant increase in CBF and ICP, and a decrease in MAP. (Key words: Anesthetics, gases; nitrous oxide. Anesthetics, intravenous; midazolam. Antagonists, benzodiazepine; Ro 15-1788. Brain; blood flow; electroencephalogram; metabolism; oxygen consumption.)

It has been proposed that anesthetic agents which diminish cerebral energy requirements do so via a reduction in cerebral function, and, hence, on a metabolic basis, could attenuate cerebral insults resulting from ischemia only to the extent that cerebral function (electroencephalogram [EEG] activity) is attenuated.¹² This has been demonstrated with thiopental,³ isoflurane,⁴ and etomidate,⁵ all of which produce dose-related decreases in cerebral metabolic rate for oxygen (CMRO₂) that are correlated with decreases in cerebral function as indicated by changes in EEG activity. Following maximal attenuation of cerebral functional activity (isoelectric EEG), these agents, even at very high doses, have no apparent direct or toxic effects on cerebral metabolism.

Midazolam is a recently released benzodiazepine, recommended for intravenous sedation, premedication, and the induction of general anesthesia.⁶ Hoffman et al.⁷ reported the effects of midazolam on CMRO₂ in rats receiving doses totaling 5.75 mg·kg⁻¹. They found dose-related decreases in CMRO₂ to 45-65% of control values. Nugent et al.⁸ studied the effects of midazolam on CMRO₂ in dogs following progressively larger bolus doses of midazolam to a cumulative total dose of 17.2 mg·kg⁻¹. They reported a dose-related decrease in CMRO₂ to a maximum of 55% of control values, and concomitant changes in the EEG reported as a decrease in frequency and an increase in amplitude. Nugent et al.⁸ suggested that even larger doses of midazolam may decrease CMRO₂ to levels approaching those seen with barbiturates.³ This suggestion is especially interesting in light of the work in progress with the benzodiazepine antagonists, as discussed below.

In the present study, we sought to determine if dose-related decreases in CMRO₂ caused by midazolam can be correlated with decreases in cerebral neuronal function (EEG activity), and whether large doses of midazolam have any direct or toxic effects on cerebral metabolic pathways. In a canine model similar to that used for the study of thiopental,³ isoflurane,⁴ or etomidate,⁵ we investigated the cerebral metabolic, vascular, and functional effects of a continuous, high-dose, intravenous infusion of midazolam. Because of differences in the reported interaction between nitrous oxide (N₂O) and benzodiazepines in their effect upon CMRO₂,⁸,⁹ we
also determined the cerebral effects of NO2 in combination with high doses of midazolam.

In recent years, a new class of drugs known as benzodiazepine antagonists has been described.10 Ro 15-1788, a benzodiazepine antagonist, is currently undergoing clinical trials. Ro 15-1788 has low toxicity, and high specificity for the benzodiazepine receptor, where it binds in a competitive manner.10 If midazolam in large doses can be shown to offer clinically beneficial cerebral effects, than the use of an antagonist such as Ro 15-1788 to reverse the anesthetic and sedative effects of midazolam would be desirable. In the current study, we have investigated the cerebral metabolic, vascular, and functional effects of Ro 15-1788, when administered following large doses of midazolam.

Materials and Methods

Nine unmedicated fasting mongrel dogs, weighing 10–15.5 kg, were studied. The protocol was approved by the Institutional Animal Care and Use Committee. The experimental protocol is summarized in figure 1. Anesthesia for the surgical preparation was induced and maintained with halothane 1% end-tidal, in 60–70% NO2 and oxygen (O2). Succinylcholine 40 mg iv was given to facilitate tracheal intubation and followed by a continuous infusion of 150 mg h−1. Ventilation (using a Harvard® pump) and inspired oxygen concentration (FiO2) were adjusted to maintain PaCO2 between 35–40 mmHg and PaO2 ≥ 150 mmHg, respectively. Cannulae were surgically inserted into a femoral artery for mean arterial pressure (MAP) monitoring (zero reference at level of mid-skeleton) and blood sampling, a femoral vein for fluid and blood administration, and the pulmonary artery via the right external jugular vein for pressure measurements (zero reference at level of right atrium). Lactated Ringer's solution was administered at a rate of 75 ml h−1 via a peripheral limb intravenous cannula. A second peripheral intravenous cannula was placed for drug administration. Sodium bicarbonate was administered as needed to maintain the buffer base near 40 mEq l−1. A percutaneous lumbar subarachnoid catheter was placed for the subsequent administration of spinal anesthesia.

A craniotomy was performed and the sagittal sinus was exposed, isolated, and, after heparinization (300–400 units kg−1 iv), cannulated as previously described.11 This technique allows direct measurement of cerebral blood flow (CBF), by a square-wave electromagnetic flowmeter,12 from the anterior, superior, and lateral portions of both cerebral hemispheres representing approximately 54% of the total brain weight.11,13 Intracranial pressure was measured using an epidural fiberoptic device (LADD Research Industries Inc.), and the cranium was rigidly closed with Surgicel® and Super Line® adhesive. Core temperature as measured by a thermistor in the pulmonary artery, and brain temperature as measured by a parietal epidural thermistor, were maintained near 37.0°C using heating lamps and blankets. The electrocardiogram was monitored using needle electrodes. A four-lead, two-channel, bifrontal and biparietal EEG was recorded continuously from electrodes glued to the skull. Arterial and sagittal sinus blood gases were determined by electrodes at 37°C (Instrumentation Laboratory 1303®). End-tidal halothane and CO2 concentrations were monitored with a mass spectrometer. Blood oxygen contents were calculated from measurement of oxyhemoglobin concentrations (CO-oximeter, IL 282) and oxygen tensions.14 Blood glucose was determined by a membrane-bound enzyme technique (Yellow Springs Instruments Model 23A Glucose Analyzer). CMRO2 was calculated as the product of CBF and the arterial-sagittal sinus blood oxygen content difference.
At the completion of the surgical procedure, all surgical wound edges were infiltrated with a total of 4 ml of lidocaine 1.0%. Spinal anesthesia was established via the previously placed subarachnoid catheter with the injection of tetracaine 20 mg in 3 ml of sterile water. The dogs’ ears were packed with cotton, and the eyes were taped closed. Halothane was discontinued and nitrogen (N₂) was substituted for N₂O in the inspired gas. A 20-min period was allowed for the end-tidal concentrations of halothane and N₂O to decrease to less than 0.05% and 1.0%, respectively. Subsequently, control measurements were obtained and the midazolam infusion begun. Measurements of CBF, ICP, and MAP were obtained initially at 2–3 min intervals, and then at 5-min intervals, while temperature, hemoglobin concentration (Hb), and blood gases were obtained at 15-min intervals. In addition, cerebral hemodynamics were again recorded more frequently following the bolus administration of Ro 15-1788.

Based upon data from pilot studies, midazolam was administered as a continuous infusion of 0.66 mg·kg⁻¹·min⁻¹ for 60 min (total dose of 40 mg·kg⁻¹). During the midazolam infusion, MAP was maintained >60 mmHg and the pulmonary capillary wedge pressure (PCWP) >8 mmHg with iv lactated Ringer’s solution and/or a low-dose phenylephrine infusion. Sodium bicarbonate was administered to maintain the buffer base near 40 mEq·L⁻¹.

In five of the nine dogs, 65% N₂O/O₂ instead of 65% N₂/O₂ was inspired for a 15-min period beginning 30 min after the start of the midazolam infusion. End-tidal N₂O concentration was recorded during and following N₂O administration. These same five dogs received a 1.0 mg·kg⁻¹ bolus (over 5–10 s) of the benzodiazepine antagonist Ro 15-1788 following the midazolam infusion (after 60 min).

Following the study period, all dogs had the dura exposed and excised, and simultaneous bilateral cortical brain biopsies were taken by a technique that deposits a sample of brain (200–400 mg) into liquid nitrogen within 1 s. The tissue was prepared for analysis in a refrigerated box (−25 °C) as described by Folbergrova et al. Tissue extracts were analyzed by enzymatic fluorometric techniques for phosphocreatine, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), glucose, lactate, and pyruvate. The energy charge (EC) of the brain tissue was expressed as EC = ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]).

**SERUM MIDAZOLAM LEVELS**

Blood samples were obtained intermittently (5–10 min intervals) throughout the midazolam infusion (prior to Ro 15-1788 administration), for later correlation of serum midazolam level with CMRO₂ and EEG activity. Serum midazolam levels were determined at the sampling periods before and after the plateau in CMRO₂ for each individual animal (determined by visual observation of the CMRO₂ versus time curve for each animal). Serum midazolam levels were also determined at the end of the midazolam infusion. Heparinized whole blood samples were initially centrifuged, and the plasma component frozen at −20 °C until later analysis. See Appendix for method of analysis.

**MIDAZOLAM AND RO 15-1788 PREPARATIONS**

Midazolam and Ro 15-1788 were both obtained from Hoffman-La Roche Inc. (Nutley, New Jersey) in a pure crystalline form. Midazolam solution contained per deciliter: 250 mg midazolam, 250 mg NaCl, 204.4 mg 25% HCl, NaOH to obtain pH 3.5 ± 0.1, and H₂O. Ro 15-1788 solution contained per deciliter: 100 mg Ro 15-1788, 10 drops Tween 80, and normal saline.

**EEG Analysis**

In two dogs, the EEG signal was recorded on magnetic tape during the midazolam infusion and subsequent reversal with Ro 15-1788. These data were later analyzed by power spectrum analysis using 2-s epochs on a Neurotrak® (Interspec Inc., Conshohocken, PA) EEG monitor and displayed using a density-modulated (DSA) display technique.

**STATISTICAL ANALYSIS**

Cerebral and physiologic variables were analyzed with repeated measures analysis of variance. When indicated, pairwise comparisons between control and subsequent values, or 60-min and subsequent values, were made using paired t tests. Data for all nine dogs have been combined at sampling points prior to the administration of N₂O. Comparisons between dogs receiving and those not receiving N₂O were made using unpaired t tests. Unpaired t tests, with a Bonferroni correction for multiple comparisons, were used to compare cerebral metabolite concentrations obtained following treatment with midazolam, or midazolam and Ro 15-1788 with laboratory normal values obtained from six dogs under spinal anesthesia. Data are reported as mean ± SEM. A P value of <0.05 was considered statistically significant.

**RESULTS**

**MIDAZOLAM**

*Cardiovascular Effects.* Midazolam produced significant changes in systemic hemodynamics (table 1).
arterial pressure decreased significantly after 15 min of the midazolam infusion, and thereafter remained stable in the 80–90 mmHg range. During the infusion of midazolam, five dogs required infusions of lactated Ringer’s solution (50–300 ml) to maintain MAP >60 mmHg and/or PCWP >8 mmHg. Three dogs additionally required a low-dose infusion of phenylephrine to maintain MAP >60 mmHg. Heart rate (HR) increased significantly in response to midazolam infusion, yet remained stable throughout the infusion.

There were no significant changes in arterial blood gases noted except for a transient, statistically significant decrease in pH and buffer base, and an increase in $P_{\text{CO}_2}$ in response to midazolam. Temperature and Hb remained stable throughout the study.

**Cerebral Effects.** The infusion of midazolam produced dose-related EEG, CMRO$_2$, and CBF changes, all of which reached a plateau after the infusion of approximately 10 mg·kg$^{-1}$ midazolam. The control state EEG (low amplitude, alpha and beta activity, fig. 2A) changed by 15 min to high amplitude, predominantly theta activity (fig. 2B). By 30 min, the EEG had stabilized and consisted of mainly high amplitude, theta and delta activity (fig. 2C). At no time was burst suppression activity or an isoelectric EEG observed.

Infusion of midazolam resulted in a significant decrease in CMRO$_2$ over 10–15 min until a plateau level was reached (table 1, fig. 3). Thereafter, administration of additional midazolam had no further effect upon CMRO$_2$. CMRO$_2$ decreased significantly from control by 15 min, and stabilized at a mean value of $4.0 \pm 0.2$ ml·min$^{-1}$·100 g$^{-1}$ (mean ± SEM) by 30 min (75% of control). CBF similarly decreased significantly from control values to a plateau level within 10–15 min of the start of the midazolam infusion (fig. 4, table 1). After 30 min of the midazolam infusion, CBF was $49 \pm 3$ ml·min$^{-1}$·100 g$^{-1}$ (75% of control). Intracranial pressure was unaffected by the infusion of midazolam (table 1).

Mean serum midazolam levels measured prior to and following the plateau of CMRO$_2$ for each individual dog were $18.4 \pm 3.8$ and $31.2 \pm 3.3$ µg·ml$^{-1}$, respectively. Serum midazolam levels increased progressively throughout the midazolam infusion, reaching a mean value of $53 \pm 5.5$ µg·ml$^{-1}$ following 40 mg·kg$^{-1}$ of midazolam (fig. 5).

**NITROUS OXIDE**

Comparing the five dogs that received 65% $N_2O$ at 30–45 min of the midazolam infusion to those that did not receive $N_2O$, there were no significant differences in physiologic variables at 45 min except for a decrease in pH and an increase in $P_{\text{CO}_2}$ in those receiving $N_2O$ (table 1). The EEG displayed a slight decrease in both amplitude and delta wave activity in response to $N_2O$ (fig. 2D). These EEG changes were not apparent 2–3 min following the discontinuation of $N_2O$ in the inspired gas. Nitrous oxide had no statistically significant effect upon CMRO$_2$ (fig. 3). CBF increased significantly with $N_2O$ administration to $68 \pm 7$ ml·min$^{-1}$·100 g$^{-1}$ at 45 min (fig. 4). This was significantly greater than the CBF at 45 min in the animals not receiving $N_2O$; however, it was not statistically different from control CBF values. When $N_2O$ was discontinued (at 45 min), CBF decreased significantly, such that, by 60 min, there were no significant differences in CBF between any of the animals. End-tidal $N_2O$ concentration at 60 min was <3% in all animals.

**Ro 15-1788**

Following Ro 15-1788 administration, MAP increased transiently to near control levels and then decreased over 15 min to $73 \pm 21$ mmHg (table 1). All animals required fluids and/or phenylephrine to maintain MAP >60 mmHg following the bolus of Ro 15-1788. Ro 15-1788 was associated with a small, statistically significant decrease in buffer base. In all dogs, immediately following the bolus of Ro 15-1788, the EEG displayed a dramatic decrease in amplitude and an increase in frequency to primarily beta activity (fig. 2E). Over the next 15 min, the EEG activity gradually returned to resemble that seen prior to the administration
TABLE 1. Cerebral and Hemodynamic Values before, during, and after Midazolam Infusion and Subsequent Ro 15-1788 Administration (Mean ± SEM) (n = 4 for Nitrogen, n = 5 for Nitrous Oxide; Data Combined at Control, 5 Min, and 30 Min for Nitrogen and Nitrous Oxide Animals)

<table>
<thead>
<tr>
<th>Midazolam Dose</th>
<th>Control</th>
<th>15 Min</th>
<th>20 Min</th>
<th>40 Min</th>
<th>60 Min</th>
<th>Ro 15-1788 After 1 Min</th>
<th>Ro 15-1788 After 15 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N₂</td>
<td>N₂₈</td>
<td>N₂₈</td>
<td>N₂₈</td>
<td>N₂₈</td>
<td>N₂₈</td>
<td>N₂₈</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>113 ± 6</td>
<td>89 ± 4*</td>
<td>88 ± 3*</td>
<td>87 ± 7*</td>
<td>86 ± 8*</td>
<td>105 ± 12</td>
<td>73 ± 9*</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>82 ± 6</td>
<td>119 ± 13*</td>
<td>118 ± 14*</td>
<td>110 ± 10</td>
<td>112 ± 10*</td>
<td>140 ± 14*</td>
<td></td>
</tr>
<tr>
<td>Pao₂ (mmHg)</td>
<td>116 ± 5</td>
<td>164 ± 5</td>
<td>170 ± 6</td>
<td>174 ± 6</td>
<td>170 ± 9</td>
<td>170 ± 7</td>
<td></td>
</tr>
<tr>
<td>Paco₂ (mmHg)</td>
<td>39 ± 0</td>
<td>42 ± 1*</td>
<td>40 ± 0*</td>
<td>39 ± 1</td>
<td>39 ± 1</td>
<td>38 ± 0</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.32 ± 0.01</td>
<td>7.28 ± 0.01*</td>
<td>7.30 ± 0.01*</td>
<td>7.29 ± 0.01</td>
<td>7.30 ± 0.01</td>
<td>7.27 ± 0.01†</td>
<td>7.28 ± 0.01*</td>
</tr>
<tr>
<td>Buffer base (mEq·L⁻¹)</td>
<td>40 ± 0.4</td>
<td>40 ± 0.4</td>
<td>40 ± 0.4</td>
<td>40 ± 0.9</td>
<td>40 ± 0.6</td>
<td>37 ± 0.5*‡</td>
<td></td>
</tr>
<tr>
<td>CMRO₂ (ml·min⁻¹·100 g⁻¹)</td>
<td>5.3 ± 0.4</td>
<td>3.9 ± 0.2*</td>
<td>4.0 ± 0.2*</td>
<td>3.5 ± 0.3*</td>
<td>3.5 ± 0.4*</td>
<td>5.2 ± 0.4‡</td>
<td>5.1 ± 0.4‡</td>
</tr>
<tr>
<td>CBF (ml·min⁻¹·100 g⁻¹)</td>
<td>66 ± 4</td>
<td>55 ± 3*</td>
<td>49 ± 3*</td>
<td>43 ± 5*</td>
<td>42 ± 5*</td>
<td>103 ± 12†</td>
<td>55 ± 4*</td>
</tr>
<tr>
<td>ICP (mmHg)</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
<td>19 ± 4*†</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>

See text for abbreviations.
* Significantly different from control values, P < 0.05.
† Significantly different from values in animals without N₂O, P < 0.05.
‡ Significantly different from values at 60 min, P < 0.05.

of Ro 15-1788. CMRO₂ increased significantly from the 60-min value of 4.2 ± 0.3 ml·min⁻¹·100 g⁻¹ to 5.2 ± 0.4 ml·min⁻¹·100 g⁻¹ after 1 min, which did not differ significantly from control values (fig. 3). CMRO₂ remained stable at this level during the 15-min measurement period following the administration of Ro 15-1788. The CBF response to Ro 15-1788 was an abrupt and significant doubling of flow to 103 ± 17 ml·min⁻¹·100 g⁻¹, after 1 min (fig. 4). This value was not significantly different from control CBF. Fifteen minutes after Ro 15-1788 administration, CBF had de-
creased to near values recorded just prior to Ro 15-1788 administration. Ro 15-1788 administration caused a statistically significant doubling of mean ICP within 1 min. The increase in ICP was not sustained, and, after 15 min, ICP had decreased to near values measured prior to Ro 15-1788 administration.

Cerebral Metabolites

The cerebral metabolic state following the midazolam infusion and following the midazolam infusion plus Ro 15-1788 was essentially normal (table 2). The ATP and phosphocreatine concentrations and the energy charge—which is a quantitative estimate of the adenine nucleotide pool and more accurately reflects the amount of energy available to the cells—were normal or above normal. Cerebral lactate was significantly decreased in both midazolam and midazolam plus Ro 15-1788 treated dogs when compared to control values.

Discussion

We have shown that midazolam produced a dose-related decrease of cerebral metabolic, vascular, and electric activity until a stable plateau was reached after a total dose of 10 mg·kg⁻¹ of midazolam had been administered. The onset of a stable plateau for these variables has not previously been recognized. Our remaining results are consistent with the known cerebral effects of midazolam as previously reported by others. Nugent et al., using the same canine model as used in the current study, reported that CMRO₂ and CBF were decreased by 45% and 71%, respectively, following a total midazolam dose of 17.2 mg·kg⁻¹. Nugent et al. administered N₂O 70% in O₂ during control measurements. Nitrous oxide has been shown to significantly increase CMRO₂ and CBF in this model. The magnitude of changes in CMRO₂ and CBF reported by Nugent et al. probably are artificially increased due to the influence of N₂O. Hoffman et al.³ reported a 35% and 40–45% decrease in CMRO₂ and CBF, respectively, in rats breathing 70% N₂O, following 5.75 mg·kg⁻¹ of midazolam. Unlike the dog, N₂O does not increase CBF and CMRO₂ in the rat, such that the percentage changes they observed closely agree with our observations. Forster et al.²⁴ used a ¹³⁵Xe inhalation technique to measure CBF changes in human volunteers in response to 0.15 mg·kg⁻¹ of midazolam. They reported a 53% decrease in CBF and a 40% increase in CVR. Midazolam appears to decrease both CMRO₂ and CBF 25–40%, with no increased effect resulting from the administration of very large doses.

There was no evidence that midazolam or midazolam plus Ro 15-1788 had any untoward effect upon metabolic pathways. Cerebral ATP, phosphocreatine, and EC were all normal or greater than normal, and cerebral lactate levels were less than normal in both sampling groups. Our results following Ro 15-1788 are not in agreement with those found by Roald et al.,²⁵ who reported that Ro 15-1788 0.15 mg·kg⁻¹ following diazepam 3.0 mg·kg⁻¹ in the dog caused a significant decrease in ATP concentration and EC, and a significant increase in AMP and lactate levels. The reasons for these differences are not clear; however, it is quite clear that the much larger dose used in the current study was without untoward effects upon cerebral metabolite concentrations.

The results of this study do not support the suggestion made previously by Nugent et al.⁸ that midazolam

![Fig. 5. Serum midazolam levels throughout the midazolam infusion. Midazolam concentration increased progressively, reaching a mean value of 53 ± 5.5 µg·ml⁻¹ after 60 min.](image-url)

**Table 2. Brain Metabolite Concentrations Following Midazolam and Midazolam Plus Ro 15-1788 (Mean ± SEM)**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control n = 6</th>
<th>Midazolam n = 4</th>
<th>Ro 15-1788 n = 5</th>
<th>Energy Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µmol/g)</td>
<td>(µmol/g)</td>
<td>(µmol/g)</td>
<td></td>
</tr>
<tr>
<td>Adenosine Triphosphate</td>
<td>2.01 ± 0.01</td>
<td>1.88 ± 0.04*</td>
<td>2.11 ± 0.07</td>
<td>0.87 ± 0.0</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>2.99 ± 0.12</td>
<td>3.18 ± 0.2</td>
<td>3.37 ± 0.12*</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.23 ± 0.04</td>
<td>0.79 ± 0.04*</td>
<td>0.75 ± 0.06*</td>
<td>0.92 ± 0.008*</td>
</tr>
<tr>
<td>Energy Charge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Normal values obtained from six dogs under spinal anesthesia (15). *Significantly different from control values, P < 0.016.
given in doses larger than 17.2 mg·kg⁻¹ would decrease CMRO₂ to levels approaching those seen with barbiturates.³ Midazolam, even at the large doses administered in the current study, did not abolish cerebral electrical activity, and did not decrease CMRO₂ to levels even close to those seen with other anesthetic agents. The minimal CMRO₂ value of 3.5 ml·min⁻¹·100 g⁻¹ observed following 40 mg·kg⁻¹ of midazolam appears to differ significantly from values found with thiopental (2.22 ml·min⁻¹·100 g⁻¹),³ isoflurane (2.05 ml·min⁻¹·100 g⁻¹),⁴ and etomidate (2.03 ml·min⁻¹·100 g⁻¹),⁵ all of which abolish cerebral electrical activity (i.e., isoelectric EEG).

From the stable plateau of CMRO₂ and EEG activity reached after a total dose of approximately 10 mg·kg⁻¹ of midazolam and the increasing serum midazolam concentration, it is apparent that additional midazolam administered did not have any demonstrable effect. Possible explanations for this plateau of the midazolam dose-response curve include: 1) midazolam was not reaching the active site, or 2) following the administration of 10 mg·kg⁻¹ of midazolam, all of the benzodiazepine specific receptor sites were occupied. The cerebral metabolic and vascular changes that occurred early during the midazolam infusion suggest that midazolam was, in fact, reaching an active site. Saturation of benzodiazepine binding sites appears to be the most likely explanation for the plateau phenomena observed. Benzodiazepine specific receptors, first described in 1977,²⁶,²⁷ have been shown to be saturable.²⁷,²⁸ The 25% reduction in CMRO₂ and CBF observed in the current study appears to be the maximal depressant effect possible when benzodiazepine receptors become fully saturated.

Ro 15-1788 1.0 mg·kg⁻¹ was effective in reversing the effects of midazolam 40 mg·kg⁻¹ on CMRO₂, CBF, and the EEG. However, two causes for concern were noted. First, the bolus administration of Ro 15-1788 caused a significant increase of CBF and ICP from 60-min values. Secondly, Ro 15-1788 was associated with a decrease in MAP requiring fluid and vasopressor treatment. The effective use of Ro 15-1788 in patients following self-poisoning with large doses of benzodiazepines has been reported.²⁹ Lauben et al.³⁰ have reported the effects of Ro 15-1788 as a 10-μg bolus administered to volunteers with steady-state, hypoxic serum concentrations of midazolam (0.6 μg·ml⁻¹). All subjects were orientated to person, time, and place within 54-120 s. No untoward effects were noted. Recently, Chiolerio et al.³¹ reported the postoperative use of Ro 15-1788 in evaluating patients following craniotomy for tumor or aneurysm surgery. Anesthesia was maintained with a midazolam infusion (0.2 mg·kg⁻¹·h⁻¹), fentanyl (1.9 μg·kg⁻¹·h⁻¹) and 70% N₂O. Following 5.5 ± 1.3 h (SD) of surgery, 16/18 patients were extubated and talking, 10 min after receiving up to 1.0 mg Ro 15-1788 in incremental doses. Seven patients developed transient, greater than 20% increases in MAP after Ro 15-1788. Intracranial pressure response to Ro 15-1788 was not reported. In a separate report, Chiolerio et al.³² describe the ICP effects of Ro 15-1788 1.0 mg iv in patients with severe head injury who had been sedated with midazolam. They found significant increases in ICP at 5 and 10 min following Ro 15-1788 administration. In two patients, they observed severe increases in ICP (greater than 40 mmHg) which required immediate treatment. Our findings and those of Chiolerio et al.³² suggest the need for further evaluation of Ro 15-1788, especially with respect to those patients with decreased intracranial compliance, in whom increases in ICP cannot be tolerated. The changes we noted in MAP in response to Ro 15-1788 may be related to the relatively large doses of midazolam and Ro 15-1788 administered. A decrease in MAP in response to Ro 15-1788 has not previously been reported.

The lowest CMRO₂ values measured by Nugent et al.⁸ (2.9 ml·min⁻¹·100 g⁻¹) were less than the values measured in the current study (4.0 ml·min⁻¹·100 g⁻¹). We thought it possible that the administration of N₂O by Nugent et al. may, in combination with midazolam, have been contributing to the lower CMRO₂ values. Carlsson et al.⁹ have reported that diazepam, in doses up to 7.5 mg·kg⁻¹ in the rat, decreased CMRO₂ 40% only when combined with N₂O. However, in contrast to the findings of Carlsson et al., we found that N₂O had no effect upon CMRO₂, but significantly increased CBF. This is also in contrast to the work of Hoffman et al., who found that, in rats given 5.75 mg·kg⁻¹ of midazolam, N₂O significantly increased CMRO₂ when compared to animals not receiving N₂O. In their study, CBF was not affected by N₂O. There are no clear explanations at present for the differences in CMRO₂ and CBF seen with different benzodiazepines, with and without N₂O, across various species.

In conclusion, midazolam administered as a high-dose intravenous infusion was hemodynamically well tolerated, but did not decrease CMRO₂ and CBF to the same degree as other intravenous and volatile anesthetics. Ro 15-1788 administration was effective in reversing all of the measured cerebral effects of midazolam. However, decreases in MAP, and increases in CBF and ICP seen in response to the bolus injection of Ro 15-1788, could be deleterious in the clinical setting. Neither midazolam nor midazolam plus Ro 15-1788 had any untoward effects upon cerebral metabolite concentrations. Whereas, on a metabolic basis, midazolam itself does not appear to offer any unique anesthetic advantage,
the combination of midazolam and Ro 15-1788 may prove useful in clinical anesthesia, if untoward effects of Ro 15-1788 on MAP and ICP can be avoided.

References
3. Michenfelder JD: The interdependency of cerebral functional and metabolic effects following massive doses of thiopental in the dog. Anesthesiology 41:231-236, 1974

Appendix

Midazolam Analysis

Reagents. Midazolam maleate was obtained from Hoffman-La Roche (Nutley, New Jersey). The internal standard, 7-ido-5-fluoro-benzophenone was obtained from Merck, Sharp, and Dohme (Montreal, Quebec). Hepane and isoproil alcohol were obtained from Burdick and Jackson (Muskegon, Michigan). All other chemical reagents were ACS grade obtained from standard chemical suppliers.

Instrument. Analysis of extracted serum samples was performed using a Hewlett-Packard 5990 capillary gas chromatograph equipped with electron capture detector. The injector was operated in the split-mode (30:1 split). The column used was a 30-m long, 0.32-mm inside diameter capillary, coated with 1 μm of SE-54 covalently bonded to the glass surface.
Helium flow through the column was 1.2 ml·min⁻¹. At the time of sample injection, the column was maintained at 80° C. Following injection, the temperature was ramped at 30° C·min⁻¹ to 220° C, followed by a slow temperature program of 10° C·min⁻¹ to 280° C. Under these conditions, midazolam eluted at 5.504 min and the internal standard eluted at 5.721 min.

Procedure. Standards of midazolam were prepared at a concentration of 1, 10, and 40 µg·ml⁻¹ in dog serum. Prior to analysis, all specimens were stored at −20° C. All specimens were analyzed simultaneously (over a 2-day time period). The instrument was calibrated at 10 µg·ml⁻¹, and standards at 1 and 40 µg·ml⁻¹ were randomly interspersed throughout the run. One milliliter of serum was mixed with 0.2 ml of internal standard and 0.5 ml of 0.5 mol·l⁻¹ sodium hydroxide. Five milliliters of hexane/isopropyl alcohol (97 parts/three parts) were added to the sample and mixed vigorously for 1 min. Following centrifugation, the organic phase was transferred to a clean glass test tube and the solvent was evaporated under a stream of nitrogen at 35° C. The remaining residue was dissolved in 0.1 ml of methanol and transferred to a sealed vial in anticipation of gas chromatographic analysis. One microliter of the resolved residue was injected in the gas chromatograph, and the chromatograph was developed over an 8-min time period. Peak area of the midazolam and internal standard peak were calculated by standard integration techniques. The ratio of the midazolam peak to the internal standard peak was used to calculate concentration.

Assay Characteristics. Prior to analysis of specimens, the procedure described was validated as follows: 1) no apparent interference was observed from dog serum subjected to the extraction and chromatography procedures; 2) the limit of sensitivity of the procedure was 0.5 µg·ml⁻¹, and the procedure was demonstrated to be linear to 200 µg·ml⁻¹; and 3) extraction recovery of midazolam and the internal standard was similar, both ranging from 90–94% recovery. The internal standard compensates for any midazolam lost during the extraction process.