Ketamine Decreases Plasma Catecholamines and Improves Outcome from Incomplete Cerebral Ischemia in Rats

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Central neuroexcitatory receptors (N-methyl-D-aspartate (NMDA), non-NMDA) may affect outcome from cerebral ischemia by altering sympathetic nervous system activity. We tested whether ketamine, an NMDA antagonist, and NBQX, a non-NMDA antagonist, improve outcome from incomplete cerebral ischemia in the rat and whether a change in outcome is related to changes in plasma catecholamines. There were five treatment groups: group 1 (control, n = 10) received a fentanyl infusion at a rate of 25 µg · kg⁻¹ · h⁻¹ and ventilation with 70% N₂O in O₂. Group 2 (n = 10) received the same anesthetic treatment and were given an intraperitoneal injection of 30 mg/kg NBQX 15 min prior to ischemia. Group 3 (n = 10) received a ketamine infusion of 1.0 mg · kg⁻¹ · min⁻¹ and ventilation with room air. Group 4 (n = 10) received a ketamine infusion of 1.5 mg · kg⁻¹ · min⁻¹. Group 5 received a ketamine infusion of 1 mg · kg⁻¹ · min⁻¹ plus a 6 ml/kg intraperitoneal injection of 40% glucose solution 15 min before the start of ischemia. Ischemia was produced by right common carotid ligation combined with hemorrhagic hypotension to 35 mmHg for 50 min. Blood gases, pH, and skull temperature were controlled during ischemia. Plasma glucose increased during ischemia in all groups but was lower in ketamine-anaesthetized rats (groups 3 and 4). Glucose-loaded ketamine-anaesthetized rats (group 5) had plasma glucose concentrations similar to the control group. Plasma epinephrine and norepinephrine concentrations were significantly less in ketamine-anaesthetized rats (groups 3, 4, and 5) during ischemia compared to controls (P < 0.05). Neurologic outcome was significantly better (P < 0.05) in all ketamine-treated rats (groups 3, 4, and 5) compared to the control group, regardless of plasma glucose concentration during ischemia. NBQX did not improve neurologic outcome. These results suggest that ketamine improves neurologic outcome from incomplete cerebral ischemia by a mechanism related to a decrease in plasma catecholamine activity. (Key words: Anesthetics, intravenous; fentanyl; ketamine. Brain: blood flow; ischemia; receptors. Receptors: excitatory; N-methyl-D-aspartate. Sympathetic nervous system, catecholamines: epinephrine; norepinephrine.)

Two types of excitatory ionotropic receptors have been described in brain tissue: N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors. Antagonists for both of these receptors may improve outcome from incomplete or focal but not complete cerebral ischemia. Another mechanism of ischemic injury is the sympathetic nervous system. Treatments that inhibit release of catecholamines, such as ganglionic blockade and α₂-adrenergic agonists, decrease ischemic brain injury. Stimulation of the NMDA receptor produces norepinephrine release in the hippocampus. This suggests that there may be a connection between the NMDA receptor and catecholamine-related ischemic injury. Ketamine is a noncompetitive NMDA antagonist that may stimulate or inhibit sympathetic activity. However, little is known about the interaction of non-NMDA excitatory receptors with catecholamines. The purpose of this study was to investigate the effects of ketamine and NBQX, a non-NMDA antagonist, on plasma catecholamines concentrations, neurologic outcome, and histopathologic damage in a rat model of incomplete cerebral ischemia.

Materials and Methods

ICHEMIA

These experiments were carried out after approval from the institutional animal care committee. Male Sprague-Dawley rats (350–450 g) were anesthetized with isoflurane in a bell jar. After tracheal intubation, the lungs were ventilated with 2% isoflurane in O₂. Catheters were inserted into the left femoral vein, the right femoral artery, and the right jugular vein for pressure recording, drug infusion, and blood withdrawal. The right common carotid artery was isolated for later clamping. At the end of surgery the isoflurane was withdrawn and the rat allowed 30 min for equilibration according to one of the following randomly assigned treatments. Group 1 (control, n = 10) received a 10 µg/kg intravenous bolus of fentanyl followed by an infusion at a rate of 25 µg · kg⁻¹ · h⁻¹ and ventilation with 70% N₂O in O₂. Group 2 (n = 10) received the same anesthetic treatment as group 1. In addition, these rats were given an intraperitoneal injection of 30 mg/kg NBQX (Novo Nordisk) 15 min before the start of the study. A previous study has shown that these doses of NBQX, given before and after isch-
emia, improved outcome in gerbils.⁸ We were limited to one dose of 30 mg/kg NBQX in each rat because of a limited availability of the drug. Group 3 (n = 10) received a ketamine infusion of 1.0 mg · kg⁻¹ · min⁻¹ and ventilation with room air. Group 4 (n = 10) received a ketamine infusion of 1.5 mg · kg⁻¹ · min⁻¹ and ventilation with room air. Group 5 (n = 10) received a ketamine infusion of 1 mg · kg⁻¹ · min⁻¹ and ventilation with room air plus a 6-mL/kg intraperitoneal injection of 40% glucose solution 15 min before the start of ischemia.

Ischemia was produced by right common carotid clamping combined with hemorrhagic hypotension to 35 mmHg for 30 min. Arterial blood samples were taken under baseline conditions, during ischemia, and at the end of a 20-min recovery period for measurement of blood gas tensions, plasma glucose, and plasma catecholamine concentrations. Blood gas tensions and pH were measured using an Instrumentation Laboratories 1303 blood gas analyzer. Plasma glucose was measured using a Yellow Springs Glucose analyzer. Plasma catecholamines were measured by radioenzymatic assay.⁹ The sensitivity and coefficient of variation of the assay was 48 pg/ml and 7.5% respectively for norepinephrine and 36 pg/ml and 10% respectively for epinephrine. In order to evaluate baseline catecholamine concentrations, plasma catecholamines were measured in an additional five rats anesthetized with fentanyl/N₂O but not made ischemic. Skull temperature was measured over the ischemic hemisphere using a Yellow Springs thermistor needle and maintained at 37°C with an overhead heat lamp controlled by servomechanism. PaCO₂ was maintained between 35 and 40 mmHg. At the end of the equilibration period, cortical and subcortical CBF were measured using cobalt-57–labeled 15-μm microspheres according to previously reported methods.¹⁴ Cortical CMRO₂ was calculated as the product of cortical CBF and the difference of arterial and sagittal sinus O₂ content. Blood O₂ content was measured from an Instrumentation Laboratory co-oximeter plus the calculated O₂ dissolved in plasma.

ELECTROENCEPHALOGRAM

Electroencephalography was used to evaluate the effectiveness of DL-α-amino-3-hydroxy-5-methyl-isoxazolopionic acid (AMPA) receptor blockade with NBQX. Rats were anesthetized with 2% isoﬂurane in O₂, and catheters were inserted into the femoral artery and vein. The skull was exposed and a 2-mm hole drilled 1 mm posterior and 1 mm to the right of the bregma. The isoﬂurane was then withdrawn and the rat allowed 30 min for equilibration with an intravenous infusion of 25 μg · kg⁻¹ · h⁻¹ fentanyl combined with 70% N₂O in O₂. AMPA (Sigma Chemicals) was dissolved in sterile saline in a concentration of 1 mg/ml. NBQX was dissolved in sterile water in a concentration of 6 mg/ml. Intrastriatal injections of AMPA were given at a depth of 5 mm from the skull. To antagonize the effect of AMPA, intraperitoneal injections of NBQX (30 mg/kg) were given 20 min before intrastriatal AMPA injections.
STATISTICS

Data are reported as mean ± standard deviation. Non-parametric data including neurologic and histopathologic scores were compared using Kruskal Wallis tests and Spearman rank-order correlations. Parametric data were compared using analyses of variance and Tukey’s test for comparison between specific treatment conditions.

Results

Arterial blood pressure, $P_{aCO_2}$, $P_{aO_2}$, $pH$, plasma glucose concentrations, and blood withdrawal in the five treatment groups are shown in Table 1. NBQX (group 2) did not alter any parameter compared to fentanyl/N_2O-anesthetized controls (group 1). Ketamine (groups 3 and 4) decreased plasma glucose concentration and blood withdrawal requirements during ischemia compared to group 1 controls ($P < 0.05$). Glucose-loaded ketamine-anesthetized rats had increased plasma glucose concentrations before and after ischemia compared to group 1. $P_{aO_2}$ was less in ketamine-anesthetized rats (groups 3, 4, and 5) compared to fentanyl/N_2O- and NBQX-treated rats ($P < 0.05$).

During ischemia, epinephrine and norepinephrine were significantly lower in all ketamine-treated groups compared to fentanyl/N_2O controls (fig. 1). Plasma epinephrine and norepinephrine in five fentanyl/N_2O-anesthetized rats without ischemia were $0.40 ± 0.22$ ng/ml and $0.38 ± 0.14$ ng/ml (mean ± SD) respectively. NBQX did not alter the increase in catecholamines produced by hypotension and brain ischemia. Neurologic outcome after ischemia is shown in figure 2. Ketamine- and glucose-loaded ketamine-anesthetized rats had significantly better outcome during the 3-day evaluation period compared to fentanyl/N_2O-treated rats. NBQX (group 2) did not improve outcome compared to group 1 controls.

The histopathologic outcome scores in the two rats that did not die of stroke in group 1 were 8 and 5, indicating moderate neuronal injury ($0 =$ no injury, $9 =$ total hemisphere infarct). In group 2, the two rats evaluated for histopathology had scores of 9 and 4. Median histopathologic scores in ketamine-anesthetized rats were as follows: group 3 = 3 (n = 10); group 4 = 6 (n = 9); and group 5 = 4 (n = 8). No neuronal damage was seen in the non-ischemic hemisphere. Too few rats survived in group 1 to compare neuronal damage statistically to that in the

<table>
<thead>
<tr>
<th>Table 1. Mean Arterial Blood Pressure, $P_{aCO_2}$, $P_{aO_2}$, $pH$, Plasma Glucose, and Blood Withdrawal during Ischemia</th>
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<tbody>
<tr>
<td><strong>Treatment</strong></td>
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<tr>
<td><strong>Group 1, Fentanyl/N_2O (n = 10)</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Ischemia (15)</td>
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<tr>
<td>Ischemia (50)</td>
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<tr>
<td>Recovery</td>
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<td><strong>Group 2, Fentanyl/N_2O plus NBQX (30 mg·kg⁻¹) (n = 10)</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Ischemia (15)</td>
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<tr>
<td>Ischemia (50)</td>
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<tr>
<td>Recovery</td>
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<tr>
<td><strong>Group 3, Ketamine (1 mg·kg⁻¹·min⁻¹) (n = 10)</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Ischemia (15)</td>
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<td>Ischemia (50)</td>
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<tr>
<td>Recovery</td>
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<tr>
<td><strong>Group 4, Ketamine (1.5 mg·kg⁻¹·min⁻¹) (n = 10)</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Ischemia (15)</td>
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<tr>
<td>Ischemia (50)</td>
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<tr>
<td>Recovery</td>
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<tr>
<td><strong>Group 5, Ketamine (1 mg·kg⁻¹·min⁻¹) + glucose (n = 10)</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Ischemia (15)</td>
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<tr>
<td>Ischemia (50)</td>
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<tr>
<td>Recovery</td>
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Data reported as mean ± SD.
* $P < 0.05$ compared to baseline.
† $P < 0.05$ compared to control.
HOFFMAN ET AL.

These results demonstrate that ketamine decreases plasma catecholamines and improves outcome from incomplete cerebral ischemia compared to fentanyl/N₂O. This effect is not dose-related from 1 to 1.5 mg·kg⁻¹·min⁻¹ ketamine and is not dependent on a decrease in plasma glucose during ischemia. The improvement in neurologic outcome occurs despite a higher CMRO₂ in ketamine-treated rats before the start of ischemia. In contrast to ketamine, NBQX, a non-NMDA antagonist, did not affect plasma catecholamine concentrations or outcome from ischemia compared to control rats. These data show that ketamine is sympatholytic during hypotensive ischemia. This may be the primary mechanism mediating a better ischemic outcome with ketamine.

Ketamine stimulates the phencyclidine receptor in the brain, producing dissociative anesthesia. This is associated with noncompetitive inhibition of the neuroexcitatory NMDA receptor. Phencyclidine receptor agonists such as ketamine also inhibit uptake of [³H]-norepinephrine, increasing catecholamine extracellular activity. This may account for the transient increase in plasma norepinephrine seen in patients following ketamine injection and the sympathomimetic effects of ketamine that have been previously reported. In this study, ketamine decreased the sympathetic response to hypotensive ischemia. This is consistent with Pfeifer et al., who showed that ketamine (2.5–10-mg/kg bolus) decreased baseline preganglionic sympathetic nerve activity as well as the sympathetic response to asphyxia compared to N₂O ventilation in cats. It also agrees with Kolka et al., who found that ketamine (0.65 mg·kg⁻¹·min⁻¹) abolished the increase in plasma epinephrine and norepinephrine produced by cold exposure in unanesthetized monkeys. Ketamine may produce sympatholytic effects in association with anesthesia. This is consistent with reports that inhalation anesthetics inhibit the sympathetic response to hypotensive ischemia and improve ischemic outcome. However, this is not true for all anesthetics, since fentanyl/N₂O anesthesia produces marked catecholamine release during ischemia and results in a poor outcome. The key factor may be the ability of the anesthetic to blunt stress-related catecholamine release.


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Fig. 1. Plasma catecholamine concentrations (mean ± SD) as determined 30 min after onset of ischemia. Number of rats in each group = 10. Number under each bar indicates treatment group. Significance indicates difference from group 1 (*P < 0.05).

- Ketamine-treated rats. The correlation between neurologic and histopathologic outcome in all rats was r = 0.67 (P < 0.05).
- Arterial blood pressure, blood gas tensions, CBF, and CMRO₂ for fentanyl/N₂O- and ketamine-anesthetized rats without ischemia are shown in Table 2. Ketamine decreased mean arterial blood pressure and increased cortical CBF and CMRO₂ compared to fentanyl/N₂O controls.

- Intrastriatal injections of 25 nM AMPA produced prolonged seizure activity starting within 10 s of injection (n = 2, Fig. 3). Pretreatment with 30 mg/kg intraperitoneal NBQX 20 min before AMPA either prevented seizure activity (n = 2) or delayed the onset of spikes on the electroencephalogram for 6 min (n = 1).

Discussion

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The importance of central sympathetic activity in the cerebral protective effects of ketamine is uncertain. Stimulation of NMDA receptors during ischemia may induce catecholamine release during ischemia. This release would be inhibited by ketamine. However, we have not tested whether the decrease in plasma catecholamines seen here during ischemia with ketamine is associated with a decrease in central sympathetic activity. Previous studies

**Table 2. Mean Arterial Blood Pressure, Cerebral Blood Flow, and Cerebral Oxygen Consumption during Fentanyl/N₂O and Ketamine Anesthesia**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MABP (mmHg)</th>
<th>PCO₂ (mmHg)</th>
<th>PAO₂ (mmHg)</th>
<th>pH</th>
<th>Cortex CBF</th>
<th>Subcortex CBF</th>
<th>CMRO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl/N₂O</td>
<td>12</td>
<td>150 ± 12</td>
<td>36 ± 2</td>
<td>150 ± 24</td>
<td>7.38 ± 0.02</td>
<td>130 ± 44</td>
<td>109 ± 40</td>
<td>7.8 ± 1.4</td>
</tr>
<tr>
<td>Ketamine (1 mg·kg⁻¹·min⁻¹)</td>
<td>8</td>
<td>83 ± 7*</td>
<td>37 ± 3</td>
<td>85 ± 12*</td>
<td>7.37 ± 0.04</td>
<td>179 ± 30*</td>
<td>99 ± 20</td>
<td>10.2 ± 1.4*</td>
</tr>
</tbody>
</table>

Data = mean ± SD.

MABP = mean arterial blood pressure; CBF = cerebral blood flow (milliliters per 100 g per minute); CMRO₂ = cerebral cortical oxygen consumption (milliliters oxygen per 100 g per minute).

* P < 0.05 compared to Fentanyl/N₂O.
suggest that circulating catecholamine activity may be important during ischemia. Peripheral ganglionic blockade decreases plasma catecholamines during ischemia and improves ischemic outcome in N2O-ventilated rats. The improvement in outcome was reversed by intravenous norepinephrine and epinephrine infusion. This suggests a direct influence of blood catecholamines in ischemic injury. Circulating monoamines may cross the blood–brain barrier, which is compromised by ischemia, or may stimulate brain regions with a reduced blood–brain barrier, such as the area postrema. These possibilities remain to be tested.

Although NMDA antagonists improve outcome from incomplete or focal cerebral ischemia, they are not effective during complete ischemia. Previous studies suggest that glutamate, an excitatory receptor agonist that stimulates NMDA receptors, may play a major role in ischemic injury. However, in a rat model of forebrain ischemia, glutamate release from ischemic brain regions is not related to neuronal injury. This suggests that other factors besides glutamate release play a pivotal role in ischemic injury. Studies of anoxic injury of neuronal tissue indicate that ketamine reduces hypoxic injury by a mechanism not directly related to the NMDA receptor. Our results show that catecholamine release may be the critical factor. This is supported by the work of Jensen and Auer, who reported that in rats pretreated with the ganglionic blocker trimetaphan, ketamine (5 mg/kg) did not improve neuropathologic outcome from forebrain ischemia compared to 70% N2O–treated ischemic rats. This indicates that ketamine may improve ischemic outcome by inhibition of catecholamine release rather than by antagonism of the NMDA receptor.

In the current studies, cortical CMRO2 in fentanyl/N2O-anesthetized rats was 25% less than with ketamine (1 mg·kg⁻¹·min⁻¹). A study of regional cerebral glucose consumption in rats demonstrated that ketamine increased cerebral glucose consumption in the hippocampus but in no other brain regions compared to unanesthetized controls. Conversely, fentanyl anesthesia may decrease cortical CMRO2. If outcome were related to preischemic metabolic demand, ketamine should have produced a worse outcome. We conclude that the cerebral metabolic effects of ketamine before the onset of ischemia were not an important factor for improving outcome after ischemia. However, during ischemia in fentanyl/N2O-anesthetized rats, increases in CMRO2 may occur and exacerbate ischemic injury.

Several studies indicate that ketamine increases CBF with little or no change in CMRO2. It is unlikely that ketamine would increase CBF in ischemic tissue, in which cerebrovasodilation is already maximal. Ketamine-induced cerebrovasodilation may worsen ischemia by promoting reverse steal and by shunting blood flow from ischemic tissue. This suggests that the cerebrovascular effects of ketamine do not attenuate and could worsen ischemic injury compared to fentanyl/N2O.

During ischemia, increased plasma and brain tissue glucose worsen outcome. Glucose-related ischemic injury is presumed to be due to anaerobic metabolism, brain tissue lactic acid production, and increased tissue acidosis, although this has been questioned. In the cur-
rent studies, ketamine infusion decreased plasma glucose during incomplete ischemia. This was probably due to decreased catecholamine release with ketamine treatment. It is known that lower plasma glucose during ischemia improves outcome in this model of incomplete cerebral ischemia. However, this is not the primary mechanism of cerebral protection with ketamine, since neurologic outcome was improved in glucose-loaded ketamine-anesthetized rats. These results also suggest that increased glucose is not an important factor in ischemic injury when the plasma catecholamine concentrations are low.

Non-NMDA excitatory amino acid receptors that activate ion channels may be divided into two categories, kainate and AMPA receptors. Our results confirm that NBQX is a potent antagonist of AMPA with respect to seizure activity. Although kainate and AMPA stimulate separate receptors, electrophysiologic evidence indicates that both receptors are associated with the same ion channel and are competitive. NBQX shows a high affinity for central [3H]-AMPA binding sites, and it also antagonizes kainate-induced [3H]-GABA release from cultured cortical neurons. This suggests that responses observed with kainate are mediated by ion channels associated with AMPA receptors in the mammalian central nervous system. NBQX has been shown to improve outcome from ischemia in the gerbil. However, the dose used, 30 mg/kg given 15 and 5 min before and 10 min after ischemia, was more extensive than that used here. We were limited to one dose of NBQX per rat in this study by the availability of the drug. This may have been inadequate if NBQX is short-acting and the protective effect was dissipated sometime before the posts ischemic period. This is consistent with the suggestion that NBQX is effective in reducing posts ischemic cerebral injury. Our results indicate that NBQX does not interact with the sympathetic nervous system and does not attenuate intraschismic injury produced by incomplete ischemia in the rat.

It is necessary to consider the possibility that ketamine did not improve outcome from incomplete cerebral ischemia but that fentanyl/N2O, or more specifically N2O, worsened ischemic outcome. The increase in catecholamines may have occurred because of inadequate anesthesia, which was unrelated to ischemia or hypotension. Fentanyl/N2O anesthesia was chosen as the control anesthetic treatment because it provides adequate anesthesia with normal sympathetic responses and CBF regulation. Data here support the conclusion that fentanyl/N2O does not increase plasma catecholamines in nonschismic brain but allows an appropriate sympathetic response to hypotension and ischemia. It is also unlikely that N2O is the primary factor of injury since previous studies have shown that N2O added to 0.5 or 1 MAC isoflurane anesthesia does not significantly worsen ischemic outcome. These results and those of previous studies support the conclusion that incomplete ischemic injury is mediated by an increase in plasma catecholamine concentrations during ischemia and not to the baseline anesthetic state.

In summary, our results indicate that ketamine improves outcome from incomplete cerebral ischemia. This effect is not related to plasma glucose but is associated with lower plasma catecholamines in ketamine-treated rats during ischemia. This is consistent with previous studies that show that circulating catecholamine activity is related to outcome from incomplete cerebral ischemia in the rat.

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