Anesthesiology
65:272–277, 1986

Preservation of Cerebral Metabolites by Etomidate during Incomplete Cerebral Ischemia in Dogs

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Changes in cerebral high-energy phosphate stores and lactate concentration (as evidence for cerebral protection) were studied in dogs treated with etomidate during incomplete global ischemia, which was of a magnitude insufficient to abolish neuronal synaptic activity (as evidenced by electrical activity on EEG). In six dogs the effects of etomidate (5 mg · kg^{-1}) on the rates of adenosine triphosphate (ATP) and phosphocreatine (PCr) depletion and lactate accumulation during 9 min of oligemic hypotension to 31 mmHg were compared with six untreated dogs. In the dogs treated with etomidate the cerebral energy stores of ATP and PCr and the cerebral energy charge were maintained at higher levels than in the untreated dogs, and the cerebral lactate accumulation was significantly less. This effect of etomidate is similar to that of other anesthetics (thiopental and isoflurane) in this model. The authors conclude that in circumstances of ischemia that are insufficient to abolish neuronal synaptic activity, etomidate may improve tolerance of the brain to ischemia by decreasing cerebral metabolism through its suppression of neuronal synaptic activity. (Key words: Anesthetics, intravenous: etomidate. Blood pressure: hypotension. Brain: metabolism; protection. Hemorrhage. Shock.)

ATTEMPTS TO PRESERVE brain function by prolonging tolerance of the brain to ischemia are desirable when the possibility of hypoxia or ischemia can be anticipated, as in some patients undergoing thoracic, cardiac, or neurosurgery. Several anesthetics have been studied for a possible role in brain protection because of their ability to decrease cerebral metabolism, thereby decreasing oxygen demand at a time when oxygen supply is decreased. Presumably, the major mechanism by which anesthetics might provide protection is by decreasing neuronal synaptic activity (electrical activity on EEG) with a parallel decrease in cerebral oxygen requirements.[1] Any such protection would occur only in those circumstances in which neuronal synaptic activity has not already been abolished by ischemia. These include instances of focal or incomplete global ischemia.

Etomidate produces a dose-related decrease in cerebral oxygen metabolism (CMR_{O_2}) that appears to be secondary to a decrease in neuronal function.[2] This action is similar to that of other anesthetics, notably thiopental[3] and isoflurane.[4] As a crude measure of cerebral protection, etomidate has been shown to prolong survival in basic animal screening tests during hypoxia or ischemia.[5,6] Evidence of potential cerebral protection can be demonstrated by measurement of cerebral metabolism, cerebral histology, and electrophysiologic measurements,[7] with the ultimate evidence of protection being improvement in neurologic function. The purpose of the present study was to investigate the effect of etomidate on cerebral metabolism (measurement of cerebral energy stores and lactate accumulation) in a model of incomplete global ischemia produced by oligemic hypotension in dogs. In this model both thiopental[1] and isoflurane[8] have demonstrated some evidence of protection by decreasing the rate of energy depletion and lactate accumulation.

Methods

This protocol was approved by the Animal Care and Use Committee and the Research Committee of the Mayo Foundation and Mayo Clinic.

Six unmedicated, fasting mongrel dogs weighing 12.0–15.5 kg were studied before and during incomplete global ischemia according to a protocol described by Michenfelder and Theye.[1] Anesthesia was induced and maintained with 0.9% end-expired halothane, 70% nitrous oxide in oxygen for the surgical preparation. Succinylcholine (40 mg) was injected intravenously to facilitate endotracheal intubation and thereafter infused at a rate of 75 mg · h^{-1} to maintain muscle paralysis. Ventilation was controlled with a Harvard pump adjusted to maintain normocarbia. A peripheral intravenous catheter was placed for the administration of drugs and maintenance fluid, isotonic saline, infused at a rate of 75 ml · h^{-1}. A catheter was placed in the left femoral artery for pressure measurements and blood sampling. A large-bore cannula was placed in the right femoral artery. This was cross-clamped and connected to a 1-l reservoir containing 5,000 IU heparin. The top of the reservoir was attached to an anaeroid manometer, which permitted precise control of the pressure within the reservoir. Esophageal and parietal epidural thermistor probes were placed to monitor temperature, which was maintained at 37° C. Biparietal electrodes were cemented to the skull to monitor the EEG continuously. End-expired halothane concentrations were measured with an infrared analyzer (Beckman Medical Gas Analyzer LB-2®).

A biparietal frontal–parietal craniectomy was performed, and the dura was excised to expose the dorsal

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Received from the Department of Anesthesiology, Mayo Medical School and Mayo Clinic, Rochester, Minnesota. Accepted for publication May 11, 1986. Supported in part by Research Grant NS-7507 from the National Institutes of Health, Public Health Service.

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TABLE 1. Arterial Blood Values in Untreated and Etomidate Dogs before and after 9 Min of Hypotension

	Before Hypotension		End of Hypotension	
	Untreated	Etomidate	Untreated	Etomidate
P_{O_2} (mmHg)	147 ± 7	151 ± 3	132 ± 6	126 ± 10*
P_{CO_2} (mmHg)	39 ± 2	40 ± 1	32 ± 4	36 ± 2
*p*H	7.39 ± 0.02	7.38 ± 0.01	7.32 ± 0.02*	7.29 ± 0.03*
BB+ (mEq/l)	44 ± 1	44 ± 0.4	37 ± 1*	38 ± 1*
Hb (g/dl)	18 ± 1	17 ± 1	16 ± 0.4*	15 ± 1*
Lactate (μmol/ml)	2.1 ± 0.9	2.2 ± 0.4	5.0 ± 0.7*	4.8 ± 0.5*
L/P	12 ± 0.3	12 ± 1	36 ± 5*	35 ± 5*
Glucose (mg/dl)		141 ± 13		172 ± 29
MAP (mmHg)	165 ± 5	125 ± 6†	31 ± 2*	31 ± 1*
Heart rate (beats/min)	135 ± 4	150 ± 10	135 ± 15	187 ± 16*†
Temperature (°C)	37.6 ± 0.2	37.3 ± 0.2	37.9 ± 0.2*	37.5 ± 0.7

Mean ± SE for six dogs.
BB+ = buffer base; Hb = hemoglobin; L/P = lactate/pyruvate ratio; MAP = mean arterial pressure.

* Significantly different from control ($P < 0.05$).
† Significantly different from untreated group ($P < 0.05$).

aspect of the cerebral hemispheres in preparation for taking brain biopsies.

Following the surgical preparation, the inspired halothane concentration was decreased and time was allowed to eliminate the halothane until an end-expired halothane concentration of 0.1% was achieved and maintained with an extremely low concentration of inspired halothane, together with 70% nitrous oxide for analgesia. Skin and muscle edges of the craniectomy were infiltrated with 1–2 ml of 1% procaine.

Prior to ischemia, control blood samples were analyzed for arterial blood gases; hemoglobin concentration; and arterial glucose, lactate, and pyruvate concentrations.[9] Following the control measurements each dog was given a bolus dose of etomidate (5 $mg \cdot kg^{-1}$), which produced either burst suppression or electrical silence on the EEG.

Thereafter, in each dog, the femoral artery cannula was opened to the reservoir and the mean arterial pressure (MAP) was decreased within 30 s to 30–32 mmHg. Arterial blood pressure and EEG were recorded continuously throughout the hypotensive period. After the desired MAP had been achieved, serial biopsies were taken from the exposed cerebral cortex (alternating hemispheres) according to the method of Kramer *et al.*[10] at 0.5, 1.5, 3, 5, 7, and 9 min. Each sample was analyzed for adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), phosphocreatine (PCr), glucose lactate, and pyruvate.[9] The energy state of the tissues was expressed as the energy charge (EC) of the adenine nucleotide pool (EC = [ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP]).[11] The cerebral metabolites of the etomidate-treated animals obtained periodically throughout the hypotensive period were compared with the metabolites of an untreated group.[8] At each sample time significant differences (denoted by ∗) in metabolite concentration between the untreated and etomidate-treated group were determined by Student's *t* test for unpaired data. In addition, the *rates* of depletion of ATP or PCr, or lactate accumulation, were calculated for each group, and then significant differences in linear regression (denoted by †) for each metabolite were compared by Student's *t* test for unpaired data.

Results

In the control period, the only difference between the etomidate-treated and untreated groups was a lower MAP in the etomidate-treated group. Because these measurements were taken before the administration of etomidate (table 1), the reason for this is unknown.

During the 9-min period of oligemic hypotension, the MAP (calculated from measurements at 1-min intervals) in both the etomidate-treated and untreated groups was maintained at 31 mmHg. A mean of 41 ± 3 $ml \cdot kg^{-1}$ blood was withdrawn to achieve this hypotension. Heart rate increased significantly only in the etomidate group. Arterial blood values for the two groups before and at the end of hypotension are presented in table 1. Adequate arterial oxygenation was maintained during hypotension, although the P_{O_2} was decreased from the control normotensive levels in both groups. P_{CO_2} also decreased in both groups. A metabolic acidosis developed in both the untreated and etomidate groups during the 9 min of hypotension. This was indicated by a significant decrease in *p*H and buffer base (BB+), and a significant increase in arterial lactate concentration and lactate/pyruvate (L/P) ratio.

In the untreated dogs, the EEG remained active, although abnormal, throughout the period of hypotension. In these dogs, within 30–45 s of the onset of hypotension, the EEG changed from a pattern of low amplitude, high-frequency, to a pattern of higher-amplitude, slow waves with intermittent bursts of low-amplitude waves indicative

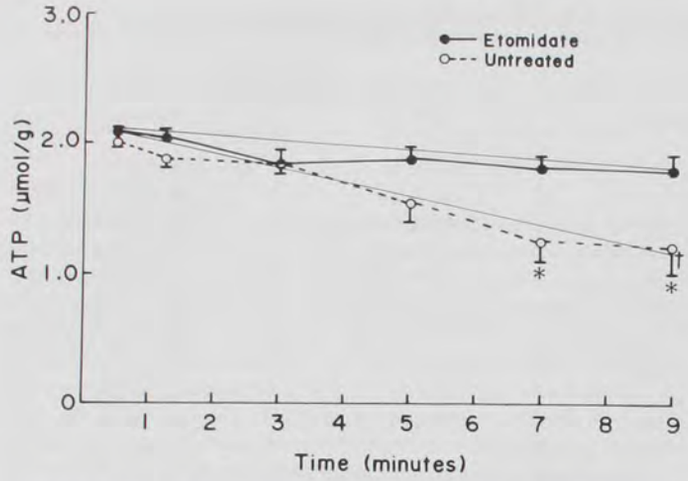

FIG. 1. Effect of etomidate (5 mg · kg^{-1}) on cerebral adenosine triphosphate (ATP) concentration during cerebral ischemia produced by oligemic hypotension in dogs. Mean (±SE) ATP concentration for six untreated[8] and six etomidate-treated dogs is shown for each time. * denotes significant difference ($P < 0.05$) at an individual time. The rate of depletion of ATP for each group is shown by the fine line. The rate of depletion of ATP was significantly less in the etomidate-treated group than in the untreated group (†) ($P < 0.05$).

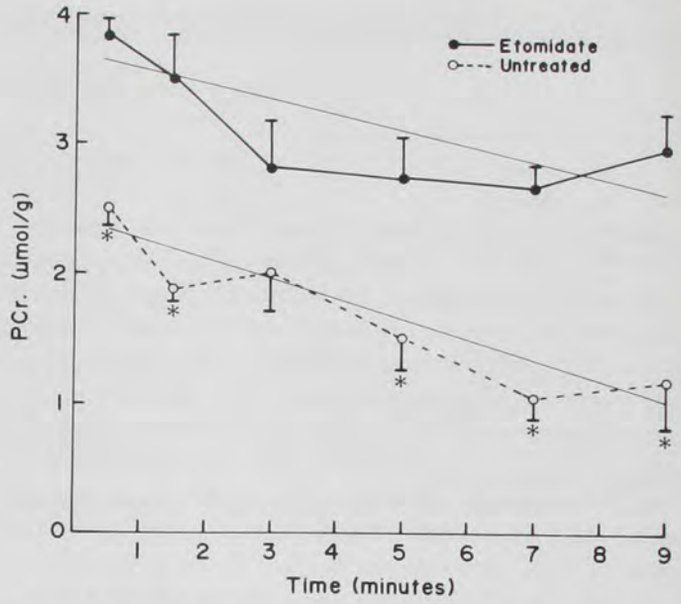

FIG. 3. Effect of etomidate (5 mg · kg^{-1}) on cerebral phosphocreatine (PCr) concentration during cerebral ischemia produced by oligemic hypotension in dogs. Mean (±SE) PCr concentration for six untreated[8] and six etomidate-treated dogs is shown for each time. * denotes significant difference ($P < 0.05$) at an individual time. The rate of depletion of PCr for each group is shown by the fine line. There was no difference in the rate by depletion of PCr between the two groups.

of ischemia. In the treated dogs, etomidate produced an EEG pattern of burst suppression or electrical silence prior to and throughout the period of ischemia.

Cerebral energy stores of ATP (fig. 1), the EC (fig. 2), and phosphocreatine (fig. 3) decreased in both groups during the period of hypotension, while the cerebral lactate concentrations increased (fig. 4).

In the untreated animals, the cerebral stores of ATP decreased progressively to 60% of normal (2.01 ± 0.01 μmol · g^{-1})[12] by 9 min (fig. 1). For the etomidate-treated

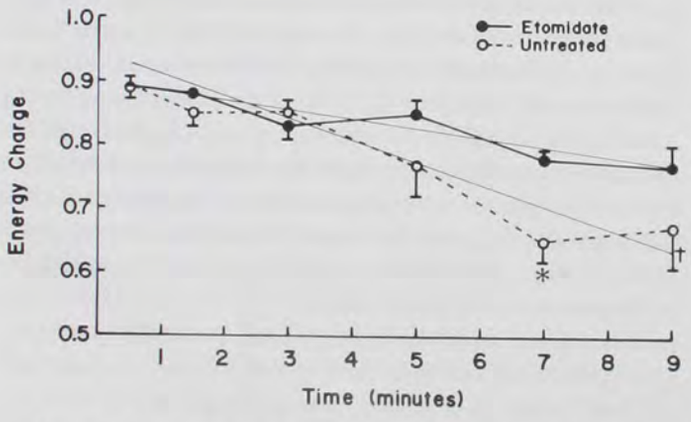

FIG. 2. Effect of etomidate (5 mg · kg^{-1}) on the calculated cerebral energy charge during cerebral ischemia produced by oligemic hypotension in dogs. Mean (±SE) for six untreated[8] and six etomidate-treated dogs is given at each time. * denotes significant differences ($P < 0.05$) at an individual time. The rate of decrease of the energy charge for each group is shown by the fine line. The rate of decrease of the energy charge was significantly less in the etomidate-treated group than in the untreated group (†) ($P < 0.05$).

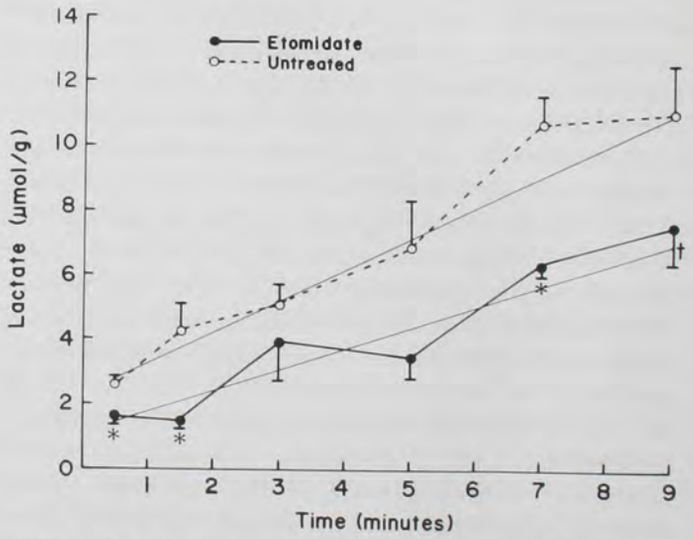

FIG. 4. Effect of etomidate (5 mg · kg^{-1}) on the cerebral lactate concentration during cerebral ischemia produced by oligemic hypotension in dogs. Mean (±SE) for six untreated[8] and six etomidate-treated dogs is shown for each time. * denotes significant difference ($P < 0.05$) at an individual time. The rate of accumulation of lactate for each group is shown by the fine line. The rate of lactate accumulation was significantly less in the etomidate-treated group than in the untreated group (†) ($P < 0.05$).

group, this *rate* of depletion was significantly less than for the untreated group, although individual comparison of mean ATP concentrations at each time point revealed significant differences only at 7 and 9 min. At 9 min the ATP concentration was maintained at 89% of normal in the etomidate-treated group.

The EC, which is an estimate of the total adenine nucleotide pool (ATP, ADP, and AMP), is calculated to evaluate any imbalance between the rate of production of ATP and the rate of utilization of ATP (increased AMP). At 9 min the EC in the untreated group had decreased to 76% of normal (0.87 ± 0.001),[12] while it was 89% of normal in the etomidate-treated group (fig. 2). Differences at individual times achieved significance only at 7 min. However, the *rate* of decrease of the EC was significantly less in the etomidate-treated group than in the untreated group.

While the *rate* of depletion of cerebral phosphocreatine concentration was similar in both the untreated and etomidate-treated groups, the phosphocreatine concentration was maintained at a significantly higher level in the etomidate-treated group throughout the period of hypotension (fig. 3). The phosphocreatine concentration in the etomidate-treated group was within normal limits ($2.99 \pm 0.12\ \mu g \cdot g^{-1}$)[12] or greater than normal throughout the hypotensive period.

In the untreated group the lactate concentration increased to approximately ten-fold the normal value of $1.23 \pm 0.04\ \mu g \cdot g^{-1}$ [12] by 9 min of hypotension (fig. 4). Although individual comparison of mean lactate concentration between the two groups at each time point reached significance only at 0.5, 1.5, and 7 min, the *rate* of accumulation of lactate was significantly less in the etomidate-treated group.

Discussion

The purpose of the present study was to determine if etomidate's ability to suppress neuronal synaptic activity, with a concomitant decrease in neuronal metabolism, might slow the rate of depletion of high energy stores and lactate accumulation and thereby provide some increased tolerance of the brain to incomplete global ischemia produced by oligemic hypotension. The EEG pattern of burst suppression or isoelectricity produced by the dose of $5\ mg \cdot kg^{-1}$ etomidate indicates near-maximal suppression of neuronal synaptic activity, so that CMR_{O_2} was presumed to be at or near the minimum that can be produced by etomidate (approximately a 50% decrease).[2]

Indeed, this dose of etomidate does slow the rate of depletion of cerebral concentration and slows the rate of accumulation of cerebral lactate concentration. With decreased metabolism, less ATP was required so that ATP concentration and the EC were maintained at higher levels in the etomidate-treated group than in the untreated group.

The effect of etomidate on the cerebral concentration of phosphocreatine was curious. While etomidate did not affect the *rate* of depletion of phosphocreatine, it produced a significant increase in the phosphocreatine concentration at 0.5 min ($3.88 \pm 0.7\ \mu mol \cdot g^{-1}$), such that the phosphocreatine concentration was maintained at or above the normal level throughout the hypotensive period. This phenomenon was also observed in a previous study in which the cerebral phosphocreatine level ($3.84 \pm 0.13\ \mu mol \cdot g^{-1}$) was significantly greater than normal following the administration of a dose of $21.4\ mg \cdot kg^{-1}$ etomidate.[2] Increases in cerebral phosphocreatine concentration can occur with general anesthesia, and this may explain the phosphocreatine levels observed in this study. However, increases in PCr of this magnitude have not been observed with other anesthetics.[8] Phosphocreatine concentration is also influenced by intracellular *p*H; an increase in intracellular H^+ shifts the creatine (Cr) kinase reaction in the direction of ATP formation ($PCr + ADP + H^+ \leftrightharpoons Cr + ATP$).[7] Therefore, an intracellular alkalosis may shift the reaction in the direction of increased PCr. While this explains the increased PCr concentration that occurs during reperfusion following ischemia, it does not explain increased PCr during ischemia.

The lower rate of lactate accumulation in the etomidate-treated group indicates that there was less cerebral anaerobic metabolism in that group, because systemic lactate levels were similar in both groups.

Changes in arterial blood gases during the hypotensive period deserve comment. Arterial oxygen and CO_2 tensions decreased during the hypotensive period in both the untreated and etomidate groups. Despite this decrease in P_{CO_2} a significant metabolic acidosis occurred in both groups. This is unlike the changes observed when isoflurane was administered prior to and during the period of oligemic hypotension.[8] In that circumstance arterial oxygen and carbon dioxide tensions decreased slightly (as in the present study), but there was no evidence of metabolic acidosis. During oligemic hypotension, profound systemic vasoconstriction will occur to limit peripheral perfusion in order to maintain cardiac output to vital organs. This can cause a decreased arterial P_{CO_2} and the metabolic acidosis. Etomidate is a potent cerebrovasoconstrictor that constricts systemic vessels as well. This may have contributed to the metabolic acidosis seen. Conversely, isoflurane, being a potent vasodilator, might offset some of the vasoconstrictive response to oligemic hypotension and thereby maintain adequate peripheral perfusion.

These observations of the effect of etomidate on cerebral metabolism during incomplete global ischemia in-

dicate that etomidate may be capable of providing some degree of cerebral protection during incomplete ischemia. Proposed mechanisms for cerebral protection by etomidate include: anticonvulsant activity (the prevention of hyperactivity following hypoxia or ischemia would minimize anaerobic metabolism and reduce lactic acidosis); immobilization, which would decrease oxygen demand; reduction in intracranial pressure, which might prevent a decrease in cerebral perfusion; and a decrease in CMR_{O_2}, reducing the rate of high-energy phosphate consumption. Etomidate has been shown to be a potent anticonvulsant against seizures induced electrically or chemically by 80 $mg \cdot kg^{-1}$ pentylenetetrazol in mice.[13] As an anesthetic it produces immobilization. As a potent vasoconstrictor, it reduces cerebral blood volume to reduce intracranial pressure in dogs with normal intracranial pressure (ICP)[2] and may casue a beneficial redistribution of cerebral blood flow (CBF). Its effect on reducing intracranial hypertension has not yet been demonstrated. The first three proposed mechanisms for cerebral protection by etomidate have been eliminated from the present study. No seizure activity was seen on EEG in either the untreated or etomidate-treated animals. Therefore, etomidate's anticonvulsant activity was not contributory. Immobilization was provided by a continuous infusion of succinylcholine so that immobilization produced by etomidate was unnecessary. Finally, in this model, cerebral vasoconstriction would more likely further decrease CBF than provide a beneficial redistribution of CBF. Therefore in this model we assume that the major mechanism of cerebral protection by etomidate is that of a decrease in CMR_{O_2}. General anesthetics affect neuronal synaptic activity to induce anesthesia by changing presynaptic membrane conductance. Any decrease in neuronal synaptic activity should be accompanied by a decrease in metabolism necessary for that activity.

In basic screening tests there is some evidence that etomidate provides some measure of protection during hypoxia or ischemia. Etomidate has been shown to prolong survival in mice exposed to hypobaric hypoxia in a decompression chamber simulating 35,000 ft.[5] Rats exposed to hypoxic hypoxia (100% nitrogen for 1 min) become agitated, convulse, then become inert and die. Etomidate has been shown to protect these rats from convulsions and death.[5] Ischemic changes in histopathology occur in the third and sixth layers of the parietal cortex in the untreated animals. Treatment with etomidate prior to the nitrogen exposure prevents these histopathologic changes.[14] Rats exposed to histotoxic hypoxia *via* an injection of 5 $mg \cdot kg^{-1}$ potassium cyanide died. These rats had extensive histopathologic damage in the CA_1 region of the hippocampus. Rats pretreated with etomidate had no morphologic changes in the hippocampus[15] and survived.[5]

In a canine model of oligemic ischemia, dogs treated with etomidate were compared with dogs treated with thiopental or pentobarbital.[6] Etomidate-treated dogs had a higher survival rate than dogs given barbiturates. While lack of cardiodepressant side effects in the etomidate-treated dogs may have contributed to the outcome, the authors postulated that an improved and sustained CBF at the cardiorespiratory centers was the protective effect demonstrated by these experiments. However, it could also be postulated that the decrease in the cerebral oxygen demand produced by etomidate's suppression of neuronal synaptic activity, as evidenced by an isoelectric EEG during the time of decreased oxygen supply, contributed to the prolonged survival.

We conclude that the primary mechanism for preservation of cerebral metabolites and, therefore, greater tolerance to incomplete ischemia demonstrated in our model is a reduction in cerebral metabolism secondary to a suppression of neuronal synaptic activity produced by etomidate. In this model of incomplete ischemia the oxygen and substrate delivery is insufficient to meet the cerebral metabolic demands for either normal neuronal synaptic activity (evidenced by alteration in EEG) or for the maintenance of cellular integrity (evidenced by high-energy store depletion and lactate accumulation). In the etomidate-treated group the decreased oxygen supply during the period of hypotension was less deleterious because oxygen demand was decreased by etomidate secondary to its suppression of neuronal synaptic activity. Etomidate preferentially suppresses the neocortex[16] and has a GABA-mimetic action on brain stem reticular formation.[17] Other contributing mechanisms of etomidate for increased tolerance to incomplete ischemia include redistribution of CBF,[6] decrease in intracranial pressure,[2] and attenuating free fatty acid liberation.[18]

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