

Electroencephalographic Burst Suppression Is Not Required to Elicit Maximal Neuroprotection from Pentobarbital in a Rat Model of Focal Cerebral Ischemia

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Background: Barbiturates have previously been demonstrated to reduce focal cerebral ischemic brain damage. However, the dose of drug required to elicit maximal neuroprotection has not been defined. The authors hypothesized that doses of pentobarbital substantially lower than those required to cause electroencephalographic burst suppression would result in maximal magnitudes of reduction of cerebral infarct volume.

Methods: Wistar rats underwent 90 min of filament occlusion of the middle cerebral artery while either awake (control), or anesthetized with intravenous sodium pentobarbital administered to preserve an active electroencephalogram ($15\text{--}23\text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) or a pattern of burst suppression ($45\text{--}60\text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$; $n = 17$). During ischemia and for the first 6 h of recirculation, brain temperature was rigorously controlled at $38.0 \pm 0.2^\circ\text{C}$. Rats were allowed a recovery interval of 7 days after which neurologic function and cerebral infarct volume were assessed. In nonischemic rats undergoing a similar anesthetic protocol, the cerebral metabolic rate of glucose utilization was measured at each anesthetic depth.

Results: Relevant physiologic values were similar between groups. Total infarct volume (mean \pm SD) was smaller in the active electroencephalogram group than in the control group ($124 \pm 68\text{ mm}^3$ versus $163 \pm 66\text{ mm}^3$; $P < 0.05$). Increasing the dose of pentobarbital (burst suppression) did not further decrease infarct volume ($128 \pm 54\text{ mm}^3$). Neurologic score and infarct volume were positively correlated ($P < 0.001$). Cerebral metabolic rate of glucose utilization was reduced by 56% in the burst suppression group versus 43% in the active electroencephalogram pentobarbital group ($P < 0.001$).

Conclusions: Sodium pentobarbital administered at either dose (active electroencephalogram or burst suppression) resulted in an $\approx 25\%$ reduction of cerebral infarct size, indicating that burst suppression is not required to elicit maximal neuroprotective efficacy. (Key words: Anesthetics, barbiturate: pentobarbital. Animals: rat. Brain: ischemia; middle cerebral artery. Metabolism: cerebral metabolic rate.)

BARBITURATES are known to reduce cerebral metabolic rate (CMR) in a dose-dependent manner. This occurs in parallel with progressive suppression of electrocortical activity. Maximal reduction in CMR is achieved simultaneously with onset of an electroencephalographic (EEG) pattern of burst suppression/isoelectricity (electrocortical quiescence).¹ It is therefore held that reduction in CMR associated with administration of this class of drugs is directly attributable to effects on synaptic neurotransmission.

Early work demonstrated that barbiturates also can increase tolerance of brain to an ischemic insult. A critical investigation demonstrating potential limitations of this therapy was performed by Michenfelder and Theye.² In their experiment, cortical high energy phosphate concentrations were better preserved in dogs treated with high-dose thiopental only when the severity of the insult caused some depression of electrocortical activity but not EEG isoelectricity. These data allowed the theoretical prediction that beneficial effects of barbiturates on ischemic outcome would be observed only under conditions of focal ischemia

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(where penumbral EEG activity persisted) whereas barbiturates would have no benefit in conditions of severe global ischemia where electrocortical activity was abolished. This prediction has been supported by subsequent investigations in both animals and humans.³⁻⁶

Another prediction made on the basis of these data is that if barbiturates provide protection by reducing CMR, then maximal protection would be expected from a dose of barbiturate that would be sufficient to produce electrocortical quiescence. Smaller doses would not be expected to provide optimal benefit. Despite the finding that barbiturates often are clinically administered to the point of EEG burst suppression, direct tests of a dose-response relationship for barbiturate efficacy are scant.^{7,8}

More recently, a variety of other anesthetic agents has become available that share properties similar to barbiturates with respect to both EEG and CMR.⁹⁻¹² Despite this, those compounds have largely failed to provide protection in a magnitude greater than that provided by other anesthetics that do not produce a large effect on CMR.^{13,14} A prime example is isoflurane, which has been shown to offer no advantage over other anesthetics including halothane or a combination of nitrous oxide/fentanyl.¹⁴⁻¹⁷ It therefore is conceivable that barbiturates possess other mechanisms of action that are critical to their protective effect. If so, it is also possible that the peak effect of such mechanisms does not parallel reduction in CMR (*i.e.*, maximal benefit might be achieved with a substantially lower dose of drug). The following experiment was designed to examine if a lower dose of pentobarbital is as efficacious in providing neuroprotection as is a dose sufficient to cause EEG burst suppression in a rodent recovery model of focal cerebral ischemia.

Methods and Materials

This study was approved by the Duke University Animal Care and Use committee. Male Wistar rats (age 8–10 weeks; body weight 250–300 g) were anesthetized with 40 mg/kg intraperitoneal sodium pentobarbital (Nembutal; 50 mg/ml, Abbott Laboratories, North Chicago, IL). Each animal was positioned in a stereotactic head frame with specialized ear bars designed to prevent damage to the tympanic membranes. Using aseptic technique, a midline scalp incision was made after local infiltration of the skin with 1% lidocaine. A burr hole was drilled over the left hemisphere at bregma = 0

mm. A radiotelemetered thermistor (Brain Probe, model XM-FH, Mini Mitter, Sunriver, OR) was advanced into the cerebral cortex to a depth of ≈ 2 mm. The probe was fixed in place with cyanoacrylate glue and the wound was closed with sutures. The animals were returned to their cages after recovery of the righting reflex.

Before completing this procedure the temperature probe was calibrated in a circulating water bath against a mercury thermometer standard within the ranges of 35.0°C–40.0°C. This allowed extrapolation of temperatures from calibration points in accordance with the radio frequency emitted by the thermistor. Analog signals from the radio thermistor were received (Telemetry Receiver Model RA1010, Data Science, St. Paul, MN), converted to digital signals, and processed through a computer (4DX-33V, Gateway 2000, North Sioux City, SD).

Three to 5 days after the above procedure, rats were fasted from food but allowed free access to water for 12–16 h. Each rat was then anesthetized with 2–3% halothane in 40% O₂/balance nitrogen. After tracheal intubation, the lungs were mechanically ventilated to maintain normocapnia. The halothane concentration was reduced to 1.0–1.5%. Via surgical incision, the tail artery was catheterized to monitor mean arterial blood pressure and sample blood. The animals were then prepared for middle cerebral artery occlusion using modifications of techniques described by Zea Longa *et al.* and Memezawa *et al.*^{18,19} A midline cervical skin incision was made and the right common carotid artery was identified. The external carotid artery was isolated and the occipital, superior thyroid, and external maxillary arteries were ligated and divided. The internal carotid artery was then dissected distally until the origin of the pterygopalatine artery was seen.

After surgical preparation, animals were randomly assigned to one of three groups:

- Control (n = 17): Halothane was continued until onset of ischemia and then abruptly discontinued.
- Active EEG (n = 17): Halothane was discontinued. Sodium pentobarbital was infused intravenously to cause mild suppression of the EEG. This typically required an infusion rate of 15–23 mg · kg⁻¹ · h⁻¹. Pentobarbital infusion was continued throughout ischemia and for the first 30 min after onset of reperfusion;
- Burst suppression (n = 17): Halothane was discontinued. Pentobarbital was infused intravenously to

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cause a pattern of EEG burst suppression. This typically required an infusion rate of $45\text{--}60\text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. Pentobarbital infusion was discontinued at termination of ischemia.

After surgical preparation, an interval of 30 min was allowed for physiologic stabilization and conversion of the anesthetic from halothane to pentobarbital in the latter two groups. Adjustments in the infusion rate of pentobarbital were allowed during the ischemic interval to maintain EEG activity as described for the respective groups. Pilot studies had identified that continuation of pentobarbital infusion for 30 min after onset of recirculation in the active EEG group would result in similar intervals until recovery of the righting reflex after ischemia in the two pentobarbital treatment groups.

After arterial blood gas/pH, plasma glucose, hematocrit, and mean arterial blood pressure determinations, all rats underwent 90 min of middle cerebral artery occlusion. This was achieved by introduction of a 0.25-mm diameter nylon filament into the external carotid artery stump. The filament was passed ≈ 23 mm distally through the internal carotid artery until a slight resistance was felt.

In the control group, the wound was loosely closed with sutures and halothane was discontinued. The endotracheal tube was removed on resumption of adequate spontaneous ventilation. The interval between introduction of the filament and recovery of righting reflex was typically 5–8 min. Rats in this group were placed in a 10-l Plexiglas box containing 40% O_2 /60% N_2 . Rats in the two pentobarbital treatment groups remained supine and the lungs were mechanically ventilated with a delivered gas mixture of 40% O_2 /60% N_2 .

During ischemia and the first 6 h of recirculation, cortical temperature was regulated as follows. Brain temperature was monitored from the implanted thermistor. If the value transmitted from the thermistor was less than 38.0°C , a heat lamp was automatically turned on. If brain temperature was higher than 38.0°C , chilled room air was blown over the surface of the animal by automated control of the gas source.

Mean arterial pressure was measured continuously throughout the ischemic interval in all groups. Arterial blood gases/pH levels were determined 45 min after onset of middle cerebral artery occlusion, at termination of ischemia, and for the two pentobarbital groups hourly during the 6-h recovery period.

After 90 min of middle cerebral artery occlusion, the filament was removed. Rats in the control group were reanesthetized with halothane 5 min before the conclusion of the ischemic interval. These spontaneously breathing rats continued to receive halothane *via* a snout cone during closure of surgical wounds. At onset of recirculation, halothane was discontinued and rats in the control group were allowed to awaken. In the pentobarbital-anesthetized rats, the tracheas were extubated when the rats resumed spontaneous ventilation, which typically occurred within 5 or 6 h after onset of reperfusion. During this 5–6-h interval, brain temperature remained servoregulated at 38.0°C . Rats in the control group were returned to the Plexiglas box for the first 6 h after reperfusion allowing continued servoregulation of cortical temperature. All animals were then returned to cages with unrestricted access to food and water.

A neurologic evaluation was made of all animals 7 days after the ischemic insult. Each rat was assigned a score of 0–3 where 0 = no observable deficit, 1 = forelimb flexion, 2 = decreased resistance to lateral push without circling, and 3 = same behavior as 2, with circling.²⁰ Neurologic tests were performed by a single observer blinded to the experimental condition of the animal.

After neurologic evaluation, animals were weighed and then anesthetized with 4% halothane in oxygen. They were then decapitated. The brains were removed and frozen at -20°C in 2-methylbutane. Using a cryotome, quadruplicate 20- μm thick coronal sections were taken at 660- μm intervals over the rostral-caudal extent of the infarct. The sections were dried and stained with hematoxylin and eosin.

Infarct volume was measured by digitally sampling stained sections with a Sony CCD Model XC-77 video camera (Japan) controlled by an image analyzer (M2 Turnkey System, Imaging Research, St. Catharines, Ontario). The image of each section was stored as an x by y matrix of pixel units. For each tissue section, the pixel units were calibrated to give values as mm^2 . The digitized image was then displayed on a video monitor. With the observer masked to experimental conditions, infarct borders in both cortex and subcortex were individually outlined (corpus callosum excluded) using an operator-controlled cursor. The area of infarct (mm^2) was determined automatically by counting pixels contained within the outlined regions of interest. Infarct volumes (mm^3) were computed as running sums of infarct area multiplied by the known interval (*e.g.*,

660 μm) between sections over the extent of the infarct expressed as an orthogonal projection.

A second experiment was performed to identify the effects of the respective doses of pentobarbital on the cerebral metabolic rate of glucose utilization (CMRglu). Rats were assigned to the same anesthetic groups as described earlier (*i.e.*, control, active EEG, or burst suppression; $n = 4$ or 5 per group). After induction of halothane anesthesia, bilateral femoral arterial catheters were surgically placed for continuous measurement of mean arterial blood pressure and intermittent measurement of arterial blood partial pressure of oxygen, partial pressure of carbon dioxide, pH, and plasma radioactivity and glucose concentrations. Bilateral femoral venous catheters were placed for infusion of isotope and pentobarbital or saline. In the control group, wounds were closed with sutures and the animal was placed in a plaster cast extending from the hindlimbs to the lower abdomen. These spontaneously breathing rats were allowed to awaken and placed in the prone position in a cylindrical chamber covered with an opaque cloth to minimize visual stimuli. Control animals received an intravenous infusion of 0.9% NaCl in water at a rate sufficient to provide an equal volume of fluid as that administered in the burst suppression group ($\approx 5 \mu\text{l}/\text{min}$). Rats in the active EEG and burst suppression groups were administered sodium pentobarbital according to the regimen outlined earlier for the outcome experiment. The tracheas in these animals were intubated and the lungs were mechanically ventilated (30% $\text{O}_2/70\%$ N_2) to maintain normocapnia. Halothane was discontinued at onset of barbiturate administration.

Forty-five minutes were allowed to establish anesthetic conditions. Rats were then given $30 \mu\text{Ci}/\text{kg}$ intravenous ^{14}C -deoxyglucose (American Radiolabeled Chemicals, St. Louis, MO) over 45 s. Arterial blood samples were collected at 0.5, 1, 2, 3, 5, 7, 10, 15, 30, and 45 min after onset of infusion of isotope. Animals were then decapitated and the brains removed. The hind brain was dissected free and discarded. The remainder was cut into six pieces similar in size, which were placed in preweighed scintillation vials and weighed. Three ml tissue solubilizer (TS-2, Research Products International, Mount Prospect, IL) was added to the vial and placed in a 50°C oven for 24 h. The solution was then neutralized with $90 \mu\text{l}$ glacial acetic acid. Ten milliliters of scintillation cocktail (4a20, Research Products International, Mount Prospect, IL) was added to each vial. Contents of the vials were allowed

to stabilize at room temperature for 24 h. Beta activity was then determined by liquid scintillation counting (Wallac 1409, Wallac, Gaithersburg, MD). Arterial blood samples were immediately centrifuged. Fifty microliters from each arterial sample was added to 1 ml of tissue solubilizer and placed in a 50°C oven for 30 min. Samples were then neutralized with $30 \mu\text{l}$ glacial acetic acid and then suspended in 4a20 scintillation cocktail. After 24 h at 50°C , and then 24 h at room temperature, β activity was determined by liquid scintillation counting. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Glucose Analyzer 2, Beckman Instruments, Fullerton, CA). Calculation of CMRglu was made using the operational equation of Sokoloff *et al.* with a lumped constant value of 0.483.²¹

In addition to the above three experimental groups, another set of animals was investigated contemporaneously. These rats were examined in anticipation of a future focal ischemia outcome study involving pentobarbital. Four pilot animals were anesthetized with halothane and had catheters surgically placed in the jugular vein. The catheter was then exteriorized on the nape of the neck. Halothane was discontinued and the animals were allowed to recover for ≈ 2 h. Each rat was then placed in a methyl methacrylate polymer box and an intravenous infusion of pentobarbital was begun. In the first three animals, it was learned that a loading dose of $8 \text{ mg}/\text{kg}$ followed by a continuous infusion of $14 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ resulted in an obviously sedated animal. This dose of drug, however, allowed the animal to ambulate when stimulated. The righting reflex was preserved. This behavioral end point was confirmed in the fourth rat that received the same dose of drug. Using halothane anesthesia, three rats were surgically prepared as described for the control group in the outcome experiment. After completion of surgery, halothane was discontinued. Thirty minutes later, an $8 \text{ mg}/\text{kg}$ intravenous bolus of pentobarbital followed by a continuous infusion of $14 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (sedated group) was given. Forty-five minutes later, these rats underwent determination of CMRglu according to the protocol described earlier.

An *a priori* power analysis was performed to define sample size in the outcome experiment. With β fixed at 0.80, and predicted variance estimated from a previous study using similar methods,²² it was calculated that 17 rats in each of the experimental groups would be required to identify a 50% reduction in cerebral infarct volume. This magnitude of infarct reduction was

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chosen based on the expected magnitude of effect obtained from a variety of other pharmacologic agents currently under investigation for neuroprotective properties.

Parametric values are expressed as mean \pm standard deviation. Physiologic values were compared between groups qualitatively to preserve statistical power for analysis of major dependent variables. Cortical and subcortical infarct volumes were also compared qualitatively. Total infarct volumes were compared between the control and active EEG groups by one-sided Student's *t* test. All statistical assumptions were satisfied and there were no outliers. Positive correlation between neurologic score and infarct size was tested using Kendall's τ . This nonparametric test of association was used because the neurologic scoring system had only four categories. A *P* value was calculated for Kendall's τ using the appropriate standard normal statistic.

Cerebral metabolic rate of glucose utilization was compared between the active EEG and burst suppression groups by a one-sided Student's *t* test. Because CMRglu characteristically follows a log-normal distribution, it was logarithm transformed for statistical analysis.

Results

Physiologic values including arterial blood gases/pH level, hematocrit, and plasma glucose were similar among groups with the exception of mean arterial blood pressure in the control group immediately before onset of ischemia (table 1). This relative hypotension is attributable to halothane anesthesia. By 10 min after onset of ischemia this difference in mean arterial blood pressure was absent. The thermoregulation technique was successful in maintaining both awake and anesthetized brain temperature at $38.0 \pm 0.2^\circ\text{C}$ throughout the 90 min ischemic interval and during the first 6 h of reperfusion.

Total dose of pentobarbital was greater in the burst suppression group (89 ± 13 mg/kg) than in the active EEG group (55 ± 11 mg/kg). Control rats received no pentobarbital. Electroencephalographic activity exhibited a modest increase in δ waves in the active EEG group. In contrast, the EEG recorded from rats receiving the largest dose of pentobarbital exhibited burst suppression with a typical interburst interval of 3–10 s (fig. 1).

Cortical and subcortical infarct volumes were similar between pentobarbital-treated groups and less than

volumes observed in the control group (fig. 2). Total infarct volumes were smaller in the active EEG group than in the control group (124 ± 68 mm³ vs. 163 ± 66 mm³; *P* < 0.05). Increasing the dose of pentobarbital did not further decrease the infarct volume (128 ± 54 mm³). Neurologic score and total infarct volume were positively correlated ($\tau = 0.337$, *P* < 0.001). See figure 3.

Cerebral metabolic rate of glucose utilization was less in the burst suppression group (27 ± 3 $\mu\text{M}/100\text{g}/\text{min}$) than in the active EEG group (35 ± 4 $\mu\text{M}/100\text{g}/\text{min}$; *P* = 0.0028). Control animals had even greater CMRglu (62 ± 6 $\mu\text{M}/100\text{g}/\text{min}$). Cerebral metabolic rate of glucose utilization was observed to be 47 ± 6 $\mu\text{M}/100\text{g}/\text{min}$ in the sedated group.

Discussion

Our motivation for performing this study can be attributed to a fundamental observation. Anesthetics that cause reduction of CMR in a magnitude similar to that caused by barbiturates do not provide a neuroprotective advantage over other anesthetics that have a more modest effect on CMR. This suggests that the neuroprotective effect of barbiturates might be attributable to a different mechanism of action than is customarily considered for this class of drugs. If the principal mechanism of action of barbiturates is owing to reduction of CMR, then a dose-dependent decrease in infarct size should be evident with maximal benefit obtained at EEG quiescence. This consideration led us to examine extant evidence for a dose-response relationship for barbiturates and focal ischemic injury.

We identified two studies that examine a dose-response relationship between barbiturate therapy and outcome from focal cerebral ischemia. In the first, baboons were subjected to permanent MCA occlusion while anesthetized with either 1.2% halothane, or three different doses of pentobarbital (60, 90, or 120 mg/kg).⁷ The EEG was recorded and showed persistent isoelectricity when the pentobarbital dose exceeded 50 mg/kg. Thus, the EEG was quiescent in all three pentobarbital groups. Doses less than those required for EEG quiescence were not examined. In the second study, dogs were subjected to permanent MCA occlusion.⁸ One hour after onset of ischemia, animals were assigned to receive varying doses of pentobarbital (10–80 mg/kg). The control group was nonconcurrent, which consisted of halothane-anesthetized animals used in a different study. A dose-dependent reduction in in-

Table 1. Physiologic Values

	Control (n = 17)	Active EEG (n = 17)	Burst Suppression (n = 17)
5 min preischemia			
MABP (mmHg)	85 ± 13	116 ± 10	121 ± 9
pH _a	7.39 ± 0.06	7.39 ± 0.05	7.42 ± 0.03
Pa _{CO₂} (mmHg)	39 ± 3	39 ± 3	38 ± 3
Pa _{O₂} (mmHg)	150 ± 17	153 ± 15	146 ± 16
Glucose (mg/dl)	128 ± 14	133 ± 17	138 ± 17
Hematocrit (%)	38 ± 2	38 ± 2	39 ± 3
45 min after onset of ischemia			
MABP (mmHg)	122 ± 8	118 ± 17	113 ± 8
pH _a	7.43 ± 0.02	7.41 ± 0.04	7.42 ± 0.05
Pa _{CO₂} (mmHg)	36 ± 3	38 ± 4	39 ± 3
Pa _{O₂} (mmHg)	114 ± 29	128 ± 32	124 ± 20
Cortical temperature (°C)			
T ₀	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1
T ₃₀	37.9 ± 0.2	38.0 ± 0.1	38.0 ± 0.1
T ₆₀	38.0 ± 0.2	38.0 ± 0.1	38.0 ± 0.1
R ₆₀	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1
R ₁₈₀	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1
R ₃₆₀	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1

Values are mean ± SD.

T₀, T₃₀, and T₆₀ = 0, 30, and 60 min after onset of ischemia, respectively; R₆₀, R₁₈₀, and R₃₆₀ = 60, 180, and 360 min after reperfusion of the middle cerebral artery respectively.

fart size was observed with maximal effect at 20 mg/kg. However, temperature was not monitored or controlled at any site in the body and no attempt was made to provide mechanical ventilation in any of the groups. Further, EEG was not monitored. Therefore, it is impossible to discern what, if any, relationship was present between the neuroprotective effect of the drug and the effect on the EEG.

Although many studies have identified a neuroprotective effect for barbiturates in focal cerebral ischemia,

to our knowledge the aforementioned studies are the only ones that have attempted to define a dose-response relationship. Despite this, the common clinical acceptance is that maximal effect is obtained at doses producing a quiescent EEG.²³ Our results are not consistent with this practice and indicate that smaller doses of the drug may be as efficacious as those required to substantially suppress electrocortical activity. Clearly, at some point, a dose-response effect for barbiturates should be demonstrable. In the filament MCA occlusion

A)



B)

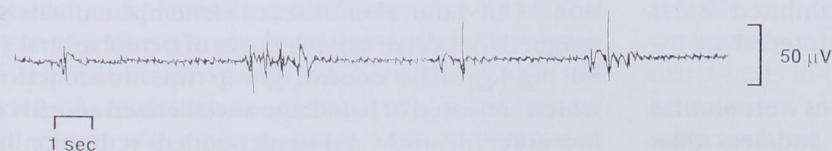


Fig. 1. Typical electroencephalographic recordings taken at the midpoint of the 90-min interval of middle cerebral artery occlusion from rats representing the active electroencephalogram (A) or burst suppression (B) groups. The electroencephalogram was not recorded in rats maintained awake during the ischemic interval.

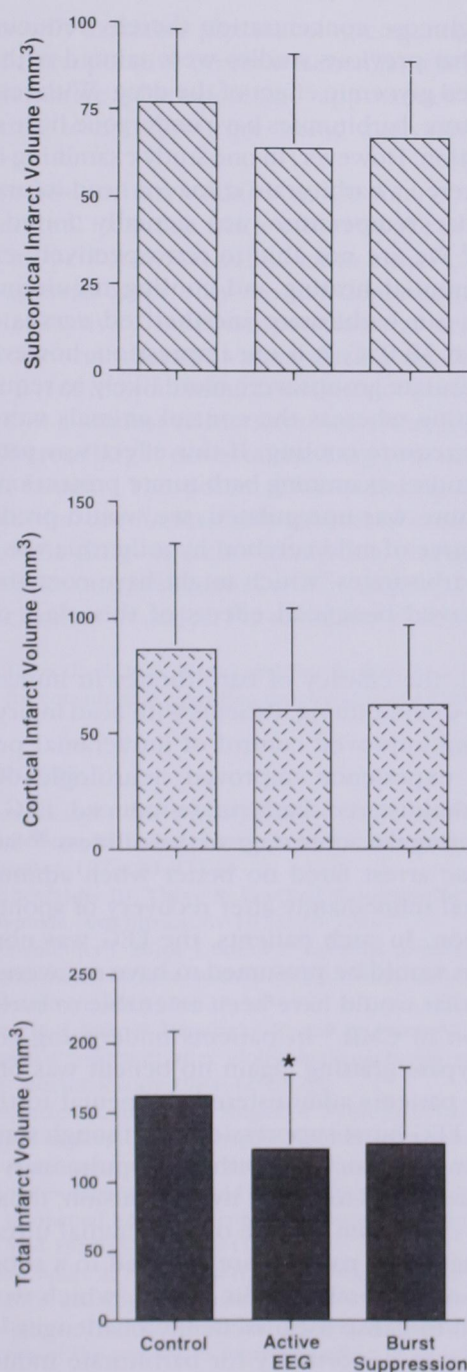


Fig. 2. Mean \pm SD infarct volumes (*top* = cortical; *middle* = subcortical; *bottom* = total) in rats maintained awake (control) or anesthetized with pentobarbital to produce a mildly active electroencephalogram or electroencephalographic burst suppression during 90 min of temporary middle cerebral artery occlusion. Low-dose pentobarbital resulted in a 25% reduction in total infarct volume compared to control rats ($P < 0.05$). A larger dose of pentobarbital provided no additional benefit. *Difference between active electroencephalogram and control groups ($P < 0.05$).

model such a relationship would be expected at doses less than those administered in the active EEG group.

In fact, there are numerous other examples where pharmacologic neuroprotection has been identified for compounds possessing anesthetic properties when administered in doses that provide little or no sedation. These drugs are those principally involved in antagonizing glutamatergic neurotransmission and include MK-801,^{24,25} CGS 19755,^{26,27} NBQX,^{28,29} and ACEA-1021.^{30,31} For the majority of these compounds, CMR has been assessed for similar doses of drug and little depression of energy requirement has been observed.^{26,32,33} Barbiturates have been identified to exhibit some degree of glutamatergic antagonism.³⁴ Further investigation will be required, however, before it can be concluded that glutamatergic antagonism constitutes an important mechanism in the protection provided by barbiturates.

Despite the similarities in ischemia outcomes for the active EEG and burst suppression groups, the CMRglu study demonstrates the difficulty in directly examining the CMR hypothesis of barbiturate neuroprotection (*i.e.*, that the magnitude of infarct size is linearly related to the magnitude of CMR reduction afforded by the drug). Although CMRglu values for the active EEG and burst suppression EEG groups were significantly different, the magnitude of CMRglu difference between the two groups was not large. Burst suppression doses

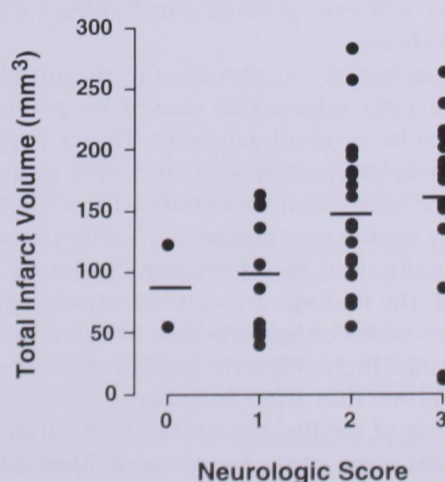


Fig. 3. Individual neurologic scores (0 = no deficit) assessed 7 days after a 90-min interval of focal cerebral ischemia plotted against total cerebral infarct volume histologically defined immediately thereafter. Neurologic scores and total infarct volume were positively correlated ($P < 0.001$). Horizontal lines depict mean total infarct values at respective neurologic scores.

of pentobarbital reduced CMRglu by 56% from the awake control values. This is consistent with other reports in the literature.^{1,35} Because EEG burst suppression has been shown to be coincident with maximal CMR reduction,¹ we can assume maximal effect was achieved in the burst suppression group. The active EEG group exhibited a 43% reduction in CMR, which amounts to 77% of the maximal reduction potentially caused by pentobarbital. Thus, if the relationship between infarct size and magnitude of CMR reduction is linear, the infarct size of the active EEG group would be expected to be 23% larger than the burst suppression group. We calculate the power to detect that difference in infarct volume to be only 11% given the variability of infarct volumes present in the experimental groups and a sample size of 17. The CMRglu values in the sedated group represented a 25% reduction from control values. This approximates the midpoint between control and burst suppression CMRglu values (*i.e.*, 44% of the potential pentobarbital-mediated depression of CMR) despite that dose causing only a sedative effect on the rat. A subsequent experiment could compare animals anesthetized with the sedated group and burst suppression group pentobarbital doses and exploit that differential in CMRglu. However, we calculate that to obtain sufficient statistical power to reject the CMR hypothesis of barbiturate neuroprotection, several hundred rats would have to be studied. It therefore seems unlikely that the stroke model used in this experimental will ever provide a truly direct test of the CMR hypothesis.

It was noteworthy to observe the magnitude of reduction of total infarct size caused by pentobarbital ($\approx 25\%$) to be as small as it was. This is particularly true when held in contrast to the 40% reduction of infarct size recently demonstrated for 1% halothane under very similar circumstances.²² Although these respective reductions in infarct size cannot be directly compared, the findings are counterintuitive given the long series of investigations that have found efficacy for barbiturate therapy but the paucity of *in vivo* studies showing protection from halothane. However, close examination of studies examining barbiturate protection present some cause for concern. Most laboratory studies defined barbiturate protection before it was made clear that factors including minor changes in brain temperature and the glycemic state of the animal are crucial in defining outcome.^{36,37} Typically, therefore, these parameters were neither monitored nor controlled. We observed no effect of barbiturates on

plasma glucose concentration thereby reducing likelihood that previous studies were tainted with an uncontrolled glycemic effect of the drug. With respect to temperature, barbiturates have undergone little specific examination. However, in one study examining humans administered a barbiturate coma for head injury, intraventricular temperature was actually found to increase.³⁸ We are not able to retrospectively examine the differential heating and cooling requirements to maintain our barbiturate anesthetized *versus* control animals at 38.0°C. It is our impression, however, that the barbiturate groups were more likely to require surface heating whereas the control animals were more likely to require cooling. If this effect was present in earlier studies examining barbiturate protection where temperature was unregulated, we would predict that some degree of mild cerebral hypothermia was caused by the barbiturates, which might have contributed to the observed beneficial effects of this class of compounds.

Indeed, the efficacy of barbiturates in human trials has been disappointing. Patients with head injury, while exhibiting improved control of intracranial pressure, did not experience improved neurologic outcome when administered barbiturate-induced EEG quiescence during the acute stage of their illness.³⁹ Survivors of cardiac arrest fared no better when administered thiopental immediately after recovery of spontaneous circulation. In such patients, the EEG was not monitored but would be presumed to have recovered some activity that would have been amenable to barbiturate reduction in CMR.⁶ In patients undergoing coronary artery bypass grafting, again no benefit was observed in those patients administered thiopental to the endpoint of EEG burst suppression.⁴⁰ Although some neurologic injury associated with cardiopulmonary bypass can be attributed to global hypoperfusion, it has since become clear from the use of transcranial ultrasonography that those patients are exposed to a substantial number of cerebral embolic events, which would be expected to cause focal ischemic challenges,⁴¹ again providing an opportunity for barbiturate induced reduction in CMR. Nussmeier *et al.* did observe a beneficial effect of thiopental on neurologic outcome from valvular heart surgery.⁵ This is perhaps our best evidence that barbiturates can reduce ischemic brain damage under specific operative conditions,⁴² but currently stands alone with respect to human data in supporting the use of barbiturates as neuroprotective agents. While barbiturates remain perhaps the best of

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the currently available pharmacologic agents for treatment of intraoperative cerebral ischemia, it is fortunate that alternative pharmacologic approaches to brain protection/resuscitation are being aggressively pursued.

In conclusion, rats underwent 90 min of transient focal cerebral ischemia while either awake or anesthetized with pentobarbital sufficient to either cause EEG burst suppression or only modest depression of electrocortical activity. Brain temperature was strictly controlled at normothermic values during the insult and the first 6 h of recirculation. Low doses of pentobarbital resulted in a modest reduction of cerebral infarct volume, whereas larger doses of the drug provided no additional benefit. The potent CMR-reducing effects of pentobarbital are inextricably related to even sedative doses of the drug. It seems likely that methods other than a stroke outcome model will be required to define the mechanism of action for this class of compounds.

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