Neuroanesthesia and Intensive Care

Thiopentone and methohexital, but not pentobarbitone, reduce early focal cerebral ischemic injury in rats

[Le thiopental et le méthohexital, mais non le pentobarbital, réduisent la lésion ischémique cérébrale focale précoce chez les rats]

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Purpose: Although barbiturates are considered to be cerebral protectants, little is known regarding the relative efficacy of different barbiturates to reduce ischemic brain injury. In a model of middle cerebral artery occlusion (MCAo), we compared the relative effects of 1.0 and 0.4 burst-suppression doses of thiopentone, methohexital, and pentobarbitone on cerebral infarct.

Methods: During isoflurane anesthesia, MCAo was achieved via a temporal craniotomy. Thirty minutes before MCAo the rats were randomized to receive one of the following which was maintained throughout the study. Halothane ($n=20$)-1.2 MAC halothane, thiopentone ($n=20$), methohexital ($n=20$), or pentobarbitone ($n=20$). The first ten animals in each barbiturate group received the respective barbiturate in a dose sufficient to maintain burst-suppression of the electroencephalogram (3–5 bursts·min^{-1}). The subsequent ten animals in each barbiturate group received 40% of the burst-suppression dose. After 180 min of MCAo and 120 min of reperfusion, cerebral injury was assessed.

Results: For the burst-suppression animals, injury volume (mm^3, mean $\pm$ SD) was less in the thiopentone group (88 $\pm$ 14) than the halothane (133 $\pm$ 17), methohexital (126 $\pm$ 19), or pentobarbitone (130 $\pm$ 17) groups (P <0.05). For 0.4 burst-suppression animals, injury volume was less for the methohexital group (70 $\pm$ 22) than the halothane (124 $\pm$ 24), thiopentone (118 $\pm$ 15), or pentobarbitone (121 $\pm$ 20) groups (P <0.05).

Conclusions: These data are inconsistent with the longstanding assumption that electrophysiologically comparable doses of the various classes of barbiturates have equivalent protective efficacy. They in turn suggest that mechanisms other than, or at least in addition to, metabolic suppression may contribute to the protective effect of barbiturates.

Objectif : *Bien que les barbituriques soient considérés comme des protecteurs cérébraux, on en sait peu sur leur efficacité relative à réduire la lésion cérébrale ischémique. Nous avons comparé, chez un modèle d'occlusion de l'artère cérébrale moyenne (OACM), les effets relatifs de doses de thiopental, méthohexital et pentobarbital, capables de suppression totale et à 40 % (1,0 et 0,4) des bouffées du tracé électroencéphalographique (EEG), sur l'infarctus cérébral.*

Méthode : *Pendant l'anesthésie à l'isoflurane, l'OACM a été provoquée au moyen d'une craniotomie temporale. Trente minutes avant l'occlusion, les rats ont été randomisés et ont reçu un des médicaments suivants, thérapie maintenue tout au long de l'étude : 1,2 CAM d'halothane (n = 20), du thiopental (n = 20), du méthohexital (n = 20) ou du pentobarbital (n = 20). Les dix premiers animaux de chaque groupe barbiturique ont reçu une dose du médicament suffisante pour maintenir la suppression des bouffées du tracé EEG (3–5·min^{-1}). Les dix animaux suivants de chaque groupe barbiturique ont reçu 40 % de la dose causant la suppression des bouffées. On a évalué la lésion cérébrale après 180 min d'OACM suivie de 120 min de reperfusion.*

Résultats : *Pour les animaux chez qui il y a eu suppression des bouffées du tracé EEG, le volume lésionnel (mm3, moyenne $\pm$ écart type) a été plus bas avec le thiopental (88 $\pm$ 14) qu'avec l'halothane (133*

± 17), le méthohexital (126 ± 19) ou le pentobarbital (130 ± 17) (P < 0,05). Pour une suppression à 40 %, le volume lésionnel a été moindre avec le méthohexital (70 ± 22) qu'avec l'halothane (124 ± 24), le thiopental (118 ± 15) ou le pentobarbital (121 ± 20) (P < 0,05).

Conclusion : Ces données contredisent l'hypothèse de longue date voulant que des doses comparables, au plan électrophysiologique, de diverses classes de barbituriques présentent une efficacité protectrice équivalente. Elles évoquent, par ailleurs, que des mécanismes de suppression métabolique différents, ou tout au moins additionnels, peuvent contribuer à l'effet protecteur des barbituriques.

S PECIFIC substitutions on the pyrimidine ring of barbituric acid have resulted in several clinically applicable barbiturates with distinct pharmacophysiologic profiles. There is abundant experimental evidence that barbiturates can decrease the extent of neurologic injury caused by an episode of temporary focal cerebral ischemia,[1–4] and seminal experiments by Michenfelder and colleagues provided data that led many to conclude that this protection was mediated primarily by reduction of cerebral metabolic rate (CMR).[4,5] More recently, however, this belief has been challenged because of the observation that a comparable reduction of CMR produced by other anesthetic agents (e.g., isoflurane, etomidate) does not have an equivalent protective effect.[3,6] Furthermore, a recent investigation by Warner *et al.*[7] demonstrated that pentobarbitone administered in the two dose regimens used in this investigation, i.e., electroencephalographic (EEG) burst-suppression and 40% burst-suppression doses, resulted in indistinguishable degrees of neuronal injury after middle cerebral artery occlusion (MCAo) in spite of significant differences in CMR.

A second widely held assumption is that, if the same metabolic end-point is established (as suggested by a quiescent EEG), there is protective equivalence among the barbiturates. This assumption seemed reasonable when the accepted dogma was that CMR suppression was the basis for barbiturate-induced cerebral protection. However, if non-CMR mechanisms contribute to barbiturate-induced cerebral protection,[7] it is not necessarily reasonable to assume that all barbiturates are equivalent with respect to these other, undefined protective properties.

In view of this potential for a therapeutic difference in the efficacy of various barbiturates in ameliorating cerebral ischemic injury, we performed a comparison of the effect of thiopentone, methohexital, and pentobarbitone on the extent of early ischemic injury following temporary MCAo in rats. Pentobarbitone is a widely used oxybarbiturate, while thiopentone is its thioanalog, and methohexital is an oxybarbiturate

with excitatory properties. Each barbiturate was given in two dose regimens to provide insight into dose-response relationships for the three barbiturates.

Methods

The protocol was approved by the Animal Investigation Committee of Loma Linda University in accordance with the standards for the care of laboratory animals of the National Institutes of Health (publication no. 96–208, 1996). Male, spontaneously hypertensive rats ($n=80$, 375–425 g, 16–20 weeks) were anesthetized with isoflurane and orotracheally intubated. Mechanical ventilation was maintained (Harvard Co., Boston, MA, USA) with isoflurane (1.44%, end-tidal) in an oxygen: air mixture (fractional inspired oxygen 0.4). The femoral vessels were cannulated for blood pressure monitoring, blood sampling, and fluid administration. Mean arterial blood pressure (MABP) was recorded using a Micro-Med blood pressure analyzer (Micro-Med, Inc., Louisville, KY, USA). The device continuously records blood pressure and averaged MABP over 15-min intervals, beginning with the moment of MCAo. Mean arterial blood pressure was supported at 120 mmHg by *iv* infusion of phenylephrine, as required.

Maintenance fluids consisted of 0.9% NaCl at 4 mL·kg^{-1}·hr^{-1}. Temperature was measured under the temporalis muscle (Mon-a-Therm temperature sensor; Mallinckrodt Anesthesia Products, St. Louis, MO, USA) and servo-controlled at 37°C by a heating blanket. At 30-min intervals, arterial blood (125 µL) was analyzed for pHa, PaCO$_2$, PaO$_2$, glucose, and hematocrit (IL-1306 pH blood gas analyzer [Instrumentation Laboratory, Lexington, MA, USA]; YSI Model 23-A glucose analyzer [Yellow Springs Instruments, Yellow Springs, OH, USA]; IEC MB centrifuge microhematocrit [DAMON/IEC Division, Needham Heights, MA, USA]). The EEG was continuously recorded between platinum needle electrodes placed in a bitemporal configuration. Thirty minutes before MCAo, the isoflurane was discontinued and each rat randomized to receive one of the following regimens, each of which was maintained for the duration of the experiment:

Part A

Control (n=10): Each rat received 1.2 MAC[8] halothane (Abbott Laboratories, North Chicago, IL, USA) while 0.9% NaCl was administered intravenously.

Thiopentone (n=10): Thiopentone sodium (Abbott Laboratories,

North Chicago, IL, USA) was infused at a dose which provided a burst-suppression (3–5 bursts·min^{-1}) pattern on the EEG.

Methohexital (n=10): Methohexital sodium (Jones Pharma, St. Louis, MO, USA) was infused at a dose which provided a burst-suppression (3–5 bursts·min^{-1}) pattern on the EEG.

Pentobarbitone (n=10): Pentobarbitone sodium (Abbott Laboratories, North Chicago, IL, USA) was infused at a dose which provided a burst-suppression (3–5 bursts·min^{-1}) pattern on the EEG.

Part B

Control (n=10): Each rat received 1.2 MAC halothane.

Thiopentone (n=10): Thiopentone sodium was infused at 40% of the dose required in Part A.

Methohexital (n=10): Methohexital sodium was infused at 40% of the dose required in Part A.

Pentobarbitone (n=10): Pentobarbitone sodium was infused at 40% of the dose required in Part A.

The volume of infused barbiturate was deducted from the maintenance fluid in each group such that all animals received equivalent amounts of fluid throughout the experiment.

A left temporal craniectomy was performed, and the middle cerebral artery was occluded in two locations with 10-O monofilament nylon suture to achieve ischemia of both cortical and subcortical tissue.[9,10] After 180 min of MCAo, the sutures were released, and a 120-min period of reperfusion ensued. During MCAo and reperfusion, the craniotomy site was bathed in mock cerebrospinal fluid at 37°C. Immediately following the 120-min period of reperfusion, perfusion fixation was performed. This was accomplished by infusion, via the ascending aorta, of 200 mL of 2% 2,3,5-triphenyltetrazolium chloride (TTC, 37°C) over 15-min followed by 50 mL of 10% buffered formalin over five minutes. The brains were immediately harvested and embedded in an egg: albumin-gelatin media and mounted on a vibratome (Vibratome Series 1000; Technical Products

International, Inc., St. Louis, MO, USA). Ten serial coronal sections were cut in 1.0-mm increments, spanning the area of middle cerebral artery distribution (2.0–11.0 mm from the frontal pole). The ten brain sections were photographed with colour slide film (Ektachrome, tungsten 160 ASA). The area of each section with deficient TTC staining was determined with a Drexel/DUMAS Image analysis system (Drexel University, Philadelphia, PA, USA), and the volume of injured tissue in the hemisphere ipsilateral to MCAo calculated from the consecutive sums of infarct area multiplied by the interval between sections (1.0 mm) over the extent of the infarct.[11]

The corpus callosum does not routinely stain with TTC in normal tissue, accordingly, the rim of tissue representing the corpus callosum was excluded from analysis. All image analyses were performed by an independent observer who was blinded to the study protocol.

The physiological data were analyzed by repeated measures analysis of variance, and volume of injury data by a one-way analysis of variance. Where differences were identified, pairwise comparisons were performed using Student's t tests with appropriate Bonferroni correction. $P < 0.05$ was considered significant. All data are presented as means ± SD.

Results

The physiologic and pharmacologic data are presented in Table I. The physiologic data were similar between groups. In general, a greater amount of phenylephrine was required during the reperfusion period, than during MCAo. For Part A, phenylephrine was required in all barbiturate groups, but not the control group. The amount of phenylephrine was greater for the methohexital group *vs* the other three groups. For Part B, phenylephrine was required only in four methohexital animals.

The volume of infarct data for Parts A and B are presented in Table II. There were no abnormalities in TTC staining for the hemisphere contralateral to MCAo.

Part A (EEG burst-suppression)

The volume (mm^3) of cerebral injury was 133 ± 17 for the control (halothane) group, and was not different for the methohexital (126 ± 19) or pentobarbitone (130 ± 17) groups; but was less ($P < 0.05$) in the thiopentone group (88 ± 14) as compared to the other three groups.

Part B (40% of the barbiturate dose required to maintain EEG burst-suppression)

The volume of cerebral injury was not different for the control (124 ± 22), thiopentone (118 ± 15) or pen-

TABLE I Physiologic and pharmacologic data (means ± SD). pH, PaO_2, $PaCO_2$, hematocrit, and glucose are the average of the values taken at 30-min intervals throughout the study period. Mean arterial blood pressure (MABP) was monitoring continuously and reported as the average over the study period. The barbiturate and phenylephrine doses given were the amount of drug given during the entire study period. The Control group received 1.2 MAC halothane, while the other groups received the identified barbiturate at either a burst-suppression dose or 40% of a burst-suppression dose.

	Control	Thiopentone	Methohexital	Pentobarbitone
PART A (burst-suppression dose)				
pHa	7.38 ± 0.03	7.37 ± 0.03	7.37 ± 0.03	7.39 ± 0.03
PaO_2 (mmHg)	35 ± 21	136 ± 17	125 ± 15	130 ± 14
$PaCO_2$ (mmHg)	40.0 ± 1.7	40.3 ± 2.5	39.6 ± 2.6	40.5 ± 2.3
MABP (mmHg)	126 ± 9	120 ± 7	118 ± 13	124 ± 6
Hematocrit (%)	46 ± 2	47 ± 2	44 ± 4	47 ± 3
Glucose (mg·dL^{-1})	109 ± 18	87 ± 12	98 ± 19	85 ± 7
Barbiturate dose (mg·kg^{-1})	—-	142 ± 15	186 ± 19	130 ± 10
Phenylephrine dose µg·kg^{-1})	0 ± 0	65 ± 45†	132 ± 46*	34 ± 24†
PART B (40% burst-suppression dose)				
pHa	7.39 ± 0.02	7.38 ± 0.02	7.39 ± 0.02	7.40 ± 0.03
PaO_2 (mmHg)	142 ± 15	141 ± 17	155 ± 14	147 ± 13
$PaCO_2$ (mmHg)	39.7 ± 0.8	39.5 ± 1.6	38.7 ± 1.8	39.2 ± 1.2
MABP (mmHg)	131 ± 7	138 ± 9	134 ± 11	131 ± 9
Hematocrit (%)	45 ± 2	47 ± 2	46 ± 3	46 ± 2
Glucose (mg·dL^{-1})	129 ± 18	109 ± 16	130 ± 20	115 ± 14
Barbiturate dose (mg·kg^{-1})	—-	52 ± 12	70 ± 15	48 ± 2
Phenylephrine dose (g·kg^{-1})	0 ± 0	0 ± 0	15 ± 19*	0 ± 0

*$P <0.05$ vs the other three groups.
†$P <0.05$ vs the control group.

TABLE II Volume of brain injury (mm³, means ± SD) as determined by TTC stain for each group. The Control group received 1.2 MAC halothane, while the other groups received the identified barbiturate at either a burst-suppression dose or 40% of a burst-suppression dose.

	Control	Thiopentone	Methohexital	Pentobarbitone
PART A (burst-suppression dose)				
Infarct volume (mm³)	133 ± 7	88 ± 14*	126 ± 19	130 ± 17
PART B (40% burst-suppression dose)				
Infarct volume (mm³)	124 ± 22	118 ±15	70 ± 22*	121± 20

*$P <0.05$ vs the other three groups.

tobarbitone groups (121 ± 20); but was less ($P <0.05$) in the methohexital group (70 ± 22) than in the other three groups.

Discussion

These data confirm previous reports that thiopentone, in a dose which induces EEG burst-suppression, results in a reduction in infarct volume following temporary focal cerebral ischemia.[1,3–5,12] However, the novel observation is that neither methohexital nor pentobarbitone, in burst-suppression doses, had a comparable effect on cerebral injury volume. Moreover, at a barbiturate dose that was 40% of a burst-suppression dose, thiopentone did not exhibit a significant effect on volume of injury while methohexital reduced injury to an extent comparable to that achieved in Part A for thiopentone. This latter observation, however, is subject to the limitation that Parts A and B of this experiment were not performed concurrently.

With few exceptions[7,13] most studies evaluating barbiturate-induced cerebral protection have been conducted on the premise that optimal outcome is dependent on maximum CMR suppression. Barbiturates are known to reduce CMR in a dose-dependent manner that occurs in parallel with suppression of the EEG.[5,7] It has been assumed that complete suppression of the EEG is required to achieve maximal cerebral protection from barbiturates. Few studies have attempted to examine the dose-response relationship between barbiturates and

neurologic outcome following focal cerebral ischemia.[7,13–15] In the most detailed of these studies, Warner *et al.*[7] assessed the effect of an active EEG dose and a burst-suppression EEG dose of pentobarbitone on infarct volume after temporary MCAo in rats. They confirmed that the degree of CMR suppression was significantly different at the two doses but, nonetheless, observed no difference in infarct volume. The results for the two pentobarbitone groups in the present study are similar to those of Warner *et al.* in that the protective efficacy was apparently not different for the burst-suppression and 40% burst-suppression doses.

The present data suggest that for some, if not all, barbiturates, mechanisms other than CMR suppression contribute to protective efficacy. The properties critical to the protective effect are yet to be identified and barbiturates have numerous effects that might be relevant. Mechanisms of note include effects on free radical scavenging, vascular tone, cellular ionic gradients, and excitotoxicity.[16–21] In most instances, there are insufficient data to conclude that these properties are shared equally by all of the available barbiturates. In addition, if these mechanisms contribute to barbiturate-mediated cerebral protection, there is no confirmation that their activity parallels the reductions in CMR caused by barbiturates.

The second assumption mentioned previously is the apparent acceptance of the protective equivalence of the various clinically available barbiturates. The present data are inconsistent with that assumption. Thiopentone and methohexital in specific doses (i.e., the former at a full burst-suppression dose and the latter at 40% of the burst-suppression dose) appeared more effective than pentobarbitone. These observations should not necessarily be unexpected because as noted above, if non-CMR mechanisms are involved, there is no basis for assuming that all barbiturates share the critical properties or that the dose-response relationship for the critical properties is such that maximal effect is achieved at complete CMR suppression. Some of the properties of barbiturates that might contribute to a protective effect are listed below.

Free radicals
There are data that suggest a differential ability of barbiturates to scavenge free radicals.[19,22,23] In a human neuronal cell preparation, Almaas, *et al.*,[19] observed that pentobarbitone, phenobarbital, methohexital, and thiopentone dose-dependently inhibited formation of hydroxyl radicals and lipid peroxidation by-products. Thiopentone was more effective than the other barbiturates in inhibiting formation of hydroxyl radicals at

equimolar concentrations; while thiopentone and methohexital were more effective than pentobarbitone and phenobarbital in inhibiting lipid peroxidation. Moreover, phenobarbital and pentobarbitone effected an increase in markers of cell damage, while thiopentone and methohexital decreased cell injury.

Nitric oxide neurotoxicity
Although controversial, there is evidence that nitric oxide contributes to ischemic brain injury.[24,25] Nitric oxide is synthesized by endothelial cells, glia and several types of neurons. Synthesis of nitric oxide from vascular endothelium is accomplished by an isoform of nitric oxide synthase that is expressed constitutively.[25] Two other isoforms of nitric oxide synthase have also been described (neuronal and inducible) which may contribute to ischemic neuronal injury.[25–27] During cerebral ischemia, neuronal nitric oxide synthase is activated[25] which can result in cytotoxicity by mechanisms which include free radical damage, inactivation of enzymes involved in mitochondrial respiration, and energy depletion subsequent to activation of poly-ADP ribose synthase.[28] In a neuronal cell culture model of nitric oxide induced cytotoxicity, Shibuta *et al.*[29] assessed the effect of thiopentone and pentobarbitone on cell death. They observed that cell death was reduced by thiopentone but not pentobarbitone. They hypothesized that it was the sulphhydryl group on thiopentone, with its augmented free radical scavenging properties, which effected this result.

Vasoactive properties
Although limited, there are data which demonstrate that specific barbiturates have unique contractile responses in cerebral vessels.[30,31] Hatano *et al.*,[30] assessed the effect of thiamylal, thiopentone, secobarbital, and pentobarbitone on helical strips of canine cerebral arteries. They observed greater vessel contraction for thiamylal than thiopentone, and a relaxation response for secobarbital and pentobarbitone. The extent to which this data applies to the present *in vivo* study is speculative. However, they raise the possibility that a barbiturate will have differential effects on vasomotor tone and therefore blood flow distribution during ischemia.

Calcium entry
It has long been known that barbiturates effect voltage-gated neuronal calcium channels[32,33] and this may have implications in the evolution of excitotoxic brain injury. Although the evidence is limited, Zhan *et al.*[34] observed, in a rat hippocampal slice model, a differential ability of barbiturates to block voltage-gated neu-

ronal calcium channels, with the potency—thiamylal > thiopentone >>> phenobarbital. Conversely, Miao *et al.*,[20] in a rat culture neuron preparation, observed a greater potency of methohexital than thiopentone in the inhibition of both the intracellular calcium peak and glutamate release in response to depolarization.

Glutamate

Barbiturates are considered to be antagonists of excitotoxic neuronal injury.[35] A potential mechanism is blockade of glutamate receptors, including the kainate, N-methyl-D-aspartate (NMDA), and -amino-3-hydroxy-5-methyl- 4-isoxazole propionic acid (AMPA) sub-types.[36] Relevant to the present results is the data of Cai *et al.*,[37] who observed a differential ability, in a neuronal culture, of barbiturates to block these receptors. Thiamylal was the most effective followed by secobarbital, while pentobarbitone and phenobarbital were without effect. In addition, in an *in vitro* preparation of rat spinal cord, Zeman and Lodge[38] observed a differential effect of barbiturates at the kainate receptor with methohexital being the most potent, followed by secobarbital, thiopentone, pentobarbitone, and phenobarbital.

Conversely, there may be properties of certain barbiturates that act to counter the overall benefit that has been observed during neurotoxic injury. Following an episode of cerebral ischemia, glutamate uptake by astrocytes is a critical function that acts to maintain neuronal survival. There is evidence that glutamate uptake by astrocytes may be inhibited in a dose-dependent manner by barbiturates.[39–42] Swanson *et al.*,[39] in rat astrocyte cell cultures, assessed the effect of barbiturates on the inhibition of glutamate uptake. They observed that thiopentone and thiamylal were the most potent in inhibiting glutamate uptake, while secobarbital, amobarbital, and pentobarbitone had negligible effects.

The preceding discussion of the differences among barbiturates does not provide a definitive explanation of the findings of the present study. While the apparent dose-related ability of thiopentone to reduce ischemic injury is intuitively reasonable, the inverse dose-response relationship of methohexital to ischemic injury is difficult to explain. The latter requires either the assumption of an inverted U-shaped dose-response for some beneficial effect or the assumption of an adverse effect that becomes apparent at higher doses. There are no data to support or refute these possibilities. The unresolved issues notwithstanding, the results of the present investigation are consistent with our initial premise that non CMR related mechanisms may contribute to barbiturate-induced cerebral protection in a manner that is not necessarily equivalent among the barbiturates.

Limitations of this study include some uncertainty as to the specificity of TTC stain to identify brain infarction. During normal aerobic metabolism, TTC is converted by mitochondrial oxidative enzymes to a formazan product which effects a red staining of brain tissue. With prolonged ischemia these enzymes are rendered dysfunctional, and because of the resulting failure of TTC conversion to its red derivative, a pale area of brain is identifiable. Thus, TTC stain defines areas of enzymatic dysfunction, not necessarily neuronal necrosis. However, our methodology is validated by data that have shown reasonable correlation between TTC stain and conventional histologic markers of infarct.[43,44] Another limitation is the delineation of cerebral infarction at an early time period following MCAo. Recent data suggests that ischemic brain injury is a dynamic process that requires at least 14 days to evolve fully.[45,46] Accordingly, the present findings should ideally be validated in a long term model of infarct assessment to confirm the outcome differences between barbiturates that we observed.

In summary, we evaluated the effect of three different barbiturates on early brain injury following temporary MCAo in rats. Two different doses of each barbiturate were administered: a dose which achieved a burst-suppression pattern on the EEG, and 40% of that dose. For the burst-suppression groups, thiopentone was the only barbiturate that significantly reduced the volume of injury as compared to a halothane anesthetized control group. For the 40% barbiturate dose groups, only methohexital reduced infarct volume. These data provoke further examination of both the commonly held tenet that a burst-suppression pattern on the EEG is necessary to achieve maximal barbiturate-induced cerebral protection, as well as tacit assumptions about the protective equivalence of different barbiturates.

References

1 *Nehls DG, Todd MM, Spetzler RF, Drummond JC, Thompson RA, Johnson PC.* A comparison of the cerebral protective effects of isoflurane and barbiturates during temporary focal ischemia in primates. Anesthesiology 1987; 66: 453–64.

2 *Warner DS, Zhou J, Ramani R, Todd MM.* Reversible focal ischemia in the rat: effects of halothane, isoflurane, and methohexital anesthesia. J Cereb Blood Flow Metab 1991; 11: 794–802.

3 *Drummond JC, Cole DJ, Patel PM, Reynolds LW*. Focal cerebral ischemia during anesthesia with etomidate, isoflurane, or thiopental: a comparison of the extent of cerebral injury. Neurosurgery 1995; 37: 742–9.

4 *Michenfelder JD, Theye RA*. Cerebral protection by thiopental during hypoxia. Anesthesiology 1973; 39: 510–7.

5 *Michenfelder JD*. The interdependency of cerebral functional and metabolic effects following massive doses of thiopental in the dog. Anesthesiology 1974; 41: 231–6.

6 *Todd MM, Warner DS*. A comfortable hypothesis reevaluated. Cerebral metabolic depression and brain protection during ischemia (Editorial). Anesthesiology 1992; 76: 161–4.

7 *Warner DS, Takaoka S, Wu B, et al.* Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia. Anesthesiology 1996; 84: 1475–84.

8 *Cole DJ, Kalichman MW, Shapiro HM, Drummond JC*. The non-linear potency of sub-MAC concentrations of nitrous oxide in decreasing the anesthetic requirement of enflurane, halothane, and isoflurane in rats. Anesthesiology 1990; 73: 93–9.

9 *Cole DJ, Drummond JC, Osborne TN, Matsumura J*. Hypertension and hemodilution during cerebral ischemia reduce brain injury and edema. Am J Physiol 1990; 259: H211–7.

10 *Cole DJ, Schell RM, Przybelski RJ, Drummond JC, Bradley K*. Focal cerebral ischemia in rats: effect of hemodilution with - cross-linked hemoglobin on CBF. J Cereb Blood Flow Metab 1992; 12: 971–6.

11 *Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR*. A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 1990; 10: 290–3.

12 *Sano T, Patel PM, Drummond JC, Cole DJ*. A comparison of the cerebral protective effects of etomidate, thiopental and isoflurane in a model of forebrain ischemia in the rat. Anesth Analg 1993; 76: 990–7.

13 *Schmid-Elsaesser R, Schröder M, Zausinger S, Hungerhuber E, Baethmann A, Reulen H-J*. EEG burst suppression is not necessary for maximum barbiturate protection in transient focal cerebral ischemia in the rat. J Neurol Sci 1999; 162: 14–9.

14 *Hoff JT, Smith AL, Hankinson HL, Nielsen SL*. Barbiturate protection from cerebral infarction in primates. Stroke 1975; 6: 28–33.

15 *Corkill G, Sivalingam S, Reitan JA, Gilroy BA, Helphrey MG*. Dose dependency of the post-insult protective effect of pentobarbital in the canine experimental stroke model. Stroke 1978; 9: 10–2.

16 *Wang T, Raley-Susman KM, Wang J, Chambers G, Cottrell JE, Kass IS*. Thiopental attenuates hypoxic changes of electrophysiology, biochemistry, and morphology in rat hippocampal slice CA1 pyramidal cells. Stroke 1999; 30: 2400–7.

17 *Zhu H, Cottrell JE, Kass IS*. The effect of thiopental and propofol on NMDA- and AMPA-mediated glutamate excitotoxicity. Anesthesiology 1997; 87: 944–51.

18 *Bickler PE, Buck LT, Feiner JR*. Volatile and intravenous anesthetics decrease glutamate release from cortical brain slices during anoxia. Anesthesiology 1995; 83: 1233–40.

19 *Almaas R, Saugstad OD, Pleasure D, Rootwelt T*. Effect of barbiturates on hydroxyl radicals, lipid peroxidation, and hypoxic cell death in human NT2-N neurons. Anesthesiology 2000; 92: 764–74.

20 *Miao N, Nagao K, Lynch III C*. Thiopental and methohexital depress Ca^{2+} entry into and glutamate release from cultured neurons. Anesthesiology 1998; 88: 1643–53.

21 *Kimbro JR, Kelly PJ, Drummond JC, Cole DJ, Patel PM*. Isoflurane and pentobarbital reduce AMPA toxicity in vivo in the rat cerebral cortex. Anesthesiology 2000; 92: 806–12.

22 *Weiss M, Buhl R, Birkhahn A, Mirow N, Schneider M, Wernet P*. Do barbiturates and their solutions suppress FMLP-induced neutrophil chemiluminescence? Eur J Anaesthesiol 1994; 11: 371–9.

23 *Smith DS, Rehncrona S, Siesjö BK*. Inhibitory effects of different barbiturates on lipid peroxidation in brain tissue in vitro: comparison with the effects of promethazine and chlorpromazine. Anesthesiology 1980; 53: 186–94.

24 *Ashwal S, Cole DJ, Osborne TN, Pearce WJ*. Dual effects of L-NAME during transient focal cerebral ischemia in spontaneously hypertensive rats. Am J Physiol 1994; 267: H276–84.

25 *Samdani AF, Dawson TM, Dawson VL*. Nitric oxide synthase in models of focal ischemia. Stroke 1997; 28: 1283–8.

26 *Clower BR, Yamamoto Y, Cain L, Haines DE, Smith RR*. Endothelial injury following experimental subarachnoid hemorrhage in rats: effects on brain blood flow. Anat Rec 1994; 240: 104–14.

27 *Iadecola C, Zhang F, Xu S, Casey R, Ross ME*. Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. J Cereb Blood Flow Metab 1995; 15: 378–84.

28 *Dawson DA*. Nitric oxide and focal cerebral ischemia: multiplicity of actions and diverse outcome. Cerebrovasc Brain Metab Rev 1994; 6: 299–324.

29 *Shibuta S, Kosaka J, Mashimo T, Fukuda Y, Yoshiya I*. Nitric oxide-induced cytotoxicity attenuation by thiopentone sodium but not pentobarbitone sodium in primary brain cultures. Br J Pharmacol 1998; 124: 804–10.

30 *Hatano Y, Nakamura K, Moriyama S, Mori K, Toda N.* The contractile responses of isolated dog cerebral and extracerebral arteries to oxybarbiturates and thiobarbiturates. Anesthesiology 1989; 71: 80–6.

31 *Yakushiji T, Nakamura K, Hatano Y, Mori K.* Comparison of the vasodilator effects of thiopentone and pentobarbitone. Can J Anaesth 1992; 39: 604–9.

32 *Heyer EJ, Macdonald RL.* Barbiturate reduction of calcium-dependent action potentials: correlation with anesthetic action. Brain Res 1982; 236: 157–71.

33 *Gundersen CB, Umbach JA, Swartz BE.* Barbiturates depress currents through human brain calcium channels studied in Xenopus oocytes. J Pharmacol Exp Ther 1988; 247: 824–9.

34 *Zhan R-Z, Fujiwara N, Yamakura T, Taga K, Fukuda S, Shimoji K.* Differential inhibitory effects of thiopental, thiamylal and phenobarbital on both voltage-gated calcium channels and NMDA receptors in rat hippocampal slices. Br J Anaesth 1998; 81: 932–9.

35 *Giffard RG, Weiss JH, Swanson RA, Choi DW.* Secobarbital attenuates excitotoxicity but potentiates oxygen-glucose deprivation neuronal injury in cortical cell culture. J Cereb Blood Flow Metab 1993; 13: 803–10.

36 *Buchan AM, Xue D, Huang Z-G, Smith KH, Lesiuk H.* Delayed AMPA receptor blockade reduces cerebral infarction induced by focal ischemia. Neuroreport 1991; 2: 473–6.

37 *Cai Z, McCaslin PP.* Acute, chronic and differential effects of several anesthetic barbiturates on glutamate receptor activation in neuronal culture. Brain Res 1993; 611: 181–6.

38 *Zeman S, Lodge D.* Pharmacological characterization of non-NMDA subtypes of glutamate receptor in the neonatal rat hemisected spinal cord in vitro. Br J Pharmacol 1992; 106: 367–72.

39 *Swanson RA, Seid LL.* Barbiturates impair astrocyte glutamate uptake. Glia 1998; 24: 365–71.

40 *Miyazaki H, Nakamura Y, Arai T, Kataoka K.* Increase of glutamate uptake in astrocytes. A possible mechanism of action of volatile anesthetics. Anesthesiology 1997; 86: 1359–66.

41 *Qu H, Faerø E, Jørgensen P, et al.* Decreased glutamate metabolism in cultured astrocytes in the presence of thiopental. Biochem Pharmacol 1999; 58: 1075–80.

42 *Patel PM, Goskowicz RL, Drummond JC, Cole DJ.* Etomidate reduces ischemia-induced glutamate release in the hippocampus in rats subjected to incomplete forebrain ischemia. Anesth Analg 1995; 80: 933–9.

43 *Hatfield RH, Mendelow AD, Perry RH, Alvarez LM, Modha P.* Triphenyltetrazolium chloride (TTC) as a marker for ischaemic changes in rat brain following permanent middle cerebral artery occlusion. Neuropathol Appl Neurobiol 1991; 17: 61–7.

44 *Isayama K, Pitts LH, Nishimura MC.* Evaluation of 2,3,5-triphenyltetrazolium chloride staining to delineate rat brain infarcts. Stroke 1991; 22: 1394–8.

45 *Du C, Hu R, Csernansky CA, Hsu CY, Choi DW.* Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J Cereb Blood Flow Metab 1996; 16: 195–201.

46 *Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM.* Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. Anesthesiology 2000; 92: 1335–42.