

Canine Cerebral Metabolic Tolerance during 24 Hours Deep Pentobarbital Anesthesia

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The impact of tolerance on cerebral metabolism during prolonged pentobarbital-induced anesthesia was evaluated in 16 dogs. Cerebral metabolic rate for oxygen was calculated from direct measurements of venous blood flow rate and the difference in blood oxygen content between arterial and venous blood during four periods of continuous and unvarying deep pentobarbital anesthesia—0–3 hours, 3–6 hours, 12–15 hours, and 21–24 hours.

During 0–3 h, the metabolic rate was $1.8\text{--}2.0\text{ ml O}_2 \cdot 100\text{ g brain}^{-1} \cdot \text{min}^{-1}$. This increased to $2.3\text{--}2.6\text{ ml} \cdot 100\text{ g}^{-1} \cdot \text{min}^{-1}$ during 3–6, 12–15, and 21–24 h. In all studies, the electroencephalogram indicated a constant deep burst-suppression level at 2–6 bursts/min, and blood pentobarbital levels were unchanged at 4.9–5.9 mg/dl throughout the entire period of anesthesia.

The sustained increase in cerebral metabolism that occurred after 3 h and continued through 24 h of pentobarbital anesthesia is presumably due to tolerance. This was a phenomenon in which metabolism increased during steady deep anesthesia with unchanged blood levels of pentobarbital, rather than one in which greater blood levels were necessary to maintain the same level of anesthesia. (Key words: Anesthetics, intravenous: pentobarbital. Brain: blood flow; electroencephalography; metabolism; oxygen consumption. Potency, anesthetic: tolerance.)

THERE IS CONSIDERABLE EXPERIMENTAL EVIDENCE suggesting that long-term high-dose barbiturate therapy may provide brain protection from ischemia.¹ However, this proposed efficacy could be diminished by the development of tolerance to depressant drugs, or curtailed if there are deleterious effects upon major organ systems of the body, *e.g.*, marked hepatic, renal, or myocardial depression of function. Tolerance is a phenomenon whereby increasingly larger drug doses are required to maintain a constant effect, or whereby a constant dose level produces a progressively lesser effect.² The present study examines the development of brain tolerance as reflected by cerebral metabolic rate and the stability of the cerebral circulation and energy stores during deep pentobarbital anesthesia for periods up to 24 hours.

Methods

Sixteen fasting unmedicated mongrel dogs weighing $12.3 \pm 0.3\text{ kg}$ (SE) were anesthetized with intra-

venous pentobarbital, 20–30 mg/kg. The trachea of each dog was intubated following muscle paralysis with intravenous succinylcholine, 20 mg, which was continued thereafter at 150 mg/h. Controlled mechanical ventilation and the inspired mixture of nitrogen and oxygen were adjusted to maintain arterial blood oxygen tension (PaO_2) between 120 and 180 torr and arterial blood carbon dioxide tension (PaCO_2) at 40 ± 2 torr (mean \pm SE). Femoral arterial and venous catheters were placed for measurement of arterial pressure, blood sampling, and drug and fluid administration (5 per cent D/W at $4\text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$); external heating with pads and lamps was used to maintain esophageal temperature at $37.0 \pm 0.1^\circ\text{C}$.

Four electroencephalographic (EEG) leads, two bifrontal and two biparietal, were placed via burr holes on the dura and the EEG was continuously monitored for estimation of depth of barbiturate anesthesia. Pentobarbital was given as needed in divided doses to maintain an EEG pattern of burst-suppression at a level of 2–6 bursts/min, or one burst every 10–30 s. This pattern was maintained as constant as possible throughout the period of observation, which ranged from 3 to 24 h. In five dogs the surgical preparation of the brain was begun immediately while constant deep anesthesia levels were being established, so that measurements of cerebral metabolic rate (CMR_{O_2}) could begin immediately and extend to 3 h. In the remaining 11 dogs, a constant deep anesthesia level was maintained for varying intervals with delay of the brain preparation for measuring CMR_{O_2} , so as to provide cerebral metabolic data for durations of pentobarbital anesthesia beyond 3 h and up to 24 h. These remaining measurements were made at 3–6 h (5 dogs), 12–15 h (4 dogs), and 21–24 h (2 dogs). In studies beyond 3 h, arterial blood gases were monitored at least every hour and the dogs were turned every 4 h. After several hours of anesthesia, and especially after the surgical preparation (when it was impossible to turn the dogs), positive end-expiratory pressure (PEEP) of 3–5 cm water was usually needed to maintain PaO_2 levels. In some dogs, after 12–15 h of deep anesthesia, this reached 5 cm water with an FI_{O_2} of 1. PEEP *per se* did not alter CMR_{O_2} . Cross-matched blood was transfused for hemoglobin $<10\text{ g/dl}$ and saline or blood given if mean arterial pressure fell below 65 torr. If buffer base decreased below 35 mEq/l, bicarbonate was given to

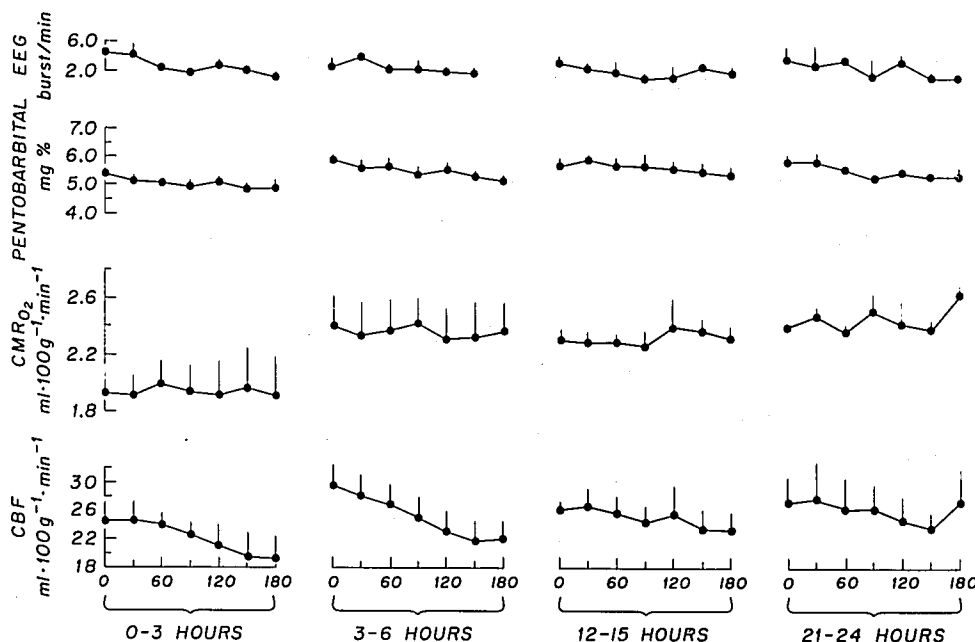
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FIG. 1. Cerebral blood flow (CBF), cerebral metabolic rate for oxygen (CMR_{O₂}), pentobarbital blood level, and electroencephalographic depth of anesthesia (EEG) during four periods of observation in dogs. Values are means \pm SE. CMR_{O₂} values are significantly less at 0-3 h.



restore it to 40 mEq/l (base excess = 0). At the end of the period of study, brain biopsies were performed in four dogs at 3 h, four dogs at 6 h, four dogs at 15 h, and two dogs at 24 h for determination of brain adenosine triphosphate (ATP), phosphocreatine, lactate, and pyruvate.

The brain was prepared as previously described for direct measurement of cerebral blood flow (CBF) from anterior, superior and lateral parts of both hemispheres (equal to about 54 per cent of total brain weight) by cannulating the posterior sagittal sinus after eliminating all relevant extracerebral connections.³ Cerebral venous blood flow rate was determined intermittently by timed collection and continuously by flowmeter. CMR_{O₂} was calculated from the Fick relationship using values for blood flow and the difference in oxygen content between arterial and sagittal sinus blood. Blood O₂ content was determined from measurements of O₂ tension, hemoglobin concentration, and oxyhemoglobin concentration (IL electrodes and CO-Oximeter).⁴ Blood gases and pH were determined by IL electrode at 37° C, arterial blood pressure by transducer, temperature from thermistor probes in the esophagus and parietal epidural space, and arterial plasma sodium and potassium by flame photometer. Brain biopsies and arterial lactate and pyruvate were assayed using standard enzymatic fluorometric techniques. Blood pentobarbital levels were determined spectrophotometrically.⁵ Results are expressed as means \pm SE and an analysis of variance was used to determine if significant differences existed among the four groups. When such differences were detected, intergroup

comparisons were performed using Student's unpaired *t* test; *P* < 0.05 was considered significant.

Results

The EEG patterns in burst/min (bursts were 1.1 \pm 0.1 s in duration) and pentobarbital blood levels (fig. 1) indicate a relatively constant and unchanging level of anesthesia during the four periods of metabolic measurements. These EEG patterns were maintained as constant as possible during the entire period of barbiturate infusion; following the induction of anesthesia, the burst suppression pattern generally stabilized in about 30-60 min after initial doses ranging from 40-60 mg/kg. It was notable that EEG bursts increased in frequency with sudden loud noises, even after 15-20 h of constant deep anesthesia.

CMR_{O₂} during the 0-3 h period was 1.8-2.0 \pm 0.2-0.3 ml O₂·100 g brain⁻¹·min⁻¹, a value consistent with that depth of barbiturate anesthesia.⁶ CMR_{O₂} increased abruptly and significantly to 2.3-2.6 \pm 0.1-0.2 ml·100 g⁻¹·min⁻¹ in the 3-6, 12-15, and 21-24 h periods, with unchanged EEG patterns or barbiturate blood levels. Patterns of CBF (fig. 1) tended to demonstrate a progressive reduction in CBF with time, which is known to occur in this preparation.³ However, reproducible constant metabolic data were obtained throughout this period, as CMR_{O₂} did not decrease in parallel with CBF even at the lowest levels of flow observed during each 3-h period. Brain biopsy values at the end of each 3-h period were similar, and the results were therefore pooled: ATP 1.99 \pm 0.05 μ mol/g, phosphocreatine 2.58 \pm 0.17

$\mu\text{mol/g}$, lactate $1.42 \pm 0.28 \mu\text{mol/g}$, and lactate/pyruvate 19.3 ± 1.5 . These values do not differ significantly from those previously reported in healthy anesthetized dogs.⁶

In all studies, during the 3-h period of measurement, mean values for arterial blood lactate remained below $1.85 \mu\text{mol/ml}$, Pa_{O_2} 140–157 torr, Pa_{CO_2} 36–40 torr, arterial pH 7.33–7.43, potassium 3.3–4.0 mEq/l, sodium 138–148 mEq/l, esophageal temperature 35.7–36.7° C, and epidural temperature 36.9–37.3° C. During the 0–3 and 3–6 h studies, mean values for mean arterial pressure varied from 85–107 torr; during the 12–15 h and 21–24 h studies, mean values varied from 60–83 torr. During the period before the brain prep was performed, blood gases, acid-base balance, and temperatures were similarly stable in all dogs.

Discussion

Prolonged deep pentobarbital anesthesia produced a significant depression of CMR_{O_2} without evidence for inadequate energy supplies, toxicity or deterioration, as brain energy stores remained normal.⁶ However, despite an unchanged burst suppression pattern on the EEG and unchanged barbiturate blood levels, CMR_{O_2} increased 25 per cent after 3 h, and this increase was maintained throughout the 24-h period of observation. This increase is presumably due to tolerance. While one might expect tolerance to be manifested by an increasing requirement for barbiturate to maintain a constant EEG level of anesthesia, this was not the case. Instead, there was an apparent alteration with time in the degree of metabolic depression at a constant deep level of anesthesia, suggesting an alteration of cerebral metabolic responses rather than functional responses to barbiturate. Prior work has demonstrated that there is a "basal" CMR_{O_2} which is achieved during deep barbiturate anesthesia when electrical activity is completely suppressed.⁷ Since EEG activity was almost completely suppressed in this study and did not change, and since CMR_{O_2} increased, this "basal" CMR_{O_2} may have increased as an expression of the development of tolerance. This suggests, in agreement with the findings of others, that tolerance is due to changes in cellular enzyme systems or membranes.^{2,8–13} While this study was limited to the brain, it is likely that other organ systems also experienced an increase in metabolism.

The apparently abrupt increase in CMR_{O_2} which occurred at three hours between the 0–3 h group and the 3–6 h group is likely an artefact related to study design. In the former group (0–3 h) there was no surgical stimulation during or after the time in which tolerance might be expected to develop. In

the latter group, and all subsequent groups, surgical stimulation occurred well after the expected onset of tolerance. We speculate that stimulation may unmask tolerance as reflected by an increase in CMR_{O_2} . In addition, the invasive nature of the surgical preparation limits the duration of individual studies to 3–4 h; thereafter artefactual decreases in CMR_{O_2} occur with deterioration of the model. Possibly this effect of time on the preparation also masked any gradual increase in CMR_{O_2} that might have otherwise occurred in the 0–3 h group.

Other studies have demonstrated alterations in the cerebral metabolic response to barbiturates that presumably are explained by the phenomenon of tolerance. Thiopental tolerance in the dog was produced by a single induction dose and was evident within two hours.¹⁰ Tolerance to pentobarbital in mice occurred following three days of treatment with phenobarbital; both the anesthetic dose and the cerebral protective dose for hypoxia were increased, the former by 56 per cent and the latter by 67 per cent.⁵ Brain levels of pentobarbital at these dose levels were likewise increased above those in non-tolerant mice. This latter study⁵ and others¹ support the proposal that brain protection by barbiturates is closely related to their depression of cerebral metabolism. Tolerance would not likely alter this protection if it were manifested by a requirement for higher doses. However, tolerance may limit the degree of protection if it involves an increase in CMR_{O_2} during barbiturate-induced stable EEG levels of electrical activity or even electrical silence. While one cannot apply these results directly to human care, one should recognize that the expected degree of protection possibly diminishes with time as tolerance develops.

The mechanism of the depressant action of barbiturates has been reported as due to reduced transmitter output presynaptically¹⁴ or to increased conductance in GABA-activated channels postsynaptically,¹⁵ and the development of tolerance may involve alterations in protein synthesis affecting the synaptic areas.¹⁶ Tolerance probably involves an alteration in various stimulus-coupled events, likely involving calcium-mediated functions¹⁷ and/or brain acetylcholine,¹⁸ and perhaps requiring central noradrenergic systems¹⁹ or serotonin.^{20,21} This central nervous system adaptation²² results in cross-tolerance to other depressant drugs.²³ However, if tolerance does involve synaptic structure and/or function, then one would expect it to also relate to cerebral activity and the EEG; to the contrary, the present data suggest that this is not essential. All of the foregoing imply, correctly, that the basic mechanism for tolerance is ultimately unknown.

The present study demonstrated depression of cerebral metabolism by prolonged deep barbiturate anesthesia without evidence of toxicity or damage. Variations in mean arterial blood pressure to values as low as 60 torr without decreases in CBF suggested that autoregulation was intact at its accepted lower limit. Tolerance developed after 3 h of deep anesthesia and was maintained, as manifested by a sustained 25 per cent increase in oxygen consumption during unchanged EEG levels and unchanged barbiturate blood levels.

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