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Barbiturates in Focal Ischemia of Primate Cortex: Effects on Blood Flow Distribution, Evoked Potential and Extracellular Potassium


SUMMARY The effect of an ultra-short acting barbiturate, methohexital, on the distribution of blood flow in baboon cerebral cortex was studied following occlusion of the middle cerebral artery under conditions of constant blood pressure. Further experiments assessed the effects of methohexital and pentobarbital on the threshold relationships (established in earlier work) between flow, cortical evoked potential amplitude and extracellular potassium activity. Regional flow was measured by the hydrogen clearance technique and the initial anesthetic was chloralose in all experiments. If flow after occlusion was > 25 ml/100g/min, so that electrical activity was sustained, methohexital reduced flow in proportion to the flow (r = -0.84, p < 0.001), but if flow was < 20 ml/100g/min, and electrical activity was reduced or absent, a significant elevation in flow occurred averaging 3.4 ml/100g/min (p < 0.01), an appreciable fraction of ambient flow. This result may be attributable to an inverse steal, blood being diverted into ischemic regions from vasoconstriction induced in relatively well-perfused areas. No statistically significant change could be demonstrated either in the flow threshold for the abolition of the evoked potential or in that for the massive increase in potassium, although methohexital tended to decrease, and pentobarbital to increase, these thresholds. However, methohexital significantly reduced the rate of decrease of the evoked potential for a given flow below the threshold. These effects may be among factors underlying any protective effect of barbiturate in focal cerebral ischemia on the neurological and neuropathological levels.

THE POSSIBILITY that barbiturates may exert a protective effect in the ischemic brain has stimulated a number of investigations using several experimental models. Protection has been suggested in terms of reduced infarct size, reduced neurological deficit and increased survival time following stroke.1-9 with both pre- and post-insult administration of the drug. However, as Michenfelder10 has pointed out, extending these models to clinical trials in order to assess the protective effect of barbiturates for the treatment of acute stroke may be difficult, chiefly because of the hemodynamic and pulmonary complications accompanying the anesthetic effect of barbiturates given at the doses suggested as necessary by experiment. More refined therapeutic regimes might nevertheless be designed which would avoid these complications. If this is to be achieved, the effects of the drugs on factors such as cerebral blood flow and its distribution, local metabolism or osmotic forces, which might be presumed to underlie any protective effect, must be measured and understood.

The present study was designed to assess the effects of 2 intravenous barbiturates, pentobarbital and ultra-
short acting methohexital, each suggested to afford protection in cerebral ischemia,1-6 on the distribution of blood flow in the cerebral cortex after occlusion of the middle cerebral artery (MCA).11 Focal flow measurements were made using the hydrogen clearance method.12 In these experiments, any reduction in systemic blood pressure produced by the barbiturate was counteracted by Aramine (metaraminol) infusion. Since barbiturates reduce cerebral metabolic rate and CMRO2,13 and may thus prolong the time in which electrical activity and ionic homeostasis are preserved following onset of a defined ischemic level, we performed other experiments to assess the effect of barbiturates on the threshold relationships between flow and neuronal function, as exemplified by the cortical evoked response (EP) and extracellular potassium activity (Ke).14,15 comparing the flow threshold relationships obtained here with those obtained in previous series14,15 without barbiturates.

Methods

Three groups of baboons (Papio cynocephalus, weights 10–16 kg), totalling 17 animals, were used. The preparation, electrode systems and experimental procedure common to all of the experiments have been described previously,13,14,15 but may be summarized as follows. Animals were maintained anesthetized with alpha-chloralose (60 mg/kg, i.v.), immobilized with repeated doses of gallamine triethiodide (1 mg/kg, i.v.) and ventilated under pure O2 to maintain normocapnia. Systemic arterial P02, Pco2 and pH were repeatedly monitored and mean systemic arterial blood pressure recorded continuously. Body temperature was maintained in the range 37-38°C.

The right cerebral hemisphere was exposed following a period of at least half an hour, and these measurements were repeated following occlusion of the MCA. The potassium electrodes were calibrated before and after each experiment.

Control measurements of flow, Ke and the EP were made during a period of at least half an hour, and these measurements were repeated following occlusion of the MCA. Details of methods specific to each group are as follows:

Group 1: Methohexital and Controlled MSBP. In this group of 5 animals, control measurements and MCA occlusion were performed with chloralose, as stated above, but without added barbiturate. After occlusion, further flow readings were obtained, following which infusions of methohexital were started at rates of 25, 50 and 100 mg/kg/h, commencing at times averaging 24, 68 and 120 min after occlusion, respectively. Finally, the infusion was discontinued 142 min (on average) after occlusion. Flows were measured within each of these 5 periods after systemic blood pressure and Pco2 had been stabilized. Great care was taken to keep the MSBP constant from one flow reading to the next throughout the whole period of occlusion, by appropriate i.v. infusion of Aramine. The difference in MSBP between any 2 of the 5 flow readings taken in the clip phase did not exceed 2 torr when averaged over the 5 animals. The difference in arterial Pco2, similarly averaged, did not exceed 1 torr.

We investigated the relationship between the degree of ischemia following MCA occlusion and the change in flow due to a subsequent dose of methohexital, in the following way. A total of 46 hydrogen electrodes were placed over the hemisphere, sampling a wide range of ischemic density. At each of the electrode sites the control flow, the flow immediately following clip (F26), and the flows during the 4 succeeding periods (F25,F50,F100, and F0, corresponding to i.v. dose rates of 25, 50, 100 and zero mg/kg/h of methohexital) were measured in units of ml/100g/min. The increases in flow relative to Fc for each of the 4 succeeding periods, namely F25-Fc, F50-Fc, F100-Fc and F0-Fc, were then calculated and plotted against Fc.

Group 2: Pentobarbital, with Preloading. These 5 animals received, in addition to chloralose, a single dose of sodium pentobarbital (14 mg/kg, i.v.) at a time averaging 73 min prior to MCA occlusion. Two of these animals also received a continuous infusion of pentobarbital (4 mg/kg/h) starting 30 min before occlusion.

Group 3: Methohexital, with Preloading. These 7 animals were given, in addition to chloralose, a continuous infusion of sodium methohexital (i.v.) commencing at a time averaging 46 min before occlusion. Five received 25 mg/kg/h, one 12 mg/kg/h and one 6 mg/kg/h. The lower doses were necessary to prevent undue depression of the mean systemic blood pressure (MSBP).

Results

Effect of Barbiturates on Blood Flow Distribution in Cortex

The results for the Group 1 animals, in which methohexital was administered with stabilized MSBP, are summarized in figure 1. There was a significant negative correlation (p < 0.001) between the clip flow (Fc) and the change in flow due to rates 25, 50 and zero mg/kg/h, and the intercept of the regression line on the Fc axis was in the range 15–25 ml/100g/min, as figure 2 illustrates for 50 mg/kg/h. In the case of the 100 mg/kg/h rate, although the regression line still had negative slope, the scatter on the data had substantially increased so that the correlation was not significant.

It appears from these data that, although methohexital depresses cerebral blood flow in regions where MCA occlusion does not initially reduce flow to below about 25 ml/100g/ml, it increases flow in regions where Fc is less than about 20 ml/100g/min. This increase may be confirmed statistically in 2 ways. First, considering the plot of F25-Fc against Fc (fig. 2) the 95% confidence limits of the mean intercept on the
by methohexital. If the flow change is averaged over all available electrodes in each animal where \( F_c < 20 \) ml/100g/min, the mean change over all animals in this range at the dose rate of 50 mg/kg/h is a statistically significant increase (3.4 ± 0.6 ml/100g/min, \( p < 0.01, N = 4 \)), which supports the hypothesis.

In Groups 2 and 3, hemispheric flow, calculated as the average over all electrodes (up to 8) in each animal, decreased significantly from 60 ± 23 ml/100g/min (mean ± sd) before barbiturate to 38 ± 15 ml/100g/min after barbiturate in the control phase (\( p < 0.001, N = 12 \)). The decreases in the 2 groups were similar and statistically significant (\( p < 0.05 \) and \( p < 0.005 \) in Groups 1 and 2 respectively). MSBP decreased significantly from 100 ± 9 mm Hg to 66 ± 21 mm Hg (\( p < 0.01, N = 12 \)) overall, but if the 2 groups are considered separately the fall was significant only in the methohexital group (\( p < 0.005, N = 7 \)).

Extracellular and Serum Potassium. Extracellular potassium activity (Ke) in the control phase decreased at all electrode sites when barbiturate was given (fig. 3). The decrease was from 4.7 ± 1.1 mM to 3.8 ± 0.7 mM (\( p < 0.01 \)) in Group 1 and, since the infusion was initially by bolus, it was possible to estimate the delay between administration of the pentobarbital and the first perceptible fall in Ke as 1–2 min. In Group 2, Ke fell similarly from 4.5 ± 0.5 mM to 3.5 ± 0.5 mM (\( p < 0.001 \)). Some recovery or adaptation toward the initial Ke level occurred subsequently in some animals. In Group 2, the sensitivity of Ke to methohexital was surprising: Ke fell perceptibly (> 0.1 mM) at a dose of only 2.0 ± 1.1 mg/kg.

Serum potassium decreased in all but 2 of the 8 animals in which it was measured, from 3.85 ± 0.64 mM before barbiturate to 3.28 ± 0.48 after (fig. 3). This decrease was significant (\( p < 0.05, N = 8 \)) but its timing relative to the corresponding decrease in Ke could not be estimated because blood sampling was not continuous. The serum potassium decrease in the pentobarbital group was higher (1.1 ± 0.3 mM, \( N = 3 \)) but not significantly so. Serum potassium was also measured in 3 animals before and after administration of chloralose. The decrease (0.4 ± 0.4 mM) was not significant.
In the clip phase, and with additional reduction in local flow produced by carefully controlled steps of exsanguination if necessary, we measured the upper and lower bounds of flow between which the massive increase in Ke occurred, as previously reported. These bounds were obtained in Group 2 animals and in 6 of Group 3 animals at a total of 12 potassium electrode sites, and are shown in figure 4. The lower bound for Group 2 was 11.0 ± 2.8 ml/100g/min and the upper was 13.0 ± 4.2 ml/100g/min. These are both higher than, but not statistically different from, the corresponding bounds found in the previous series, namely 7.6 ± 2.2 and 11.4 ± 2.6 ml/100g/min respectively.

The bounds for the Group 3 animals, on the other hand, were lower than those of the previous series: 6.5 ± 1.8 and 9.5 ± 1.5 ml/100g/min, but again the differences were not statistically significant (fig. 4).

These trends in the data may be summarized in terms of the mid-points of the brackets: 12.0, 9.5, and 8.0 ml/100g/min for the pentobarbital, previous and methohexital groups respectively.

**Modifications to the Ischemically Induced Rate of Disappearance of the EP.** In the control phase, following the administration of barbiturate, the EP amplitude decreased to a minimum (mean = 32% of control) and then recovered steadily until, just before the occlusion, it had reached a mean steady value of 74% of control. With 2 animals the EP increased 10-30% above pre-barbiturate level within a few minutes of the start of the barbiturate infusion (methohexital) before decreasing. There was a significant correlation (r = 0.7, p < 0.05) between the reduction in the amplitude of the primary EP and the reduction in cortical flow measured in the same region, both variables being expressed as percentages of the pre-barbiturate values, and averaged over the control period. This relationship is shown in figure 5. The slope of the regression line is practically unity.

In the clip phase, with 4 animals of Group 2 and 6 of Group 3, the rate of decrease of EP amplitude was measured during steady flow conditions. These data...
were compared with corresponding data of a previous study\textsuperscript{14} in which a significant correlation had been demonstrated between rate of decrease and steady flow during occlusion. Figure 6 illustrates this comparison, the data of the previous series having been augmented to include results from a total of 18 animals in which chloralose alone was used to give the regression line shown.

The 4 Group 2 points all lie to the right of the regression line, but with no significant deviation from it. In contrast, the 6 Group 3 points all lie to the left of the regression line, indicating a significant deviation for the group \((p < 0.03, 2\text{-tailed sign test})\). The Group 3 line, in addition, has a significantly lower intercept on the vertical axis \((p < 0.01)\) than that of the chloralose-alone data. These differences are interpreted as implying that, for a given flow, the rate of decrease of the EP was less in the methohexitol Group 3 than in the chloralose-alone group. The data of figure 6 suggest also that the flow threshold, below which the EP starts to become attenuated, was not significantly changed by barbiturates.

**Discussion**

**Changes in Blood Flow Distribution.**

The well-known observation that cerebral blood flow is depressed by some anesthetics, including barbiturates, has been confirmed in the present study. However, the multiple micro-regional measurements of flow, with stabilization of the BP, have now demonstrated that the flow depression induced by barbiturate in a given region of cerebral cortex depends on the degree of ischemia initially present in that region. The significant correlation between these variables (fig. 2) suggests a linear relationship and shows the flow-homogenizing effect of barbiturate, higher flows being reduced more than lower flows and differences in flow between cortical regions tending to be reduced.\textsuperscript{14} It is of interest that the percentage reduction in flow by barbiturate, averaging about 40\% as expressed by the slope of the line in figure 2, is similar in value to the depression of CMRO\textsubscript{2} by barbiturate as reported by Michenfelder et al.\textsuperscript{1, 13}

The regression line in figure 2 does not pass through the origin but crosses the axis of \(F_c\) (the initial degree of ischemia established by MCA occlusion) at some positive value. The 95\% confidence band of this crossover point includes the threshold value of flow associated with abolition of the cortical EP and other electrical activity of cortex.\textsuperscript{14} This result suggests that flow is depressed by the barbiturate only down to the EP threshold; if electrical activity is abolished by the initial ischemia in a given region, then barbiturate will not reduce the flow further. It also suggests that barbiturates reduce flow, not by direct action on the vessel wall, but indirectly by reduction of electrical activity and metabolic rate.

**Potassium and the EP**

The results from Groups 2 and 3 confirm the earlier results of others\textsuperscript{15, 16} that plasma potassium values fall significantly upon administration of anesthetics, such as barbiturates. The reason for this systemic effect is not clear. It has been attributed to decreased muscular activity, leading to reduced potassium release,\textsuperscript{18} or to an increase in extracellular fluid volume which dilutes extracellular, and thereby plasma, potassium.\textsuperscript{19} In our experiments the animals were immobilized and decreased muscular activity could not have been responsible.

In the normally-perfused brain, a clearly discernible decrease (averaging 1 mM) in extracellular potassium activity \((Ke)\) occurred promptly following intravenous barbiturate injection (fig. 3). This decrease was on average greater than the corresponding fall in plasma potassium, suggesting that the fall in Ke was not caused by the drop in plasma potassium but originated from changes in brain metabolism. The decrease in electrical activity associated with barbiturate anesthesia may underly the observed Ke decrease. It is remarkable that an average of only 2 mg/kg of methohexitol was sufficient to produce a significant fall in Ke.

Although the flow threshold for the massive Ke increase in ischemia was reduced (fig. 4) by methohexitol to 8.0 ml/100g/min, on average, from the value of...
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down to a lower flow threshold, the effect is probably not a marked one. These results do not necessarily conflict with those of Astrup et al., who showed that a reduction in pre-ischemic CMRO₂ increased the time to terminal membrane depolarization (the massive Ke increase) following abrupt onset of total ischemia, since our results pertain to a steady state of maintained low flow.

Our conclusion regarding the threshold of failure of the EP is similar. There is no indication (fig. 6) that the EP threshold is reduced by methohexital, but again there is a suggestion of an increase by pentobarbital. Our data do, however, show that once the EP has started to diminish in amplitude, methohexital reduces significantly the rate of amplitude decrease (for a given flow value) in comparison with the rate found in a previous study where chloralose alone was used. The explanation may be that methohexital reduces the rate of depletion of synthetically-based ATP and the local metabolic consumption of O₂ through the reduction in electrical activity. This result is analogous to the reduced rate of increase of Ke found by Astrup et al. Such an effect was not found with pentobarbital in our experimental format, possibly because it has less lipid solubility, and thus less cell penetration, than methohexital.

Hemodynamic Protective Factor (Inverse Steal)

Our data show that methohexital produces a significant elevation of flow in regions of cortex where the flow following MCA occlusion is below the EP threshold, provided the blood pressure is held steady. This elevation, although small (averaging 3.4 ml/100g/min), is statistically significant and a substantial fraction of the pre-treatment flow itself. The flow increase most likely results from a reduction in flow occurring in relatively well-perfused cortical regions following the reduction in metabolic rate and vasoconstriction by the barbiturate; blood is thereby diverted into the relatively ischemic regions (an inverse steal). Previous work indicates, further, that the elevation in flow found in the present study was not likely to have resulted from a presumed spontaneous improvement in collateral cortical flow unrelated to the effect of barbiturate (fig. 1, right).

An inverse steal effect would enlarge the size of the viable region of brain in which electrical activity had been silenced but irreversible structural damage had not occurred, and thus would tend to reduce the size of a developing infarct. It may, therefore, be one factor in the protective effect of barbiturate in focal cerebral ischemia on the neurological and neuropathological levels. Further work should clearly include the assessment of sub-anesthetic doses to establish whether, under conditions of unsupported blood pressure, the inverse steal can still be obtained: a major fraction of the effect of pentobarbital on glucose utilization can be obtained at sub-anesthetic doses. It is also possible that substances other than barbiturates might produce the desired local hemodynamic effect without producing anesthesia or hypotension. Such drugs might have to be region-specific in their action, perhaps depressing cortical metabolism and reducing flow in relatively normal cortex but not affecting, for example, the brainstem.

References

10. Michenfelder JD, Milde JH: Cerebral protection by anaesthetics during ischaemia (a review). Resuscitation 4: 219-233, 1975

Influence of Various Agents on the Development of Brain Edema in the Rat Following Microembolism

Protective Effect of Gamma-Butyrolactone

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SUMMARY Brain edema was induced in rats by injecting 50 μ microspheres, labelled with 85Sr, into the internal carotid artery. The use of radioactive microspheres as embolic agents enabled the number of microspheres to be determined in each cerebral hemisphere. Edema was assessed 12 or 24 h after embolization by measuring brain water content and, in some experiments, sodium and potassium.

Pretreatments with dexamethasone, parachlorophenylalanine (an inhibitor of 5-hydroxytryptamine synthesis), mepyramine and metiamide (H, and H, histamine receptor antagonists) or aminophylline did not influence significantly the development of brain edema evaluated 24 h after embolization. Aminophylline treatment (100 mg/kg) markedly increased mortality following embolization.

Gamma-butyrolactone (300 mg/kg, every 2 h) inhibited significantly the development of brain edema evaluated 12 hours after embolization. Increases in water and sodium in the embolized cerebral hemisphere were reduced by about 50%. This protective effect may be related to the known depressant action on brain metabolism.

BRAIN EDEMA is an important clinical problem and a number of experimental models have been described for producing brain edema with infarction. Among them, embolic methods have been proposed which consist of injection of microemboli into the carotid arterial system.1-8 These methods have not been used to study the effect of anti-edema therapy, perhaps due to the difficulty of obtaining reproducible embolization.

In previous work,9 we described a brain edema model using radioactive calibrated microspheres as the embolic agent permitting determination of the degree of brain embolization. It was demonstrated that the amount of brain edema and degree of increase in the blood-brain barrier permeability was proportional to the number of microspheres present in a cerebral hemisphere. In the present investigation, this model has been used to study the effect of various agents on brain edema development.

Methods
Male Iffa-Credo rats (220–300 g) were anesthetized with chloral hydrate (360 mg/kg, i.p.). About 4,000 carbonized microspheres (3M, 50.6 ± 2.5 μ in diameter, labelled with 85Sr) were injected into the left internal carotid artery as previously described.9 Rats were decapitated 12 or 24 h after embolization. The brain was removed, the cerebellum and the brain stem were discarded, both hemispheres were placed into tared vials and their radioactivity was determined in a scintillation crystal well counter (Nuclear Chicago). In order to calculate the number of microspheres contained in each sample, the mean radioactivity in one microsphere (about 3-6 cpm) was determined by simultaneous counting of a measured microsphere suspension in a hemocytometer. Animals which showed an abnormal distribution of microspheres were discarded.

Water content (g H₂O/100 g, fresh weight) was determined by difference after drying at 95°C to a
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