Effect of pentobarbital on the reactivity of isolated human cerebral arteries

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The authors have analyzed the effect of pentobarbital (10^-5M to 10^-3M) on the contractile activity of isolated human cerebral arteries. Pentobarbital was found to inhibit both the spontaneous mechanical activity and the basal tone of these vessels. Relaxation induced by this drug was dose-dependent, and was more marked when the arterial tone was previously increased with noradrenalin, potassium chloride, or 5-hydroxytryptamine. In addition, pentobarbital inhibits, in a dose-dependent manner, the contractions elicited by these vasoconstrictor agents. The present findings indicate that barbiturates decrease cerebrovascular reactivity, and disagree with the hypothesis that these drugs reduce raised intracranial pressure by means of exerting a direct constrictive effect on the cerebral arteries.

KEY WORDS • cerebral artery • barbiturate • noradrenalin • serotonin • intracranial pressure

It is widely accepted that barbiturates are effective in lowering raised intracranial pressure (ICP), and several reports indicate that the use of barbiturate-induced coma improves the final outcome of patients suffering from intracranial hypertension. However, it is still doubtful whether these drugs greatly influence outcome, and a randomized trial of barbiturate therapy in head-injury patients is needed.

It has been hypothesized that pentobarbital exerts a direct constrictive effect on cerebral vessels. This action would decrease the total cerebral blood volume (CBV), allowing a reduction in ICP. By the same proposed mechanism, barbiturates would offer protection in stroke by diverting blood flow from the healthy areas of the brain to ischemic areas. However, it is still not known how barbiturates work or if their beneficial effects in different pathological states are due to reduction in ICP, to reduction in the metabolic requirements of the brain, or a combination of both. In addition, the side-effects of these drugs and their interaction with other forms of therapy should be investigated.

The present experiments were designed to clarify the effect exerted by different doses of pentobarbital on the contractile activity of human cerebral arteries in vitro.

Materials and Methods

Cerebral Arteries and Recording System

Twenty-four cerebral artery segments (branches of the middle cerebral artery) were obtained from humans who had died of acute myocardial infarction (four cases) or infection of the small bowel (one case). The brains were removed within 6 to 10 hours of death, and the arteries were carefully dissected free of arachnoid tissue. These vessels were placed in a Petri dish containing fresh Krebs-Henseleit solution, and cut into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric recording in an organ bath containing 6 ml of Krebs-Henseleit solution at 37°C, continuously bubbled with a mixture of 95% O₂ and 5% CO₂, which gave a pH of 7.4 to 7.5. Two stainless steel pins, 150 μm in diameter, were introduced through the arterial lumen. One pin was fixed to the bath wall and the other was connected to a strain gauge for isometric tension recording. The latter pin was parallel with the former and movable,
FIG. 1. Effect of pentobarbital (PB) on the basal tone and the contractile responses of human cerebral artery segments induced by noradrenalin (NA), 5-hydroxytryptamine (5-HT), and potassium chloride (K+). After an equilibration period, PB (10^-5M to 10^-1M) was added to the bath cumulatively to observe its effect on the basal tone. After a period of repeated washing (W), contractions with NA, K+, and 5-HT were elicited (D). When sustained contractions were reached, PB (10^-4M to 10^-3M) was again added to the bath in a cumulative manner to observe its effect on the maximum contractions elicited by these three agents. After another period of repeated washing (W), PB (10^-4M solid line, and 10^-3M broken line) was added 10 minutes before NA, K+, and 5-HT were placed in the bath (D).

Thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The recording system included a force-displacement transducer and a Grass polygraph.* A resting tension of 1 gm was applied to the cylinders and readjusted every 15 minutes during a 90- to 120-minute equilibration period in which the basal tension became stable.

Analysis of Drug Effects

When the baseline tone was stable, three doses of pentobarbital (10^-4M to 10^-1M) were added to the bath cumulatively in order to test the effect of this drug on the basal tension. The preparations were then repeatedly washed with normal Krebs-Henseleit solution for 30 minutes until the arterial tone returned to the baseline. In separate preparations, 5-hydroxytryptamine (5-HT, serotonin, 10^-5M), noradrenalin (10^-3M), and potassium chloride (K+, 120 mM) were then added to the organ bath and, when maximum contraction was reached, pentobarbital (10^-4M to 10^-1M) was administered to the medium to test the effect of this agent on the contractions. The bath was then washed repeatedly for 20 minutes with Krebs-Henseleit solution until the baseline tone was again reached, and pentobarbital (10^-4M or 10^-3M) was then added to the medium. Ten minutes later, noradrenalin, K+, and 5-HT were administered so that the effect of pentobarbital on the contractions elicited by these agents could be observed. The influence of the suppression of calcium from the medium on the spontaneous mechanical activity of the arteries and the K+-induced contractions was also analyzed.

Solutions and Drugs

The composition of Krebs-Henseleit solution was (mM): NaCl 115; KCl 4.6; CaCl2 2.5; KH2PO4 1.2; MgSO4 0.7; H2O 1.2; NaHCO3 25; glucose 11.1, and disodium salt of ethylenediamine tetra-acetic acid (Na2 EDTA) 0.03. Calcium-free Krebs-Henseleit solution was prepared by omitting CaCl2. These solutions were prepared on the day of the experiment, and the chemicals used were of analytical grade.

The drugs used were 5-hydroxytryptamine creatinine sulfate, noradrenalin bitartrate, potassium chloride, and sodium pentobarbital.

Results

Several arteries showed spontaneous contractile activity that was calcium-dependent because it was abolished in a calcium-free medium. A 10^-4M dose of pentobarbital inhibited these spontaneous contractions, and a 10^-3M dose completely blocked them. Figure 1 shows the effect of three doses of ponto-
Pentobarbital effect on cerebral arteries

Pentobarbital (10^{-6}M to 10^{-3}M) on the basal arterial tone and the relaxing action of these doses on arteries contracted by noradrenalin, K^{+}, and 5-HT. This figure represents a typical recording from an experiment. The results of similar experiments performed in six to 10 vascular segments are summarized in Figs. 2 and 3. Pentobarbital produced a dose-dependent lowering of both the basal arterial tone and contractions induced by noradrenalin, K^{+}, and 5-HT that reached a maximum at 10^{-3}M. The contractions induced by noradrenalin, K^{+}, and 5-HT were (mg ± SEM): 1468 ± 253, 1900 ± 379, and 1585 ± 278, respectively.

The addition to the organ bath of pentobarbital (10^{-4}M or 10^{-3}M at 10 minutes preincubation) caused a dose-dependent inhibition of the contractions induced by noradrenalin, K^{+}, and 5-HT. The order of magnitude of this inhibitory effect was K^{+} > 5-HT > noradrenalin. A typical experimental tracing is shown in Fig. 1, and the results obtained in six to eight arterial segments are summarized in Fig. 4. The reduction induced by pentobarbital in the contractions caused by K^{+} was remarkable: 10^{-3}M barbiturate practically abolished the tension caused by this ion. The contraction induced by K^{+} was shown to be calcium-dependent, because this agent did not elicit any contractile response in a calcium-free medium.

Discussion

It has been reported by other authors that pentobarbital in anesthetic concentrations directly inhibits the spontaneous mechanical activity of the peripheral arteries and veins of the rat, as well as the contractions induced by noradrenalin, K^{+}, and serotonin in these vessels. In addition, the observation that pentobarbital reduces release of noradrenalin caused by vasoconstrictor agents or electrical stimulation helps to explain the depressant effect of this drug on peripheral vascular tone.

The findings reported here show that pentobarbital inhibits the spontaneous mechanical activity of human cerebral arteries and causes a dose-dependent relaxation of these vessels. This last effect was more marked when the arterial baseline tension had previously been increased with noradrenalin, K^{+}, or serotonin than when the vessel showed only its basal tone. On the other hand, pentobarbital was seen to inhibit in a dose-dependent manner the contractions elicited by these three agents. The evidence that both the K^{+}-induced contractions and the spontaneous mechanical activity of human cerebral arteries are calcium-dependent, added to the fact that pentobarbital decreases these responses in a dose-dependent manner, suggests that barbiturates interfere with calcium movement (probably calcium entry into the vascular smooth-muscle fiber). Both the calcium dependence of K^{+}-induced contractions and the interference by pentobarbital with calcium movements have been previously observed in other preparations.

The above findings disagree with the hypothesis that barbiturates directly constrict the cerebral arteries, thereby decreasing CBV and causing the rapid fall in ICP observed in patients treated with an intravenous bolus of pentobarbital or thiopental. In fact, our results show that pentobarbital does not constrict, but relaxes cerebral arteries in vitro, and it may be assumed that the effect is the same in vivo. If this assumption holds true, it is difficult to understand how barbiturates decrease CBV. A possible explanation for the pentobarbital-induced fall in ICP might be that this agent relaxes the major cerebral arteries, as in the present experiments, but not the arterioles; in this way, the increased hydrostatic pressure at the arteriolar level would trigger a vasoconstriction of this particular sector (the Bayliss effect), thereby decreasing total CBV. Whatever the mechanism, further in vivo studies are needed to elucidate how barbiturates
modify cerebrovascular dynamics in order to achieve a reduction of intracranial volume.

The present experiments indicate that high doses of barbiturates used in iatrogenic coma (between $10^{-4}$M and $10^{-3}$M or even greater) decrease cerebrovascular reactivity. This evidence is indirectly supported by the observation that these agents not only reduce the resting levels of ICP in head-injured patients but also stabilize the pressure recording, blunting the peaks induced by stimuli liable to increase ICP by means of a vascular-mediated response (Marshall, et al., and our unpublished observations).

Finally, because of the well known depressant cardiovascular effects of barbiturates and the impairment of cerebrovascular reactivity caused by these drugs, it seems critical to avoid extreme decreases of systemic blood pressure which may compromise cerebral perfusion in patients exposed to barbiturate coma.

References


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