J. Pharm. Pharmacol. 1981, 33: 357–361
Received October 27, 1980

0022–3573/81/060357–05 $02.50/0

Interference of pentobarbitone with the contraction of vascular smooth muscle in goat middle cerebral artery

JESUS MARÍN*, MERCEDES L. RICO AND MERCEDES SALAICES

Departmento de Farmacologia y Terapéutica, Facultad de Medicina Universidad Autónoma, C/Arzobispo Morcillo 4 Madrid-34, Spain

Pentobarbitone (10^{-5} to 10^{-3} M) decreased the basal tone of vascular smooth muscle of goat middle cerebral artery in a dose-dependent manner as well as relaxing established contractions induced by noradrenaline (NA) (10^{-5} M), 5-hydroxytryptamine (5-HT) (10^{-5} M) and KCl(120 mM). Preincubations with pentobarbitone reduced the contractions evoked by these three agents in a dose-dependent way. It also decreased Ca^{2+}-induced contractile responses in K^+-depolarized arteries and 5-HT-Ca^{2+} and NA-Ca^{2+} contractions dose-dependently. Contractions induced by K^+ were more sensitive to the depressant actions of the drug than those produced by NA and 5-HT. The small contractions evoked by K^+ and 5-HT in Ca^{2+}-free medium were also reduced in its presence. The antagonism Ca^{2+}-pentobarbitone was insurmountable. These results suggest that the drug interferes with Ca^{2+} entry and Ca^{2+} release from cell stores, and therefore with the smooth muscle contractions.

Barbiturates depress Na^+ conductance (Blaustein 1968; Seeman 1972), Ca^{2+} permeability of cell membrane (Blaustein 1976; Blaustein & Ector 1975; Altura & Altura 1975), and Ca^{2+} uptake activities of microsomal (Nayler & Szeto 1972), and mitochondrial fractions (Dransfield et al 1969). They interfere with transmission in sympathetic ganglia, in part at least, by blocking the Ca^{2+} entry and the subsequent transmitter secretion in presinaptic endings (Blaustein 1976). Pentobarbitone depresses the vascular smooth muscle of peripheral vessels (Altura & Altura 1975).

Barbiturates have been employed in reducing raised intracranial pressure (Marsh et al 1977; Marshall et al 1979a), but little is known of their effects on the brain vessels. Marsh et al (1977) suggested that the initial reduction in intracranial hypertension is due to that having a direct vasoconstrictor effect on brain vessels. This hypothesis has not been proved. We have attempted to clarify the direct effect of pentobarbitone on the smooth muscle of cerebral vessels, for this we used isolated segments of goat middle cerebral arteries.

MATERIALS AND METHODS

Brain vessels and recording system

Female goats (30–45 kg) were killed by injecting i.v. 30 ml of saturated solution of potassium chloride. The brain was removed and the middle cerebral arteries dissected and cut into cylindrical segments 4 mm in length. Each cylinder was set up in an organ bath containing 6 ml of Krebs-Henseleit solution (KHS) at 37 °C continuously bubbled with a 95% O_2–5% CO_2 mixture which gave a pH of 7·4–7·5. Two stainless steel pins, 150 μm diameter, were introduced through the lumen of each segment. One pin was fixed to the bath wall, the other, connected to a strain gauge for isometric recording, was in a parallel position and movable, 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 (Grass FTO3C) and a Grass Model 7D Poligraph. A resting tension of 1g was applied to this tissue and readjusted every 15 min during 90 to 120 min equilibration in which the basal tension became stable; the basal tension was readjusted throughout the experiment. Then contractile responses were evoked by noradrenaline (NA), 5-hydroxytryptamine (5-HT) or potassium chloride (K^+).

Analysis of drug effect

The ability of pentobarbitone to antagonize the contractions caused by 5-HT (10^{-5}M), NA(10^{-5}M) and K^+ (120 mM) as well as its action on the basal tension was tested. When the baseline was stable three doses of drug 10^{-5} to 10^{-3} M, were added to the bath cumulatively. After, the preparations were

* Correspondence.

washed repeatedly with normal KHS until the tonus of the artery returned to the basal tension in 30 min. Then the vasoconstrictor agents were added to the bath and at the height of the contraction the barbiturate (10^{-5} to 10^{-3} M), to test the influence of vascular tone on the effects of these agents. Twenty min later, during which time the bath was washed several times with KHS and the baseline recovered, pentobarbitone (10^{-4} or 10^{-3} M) was added, this was followed 10 min later by the vasoconstrictor agents to observe the inhibitory effects of the barbiturate on the contractions.

To allow analysis of the influence of extracellular Ca^{2+} on the contractions induced by NA, 5-HT and K^+ and the effect of pentobarbitone on Ca^{2+} evoked contractions, the arterial segments were exposed for 30 min to Ca^{2+}-free medium, and later, cumulative dose-response curves to $CaCl_2$ were determined in the absence and in the presence of pentobarbitone (10^{-4} or 10^{-3} M, 10 min preincubation).

Solutions, drugs and statistical evaluation

The composition of the Krebs-Henseleit solution was (mM): NaCl 115; KCl 4·6; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4 \cdot 7\ H_2O$ 1·2; $NaH\text{-}CO_3$ 25; glucose 11·1; disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA) 0·03. The solution for K^+-depolarization contained in total 120 mM of KCl and no NaCl. Ca^{2+}-free KHS was prepared by omitting $CaCl_2$ and 1 mM ethyleneglycol-bis (β-aminoethylether) *NN'*-tetraacetic acid (EGTA) was added to reduce contaminating Ca^{2+}. These solutions were prepared on the day of use and the chemicals were of analytical grade.

The drugs used were: 5-hydroxytryptamine creatinine sulphate (Sigma), noradrenaline bitartrate (Sigma), sodium pentobarbitone (Abbot). Statistical analysis was done according to conventional procedures (Snedecor & Cochran 1967). The inhibitory effects of pentobarbitone on the contraction evoked by the agonists were expressed as a percentage of the response caused by the agonists in the absence of barbiturate. Each point of the curves was calculated as the mean of 8–10 experiments and expressed as mean ± s.e.m.

RESULTS

Influence of pentobarbitone on the resting tension and its relaxant effects on NA, 5-HT and K^+ evoked contractions

The relaxing effects induced by three doses of barbiturate (10^{-5}, 10^{-4}, 10^{-3} M) on the resting tension and on the contraction evoked by NA, 5-HT and K^+ are shown in Figs 1 and 2, respectively. The vasodilator responses were greater with arteries contracted by the agonists than with those having only basal tension.

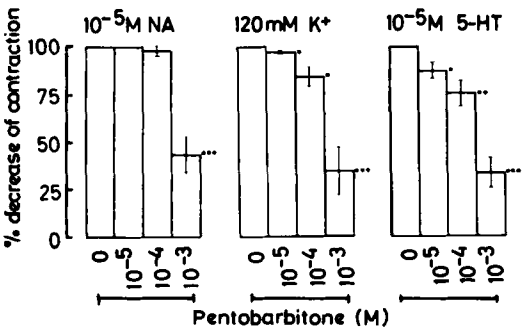

FIG. 1. Effect of pentobarbitone (10^{-5} to 10^{-3} M) added in a cumulative manner on the basal tonus of 8 to 10 segments of goat middle cerebral artery. Each point and vertical bars represent the means values and s.e.m., respectively.

Comparison of the inhibitory effects of pentobarbitone on the contractions evoked by NA, 5-HT and K^+

Pre-incubation (10 min) of segments with pentobarbitone (10^{-4} or 10^{-3} M) caused a dose-dependent inhibition of contractions induced by NA, 5-HT and K^+ on the order $K^+ > NA > 5\text{-HT}$ (Fig. 3). At 10^{-3} M the barbiturate practically abolished the tension

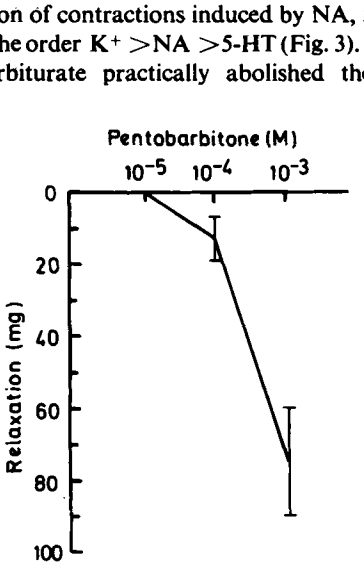

FIG. 2. Relaxant effects of pentobarbitone (10^{-5} to 10^{-3} M) on NA, 5-HT-and K^+-induced contractions in segments of goat middle cerebral artery. After the height of the contraction was reached with these agonists, pentobarbitone was added accumulatively. Mean 100% isometric responses for the three agonists were in mg: 489 ± 54, 1984 ± 362 and 1976 ± 500, respectively, values are mean ± s.e.m. 8 to 10 different arterial segments were used (* $P < 0.05$; ** $P < 0.01$; *** $P > 0.005$).

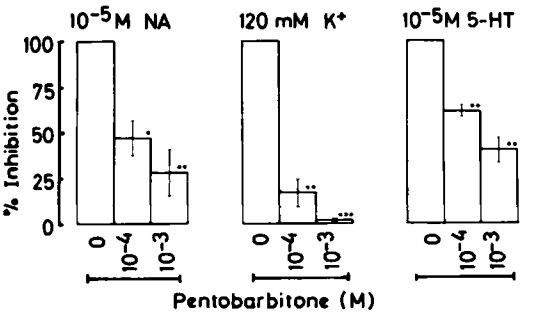

FIG. 3. Different sensitivities to the inhibition by pentobarbitone (10^{-4} and 10^{-3} M) of the contraction induced by NA, 5-HT and K^+. Mean 100% isometric responses for each agonist were in mg: 489 ± 54, 1984 ± 362 and 1976 ± 500, respectively, pentobarbitone was added 10 min before exposure to the agonists. 8 to 10 different arterial segments were used (*$P < 0.05$; **$P < 0.025$; ***$P < 0.001$).

caused by K^+. This sensitivity is probably because K^+-induced contractions depend essentially on the influx of extracellular Ca^{2+} which the drug blocks.

Influence of pentobarbitone and extracellular Ca^{2+} on NA, 5-HT and K^+ induced contractions

5-HT and K^+ caused small contractions of the vessels in a Ca^{2+}-free medium while NA did not (Fig. 4). When 2·5 mM Ca^{2+} was added to the bath the arteries began to contract. Pentobarbitone (10^{-4}M) did not significantly diminish the magnitude of Ca^{2+}-evoked contractile responses except in the case of K^+, while 10^{-3} M pentobarbitone clearly depressed these contractions above all those induced by K^+ (Fig. 4).

The Ca^{2+}-pentobarbitone antagonism was insurmountable, especially at 10^{-3} M, since even when the Ca^{2+} concentration in the bath was increased (from 8 to 10 mM) the arteries did not recover the initial contraction obtained in the absence of the barbiturate.

The inhibitory effect of pentobarbitone practically disappeared (over a 10 min period) when the arteries were repeatedly washed with normal KHS. With six control arteries to which no addition of pentobarbitone was made, the response induced by each drug was maintained during three applications of each agent, made at 30 min intervals. The arteries were then exposed to 0 Ca^{2+}-KHS for 30 min and after administration of each drug and an addition of 3·5 mM Ca^{2+} to the bath, the vessels did not completely re-establish their initial contraction as obtained in normal KHS. This seems to be typical of these vessels because in peripheral arteries 0·3 to 0·5 mM Ca^{2+} re-established the initial response in similar experimental situations (McLean et al 1978; Hester & Carrier 1978). This indicates that the lower response caused by the three agents in brain vessels previously exposed to Ca^{2+}-free solution is not due to a residual effect of pentobarbitone remaining after washing.

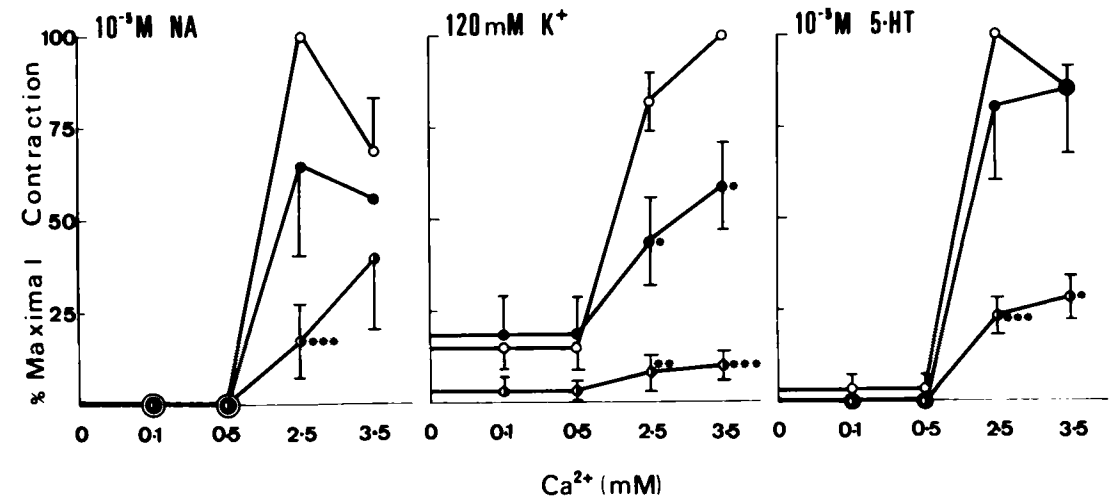

FIG. 4. Influence of pentobarbitone (10^{-4} and 10^{-3} M) on $CaCl_2$-induced contractions in 8 to 14 different segments of goat middle cerebral artery. After 30 min of exposure to Ca^{2+}-free medium NA, 5-HT and K^+ were added and then graded doses of Ca^{2+}. In this manner control contractions were obtained. The process was repeated but with previous addition of pentobarbitone. Values are mean ± s.e.m. Mean 100% isometric response in mg were: 287 ± 57; 1512 ± 203; 1196 ± 244, respectively (*$P < 0.05$; **$P < 0.025$; ***$P < 0.001$). ○ Control. ● Pentobarbitone 10^{-4} M. ◑ Pentobarbitone 10^{-3} M.

DISCUSSION

Anaesthetic doses of barbiturates can produce a fall in mean arterial pressure (Price 1975; Marshall et al 1979b) and relax isolated peripheral arteries (Altura & Altura 1975). Pentobarbitone reduces NA release induced by drugs or electrical stimulation (Göthert 1979; Lindmar et al 1979). High doses of the drug are used to reverse raised intracranial pressure (Marshall et al 1979a, b), an effect suggested to be a result of increasing cerebral vascular tone (Marsh et al 1977). Our findings do not support this since the drug produced in goat brain vessels dose-dependent relaxing responses that were greater when the arteries were given previous tone with NA, 5-HT and K^+. Therefore, pentobarbitone in anaesthetic plasma concentrations (around 2×10^{-4} M, Altura & Altura 1975; Lindmar et al 1979), caused vasodilatation of the cerebral arterial bed by direct depression of vascular tone.

The experiments NA-Ca^{2+}, 5-HT-Ca^{2+} and K^+-Ca^{2+} showed that extracellular Ca^{2+} is a necessary requirement for contraction of these arteries by the three agents. Ca^{2+} has been reported to be the final activator of contractile proteins (Bohr 1973; Allen 1977). The fact that in Ca^{2+}-free medium none of the three agents attained the same contraction obtained in normal KH5 when 3·5 mM Ca^{2+} was added to the bath indicated that extracellular Ca^{2+} seems to reduce its own permeability by an autoinhibitory effect similar to that reported by Allen et al (1976) in canine basilar artery.

The contractions induced by noradrenaline, in our case, did not show the classical two components observed in peripheral vessels, i.e., phasic (Ca^{2+}-independent) and tonic (Ca^{2+}-dependent) (McLean et al 1978; Hester & Carrier 1978; Broekaert & Godfraind 1979). 5-HT and K^+ showed a small contraction in Ca^{2+}-free solution.

The reduction of the response to added Ca^{2+} by pentobarbitone suggests it to interfere with the contraction evoked by these agonists by preventing the Ca^{2+} influx to the cell (Blaustein 1976) or Ca^{2+} access to contractile proteins. That pentobarbitone decreased the Ca^{2+}-independent contractions of K^+ and 5-HT (according to the dose) indicates that the drug interferes with Ca^{2+}-release of cellular stores or more probably displaces Ca^{2+} binding from superficial sites of the cell membrane as was reported by Nayler & Szeto (1972), this superficial pool of Ca^{2+} being important for excitation-contraction coupling (Goodman & Weiss 1971; Bohr 1973; Bolton 1979).

The different responses to Ca^{2+} of the three agonists is probably the result of differing effects by them on the supply of Ca^{2+} to the mechanism controlling vascular tone (Bohr 1973). This could explain the different sensitivities of pentobarbitone towards inhibiting the responses of each agent, NA and 5-HT contractions being less sensitive to the barbiturate than those induced by K^+, possibly because K^+-induced contractions are dependent on extracellular Ca^{2+}, and in this case the cell membrane is depolarized, making it highly permeable to Ca^{2+} which enters through the potential-sensitive channels (Bolton 1979). These channels seem to be more affected by pentobarbitone than other channels controlled by a receptor (this is the case for NA and 5-HT) probably having a higher permeability to ions others than Ca^{2+} (Bolton 1979). It has been suggested that amongst other effects, the barbiturates produce an expansion and fluidization of cell membrane, this mechanism being that which interferes with Ca^{2+} entry into the cell (Seeman 1972).

The fact that after addition of high concentrations of Ca^{2+} the arteries did not revert back to the contraction evoked by the three agonists, suggests that the effect of the barbiturate on Ca^{2+} entry seems to be unsurmountable. This finding has also been observed in other tissues (Blaustein & Ector 1975; Lindmar et al 1979).

Acknowledgements

This work was supported in part by Ministerio de Sanidad and Comisión Asesora de Investigación Científica y Técnica.

REFERENCES

Allen, G. S. (1977) Neurosurgery 1: 142–148

Allen, G. S., Gross, C. J., Henderson, L. W., Chou, S. N. (1976) J. Neurosurg. 44: 585–593

Altura, B. T., Altura, B. M. (1975) Am. J. Physiol. 229: 1635–1640

Blaustein, M. P. (1968) J. Gen. Physiol. 51: 293–307

Blaustein, M. P. (1976) J. Pharmacol. Exp. Ther. 196: 80–86

Blaustein, M. P., Ector, A. C. (1975) Mol. Pharmacol. 11: 366–378

Bohr, D. F. (1973) Circ. Res. 32: 665–672

Bolton, T. B. (1979) Physiol. Rev. 59: 606–718

Broekaert, A., Godfraind, T. (1979) Eur. J. Pharmacol. 53: 281–288

Dransfield, H., Greeff, K., Schorn, A., Ting, B. (1969) Biochem. Pharmacol. 18: 1335–1345

Goodman, F. R., Weiss, G. B. (1971) Am. J. Physiol. 220: 759–766

Göthert, M. (1979) in: Paton, D. M. (ed) The Release of Catecholamines from Adrenergic Neurons. Pergamon Press, Oxford, pp 241–261

Hester, R. K., Carrier, O. Jr. (1978) Arch. Int. Pharmacodyn. Ther. 233: 21–41

Lindmar, R., Löffelholz, K., Weide, W. (1979) J. Pharmacol. Exp. Ther. 210: 166–173

Marsh, M. L., Marshall, L. F., Shapiro, H. M. (1977) Anesthesiology 47: 149–163

Marshall, L. F., Smith, R. W., Shapiro, H. M. (1979a) J. Neurosurg. 50: 20–25

Marshall, L. F., Smith, R. W., Shapiro, H. M. (1979b) Ibid 50: 26–30

McLean, A. J., Barron, K., Du Souich, P., Haegele, K. D., McNay, J. L., Carrier, O., Briggs, A. (1978) J. Pharmacol. Exp. Ther. 205: 418–425

Nayler, W. G., Szeto, J. (1972) Am. J. Physiol. 222: 339–344

Price, H. L. (1975) in: Goodman, L. S., Gilman, A. (eds) The Pharmacological Basis of Therapeutics. 5th edn Macmillan, New York, pp 102–123

Seeman, P. (1972) Pharmacol. Rev. 24: 583–655

Snedecor, G. M., Cochran, W. G. (1967) Statistical Methods. 6th edn Ames, The Iowa State University Press, Iowa