Biochimica et Biophysica Acta, 595 (1980) 309—312

BBA Report

BBA 71423

PENTOBARBITAL SODIUM INHIBITS CALCIUM UPTAKE IN VASCULAR SMOOTH MUSCLE

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(Received July 5th, 1979)

Key words: Pentobarbital inhibition; Anesthetic action; Ca^{2+} uptake; (Vascular smooth muscle)

Summary

This report demonstrates that the commonly used anesthetic agent, pentobarbital sodium, in concentrations of $1 \cdot 10^{-4}$ to $2 \cdot 10^{-3}$ M inhibits calcium (Ca^{2+}) uptake in both rat aortic and portal venous smooth muscle. The data indicate that total exchangeable Ca^{2+} in portal vein is reduced by about 15% in $1 \cdot 10^{-4}$ M pentobarbital sodium, while the intracellular exchangeable Ca^{2+} is reduced by 24%. On the other hand, in aortic smooth muscle, while $5—20 \cdot 10^{-4}$ M pentobarbital sodium reduces total exchangeable Ca^{2+} by about 15%, intracellular Ca^{2+} is reduced by 22% in $5 \cdot 10^{-4}$ M pentobarbital sodium and by 38% in $2 \cdot 10^{-3}$ M pentobarbital sodium. The present studies thus reveal that concentrations of pentobarbital sodium known to be present during induction of surgical anesthesia can exert significant inhibitory effects on exchangeability and transmembrane movement of Ca^{2+} in at least two different types of blood vessels.

Barbiturate anesthetic agents, such as pentobarbital sodium are known to lower blood pressure. Recently, concentrations of pentobarbital known to be present during induction of surgical anesthesia (i.e. $1—5 \cdot 10^{-4}$ M) have been shown to inhibit spontaneous mechanical activity of vascular smooth muscle [1--3] and to inhibit spontaneous vasomotion in mammalian arterioles [4, 5]. Besides its direct action on blood vessels, these anesthetic concentrations of pentobarbital were also demonstrated to attenuate arterial, arteriolar, venular and venous smooth muscle responsiveness to several vasoactive substances [1—5]. These direct and indirect effects of pentobarbital have been hypothesized to be brought about via its antagonistic or inhibitory

effects on Ca^{2+} flux in vascular smooth muscle [2—5]. However, no direct evidence is, as yet, available for the latter tenet.

In view of these reports which link inhibitory effects of barbiturates on blood vessel tone to their blood pressure lowering action, and the common usage of pentobarbital as the anesthetic of choice for laboratory animals, there was a need to evaluate precisely the effects of pentobarbital sodium on Ca^{2+}, if any. We now report that concentrations of pentobarbital known to be present in the blood during induction of surgical anesthesia can inhibit exchangeability and uptake of radiocalcium in two types of vascular smooth muscle.

Aortic strips and portal veins obtained from male Wistar rats (300—400 g), were set up isometrically in vitro as described previously [2] and equilibrated for 2 h in normal Krebs-Ringer bicarbonate solution [2]. The tissues were aerated with a 95% O_2/5% CO_2 mixture and kept at 37° C at a pH of 7.4. The tissues were then incubated for 30 min in an identical Krebs-Ringer solution containing either 0, $1 \cdot 10^{-4}$, $5 \cdot 10^{-4}$ or $20 \cdot 10^{-4}$ M pentobarbital sodium to which was added 148 Bq/ml ^{45}Ca. (For the portal veins, we chose the lower concentration of pentobarbital (i.e., $1 \cdot 10^{-4}$ M), since this concentration of barbiturate produced the same degree of inhibition as did five times this amount on the aortas [2, 3].) After this time, each tissue was either rinsed in cold Krebs-Ringer bicarbonate for 10 s (conventional method) or for 2 or 5 min in 50 mM La^{3+}, Ca^{2+}-free, 5 mM Tris-Ringer medium [6, 7] (lanthanum method, as modified by Godfraind [8]). The strips were then blotted on ash-free filter paper and weighed, and the ^{45}Ca uptake was counted in a Searle Mark III liquid scintillation counter after solubilization with 1 ml NCS solubilizer at 50° C for 6 h. The 'conventional method' reveals the total exchangeable Ca^{2+} of the tissues (as verified by uptake experiments for different time periods) [9, 10]. With the 'lanthanum method'.the ^{45}Ca content is proposed to represent exchangeable (a) membrane-bound calcium (2 min wash); and (b) intracellular La^{3+}-resistant calcium (5 min wash), as the concentration of lanthanum used (50 mM) has been shown (i) to replace Ca^{2+} at superficial binding sites and (ii) to block influx and markedly retard efflux of Ca^{2+} within the 5-min contact time [6—8, 11].

Table I summarizes the results obtained in the presence and absence of

TABLE I

EFFECTS OF PENTOBARBITAL SODIUM ON TOTAL EXCHANGEABLE AND INTRACELLULAR CALCIUM CONTENT OF RAT PORTAL VEIN

Values are given as mmol/kg wet wt. (mean ± S.E.) Numbers in parentheses represent number of different tissues used for each experiment. The composition of Krebs-Ringer bicarbonate is: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 10.0 mM glucose.

Treatment	Total exchangeable calcium	2-min La^{3+} wash calcium fraction	5-min La^{3+} wash (cellular calcium)
Control	2.99 ± 0.14 (45)	2.18 ± 0.09 (31)	1.69 ± 0.14 (26)
Pentobarbital ($1 \cdot 10^{-4}$ M)	2.48 ± 0.10 (5)*	1.88 ± 0.20 (7)	1.29 ± 0.10 (4)*

*Significantly different from paired control values ($P<0.02$).

TABLE II

EFFECTS OF PENTOBARBITAL SODIUM ON TOTAL EXCHANGEABLE AND INTRACELLULAR CALCIUM CONTENT OF RAT AORTA

Values are given as mmol/kg wet wt. (mean ± S.E.). Numbers in parentheses represent number of different tissues used for each experiment.

Treatment	Total exchangeable calcium	2-min La^{3+} wash calcium fraction	5-min La^{3+} wash (cellular calcium)
Control	4.11 ± 0.10 (48)	2.09 ± 0.09 (34)	0.88 ± 0.06 (31)
Pentobarbital			
$5 \cdot 10^{-4}$ M	3.52 ± 0.14 (6)*	1.54 ± 0.13 (8)*	0.69 ± 0.04 (9)*
$2 \cdot 10^{-3}$ M	3.39 ± 0.34 (5)**	1.34 ± 0.07 (7)*	0.55 ± 0.06 (4)*

*Significantly different from paired controls ($P<0.01$).
**Significantly different from paired controls ($P<0.05$).

$1 \cdot 10^{-4}$ M pentobarbital sodium in portal venous smooth muscle with the conventional and lanthanum methods. The data indicate that while pentobarbital reduces total exchangeable Ca^{2+} by about 17% intracellular Ca^{2+} is reduced by 24% in $1 \cdot 10^{-4}$ M pentobarbital. The marked inhibition by pentobarbital sodium at this concentration, of spontaneous mechanical activity and drug-induced contractions in this tissue [2, 3] could be explained solely by a reduction in the availability of Ca^{2+}.

In aortic smooth muscle (Table II), the data indicate that while $5 \cdot 10^{-4}$ and $2 \cdot 10^{-3}$ M pentobarbital reduces total exchangeable Ca^{2+} by about 15%, intracellular Ca^{2+} is reduced by 22% in $5 \cdot 10^{-4}$ M pentobarbital and by 38% in $2 \cdot 10^{-3}$ M pentobarbital sodium. Membrane-bound Ca^{2+} is also reduced in a concentration-dependent manner by 26 and 36%, respectively, in the two different concentrations of barbiturate. Contractile inhibition seen with these concentrations of pentobarbital sodium in this tissue [2, 3] can also be explained by these results, since both intracellular and membrane-bound Ca^{2+} are thought to play important roles in agonist-induced contraction in aortic smooth muscle [11, 12]. The present results thus reveal that pentobarbital sodium exerts significant inhibitory effects on exchangeability and transmembrane movement of Ca^{2+} in at least two different types of smooth muscle.

Pentobarbital sodium has been shown by others, indirectly, to have effects on the cellular uptake of this ion. For example, it was seen to (i) decrease Ca^{2+} uptake in isolated mitochondria [13] and microsomes [14] of heart muscle (ii) be capable of uncoupling mitochondrial oxidative phosphorylation [15] (iii) exert a depressant effect on the force-frequency response in heart muscle, which is similar to that of manganese-blocked Ca^{2+} entry [16] and (iv) inhibit, Ca^{2+}-induced contractions of potassium-depolarized rat aorta and portal vein [2, 3]. It has also been shown, recently, to inhibit Ca^{2+}-ATPase activity of rat heart sarcolemmal preparations [17]. At least one other type of anesthetic molecule, namely ethanol, has also been shown to exert in the vascular smooth muscles used here, similar depressant actions

on spontaneous mechanical activity, drug-induced contractions [18, 19] and Ca^{2+} uptake [7, 10].

In view of the evidence obtained, in this study, one must seriously consider the possibilty that the reductions in arterial blood pressure and vasodilatation associated with administration of anesthetic doses of pentobarbital sodium to mammals are due, at least in part, to barbiturate-induced inhibition of Ca^{2+} exchange and movement across vascular smooth muscle cell membranes.

This research was supported by National Heart and Lung Institute Grants HL-18002 and HL-18015, National Institute of Mental Health Grant MH-26236 and National Institute of Drug Abuse DA-02339.

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