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On the local anaesthetic effect of barbiturates

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Controversy exists about whether the free acid (non-ionized) or the anionic (ionized) form of barbiturates abolish the excitation of nerves. The experiments made showed that sodium pentobarbitone and sodium phenobarbitone are more effective at pH 6·8 than 8·8 in blocking the action potential in the desheathed frog nerve. The experimental procedure employed excludes the possibility that this difference in activity is due only to a more effective rate of penetration of the non-ionized form across the nerve membrane. In the same preparation these barbiturates at both pH 6·8 and 8·8 did not interfere with the uptake and release of radiocalcium. These data suggest that barbiturates block the action potential by increasing the surface pressure of the lipid layer of the excitable membrane and do not interfere with the calcium binding to sites which govern the increased membrane conductance during excitation.

Barbiturates are known to block excitation in nerves (Heinbecker & Bartley, 1940). On the basis of extrapolated data from studies with *Arbacia* eggs (Clowes, Keltch & Krahl, 1940) and cardiac muscle (Hardman, Moore & Lum, 1959), it has been assumed that in contrast to tertiary amine local anaesthetics the unionized barbiturate is the most active form (Maynert, 1965; Sharpless 1968). Recently, however, Blaustein (1968) reported that sodium pentobarbitone blocks the action potential in the voltage-clamped lobster axon more effectively at pH 8·5 than at pH 6·7. From these results he concluded that the anionic form of these drugs appears to be more potent, and proposed that the ionized form increases the calcium binding to the excitable membrane.

In view of these contradictory results, we decided to re-evaluate the influence of pH on the local anaesthetic activity of phenobarbitone and pentobarbitone and to investigate the effect these barbiturates have on calcium binding to the nerve.

EXPERIMENTAL

Methods

Desheathed sciatic nerves of *Rana pipiens* were used. The effects of sodium pentobarbitone and sodium phenobarbitone on the action potential were examined using the sucrose gap method (Stämpfli, 1954). The nerves were mounted on a bipolar electrode and single supramaximal stimuli were applied every 2·5 min. However, when the bathing medium was changed, the nerves were stimulated every 5 s for 2 min. The evoked action potentials were recorded with an oscilloscope camera. The following schedule was observed in these experiments. The nerves were allowed to equilibrate in Ringer solution at pH 7·2 for 60 min. They were then exposed to 5 mM of either barbiturate in Ringer solution, first at pH 6·8 for 10 min, then at pH 8·8 for 10 min and finally again at pH 6·8 for 10 min. In control experiments the same schedule was followed, but no drug was present in the Ringer solution.

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The experiments on the effects of sodium pentobarbitone and sodium phenobarbitone on the uptake and kinetics of ^{45}Ca release were made on paired desheathed sciatic nerves of the same frog. One nerve was treated with either barbiturate and the other served as a control. The two procedures followed in these experiments are illustrated in Table 1. The technique for the collection of samples during the 1 h washout period was similar to that described by Bianchi & Bolton (1966). The samples were taken at 5- or 10-min intervals. The amount of ^{45}Ca remaining in the nerve after the washout period was determined in the nerve ash. The radioactivity of the collections and the remaining radioactivity of the ashed preparations were determined after adding Bray solution. The samples were counted in a Packard-Tri-Carb liquid scintillation counter.

Table 1. *Experimental procedures.* In both procedures the experimental nerves were loaded with a barbiturate at pH 6·8. In procedure I the nerve was then equilibrated with ^{45}Ca Ringer solution at pH 6·8 so no further ionization could occur. In procedure II, however, the pH of the ^{45}Ca Ringer solution was 8·8 in order to increase the degree of ionization of the barbiturates bound to the nerve membranes

		Equilibration periods (min)			Washout period
Procedure	Barbiturate	0–60	60–70	70–75	75–135
I	Pentobarbitone	Ringer pH 7·2	Ringer pH 6·8 + Barbiturate (5 mM)	^{45}Ca Ringer pH 6·8	Ringer pH 6·8
	Phenobarbitone				
	Control	Ringer pH 7·2	Ringer pH 6·8	^{45}Ca Ringer pH 6·8	Ringer pH 6·8
II	Pentobarbitone	Ringer pH 7·2	Ringer pH 6·8 + Barbiturate (5 mM)	^{45}Ca Ringer pH 8·8	Ringer pH 8·8
	Phenobarbitone				
	Control	Ringer pH 7·2	Ringer pH 6·8	^{45}Ca Ringer pH 8·8	Ringer pH 8·8

The total amount of ^{45}Ca taken up by the nerve (total ^{45}Ca content), was calculated according to Bianchi (1965), and expressed in terms of μmol ^{45}Ca/g of nerve dry weight.

The Ringer solutions consisted of 111 mM NaCl, 1·6 mM KCl, 1·0 mM $CaCl_2$ and 10 mM tris(hydroxymethyl)aminomethane at pH 6·8, 7·2 and 8·8.

The means and standard errors of the results were calculated and the level of significance determined using Student's *t*-test. The experiments were made at 20° to 22° during the winter of 1968–69.

RESULTS

Effects of pentobarbitone and phenobarbitone on the action potential

Both pentobarbitone and phenobarbitone (5 mM) markedly decreased the amplitude of the action potential (Fig. 1). After 10 min exposure to the barbiturate at pH 6·8, the size of the action potential was reduced to 41·9 $\pm$ 0·91% by pentobarbitone and to 22 $\pm$ 5·1% by phenobarbitone of their original values. On replacing this barbiturate-Ringer solution at pH 6·8 by a Ringer solution containing the same concentration of the barbiturate at pH 8·8, an immediate increase in the amplitude of the action potential was observed. In the nerves treated with phenobarbitone this

recovery occurred at a faster rate and was more pronounced (77·1 ± 6·6% after 10 min) than in those treated with pentobarbitone (67·2 ± 8·9%), although phenobarbitone had originally produced a greater degree of blockade.

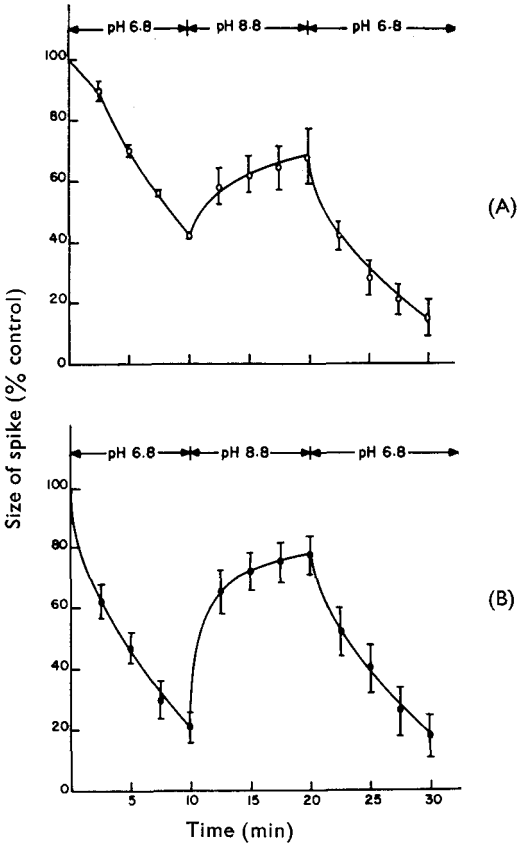

FIG. 1. Depression of the action potential of desheathed sciatic frog nerves by sodium pentobarbitone (A) and sodium phenobarbitone (B) at different pH. The preparations were successively exposed to a Ringer solution containing 5 mM of either barbiturate first at pH 6·8, then at pH 8·8 and then again at pH 6·8. The amplitude of the action potential is expressed as a percentage of its original value in barbiturate-free Ringer solution at pH 7·2 (pentobarbitone n = 8; phenobarbitone n = 6).

In no experiments, however, did the action potential return to its original size during the 10 min treatment with the barbiturate-Ringer solution at pH 8·8. When the barbiturate-Ringer solution at pH 6·8 was once more added, the size of the action potential decreased again markedly. After 10 min, the amplitudes were reduced to 14·7 ± 6·1% (pentobarbitone) and 18·0 ± 6·9% (phenobarbitone) of their original values.

Control experiments showed that the amplitude of the action potential was unaffected when the pH of the external bathing medium was varied between 6·8 and 8·8.

Effects of pentobarbitone and phenobarbitone on the uptake and kinetics of ^{45}Ca

The effects of the two barbiturates studied on the uptake of ^{45}Ca are described in Table 2. It can be seen that the total ^{45}Ca content was not significantly modified by any treatment with either pentobarbitone or phenobarbitone. Even in the nerves

Table 2. *Effects of pentobarbitone and phenobarbitone on the uptake of ^{45}Ca in paired nerves**

Experimental procedure	Total ^{45}Ca content (μmol/g)	^{45}Ca uptake in the slow component (μmol/g)	^{45}Ca uptake in the fast component (μmol/g)
Procedure I			
Pentobarbitone	0·77 ± 0·04	0·05 ± 0·004	0·72 ± 0·03
Control	0·74 ± 0·06	0·07 ± 0·005	0·67 ± 0·05
Procedure II			
Pentobarbitone	0·55 ± 0·02	0·06 ± 0·002	0·49 ± 0·02
Control	0·64 ± 0·05	0·06 ± 0·005	0·58 ± 0·04
Procedure I			
Phenobarbitone	0·67 ± 0·05	0·04 ± 0·003	0·63 ± 0·05
Control	0·65 ± 0·05	0·04 ± 0·004	0·61 ± 0·04
Procedure II			
Phenobarbitone	0·75 ± 0·05	0·05 ± 0·004	0·70 ± 0·05
Control	0·84 ± 0·04	0·07 ± 0·004	0·77 ± 0·04

* = mean and standard error (n = 5); in all experiments $P > 0·05$.

in which the uptake of radiocalcium occurred in the presence of the ionized form of the barbiturates (procedure II), in no experiment did the treatment lead to an increase in the uptake of ^{45}Ca.

The average desaturation curves obtained from nerves under control conditions and after treatment with pentobarbitone (procedure I) are shown in Fig. 2. The treatment of the nerves with pentobarbitone according to procedure II yielded almost identical desaturation curves. Analogous results were obtained with phenobarbitone (procedures I and II).

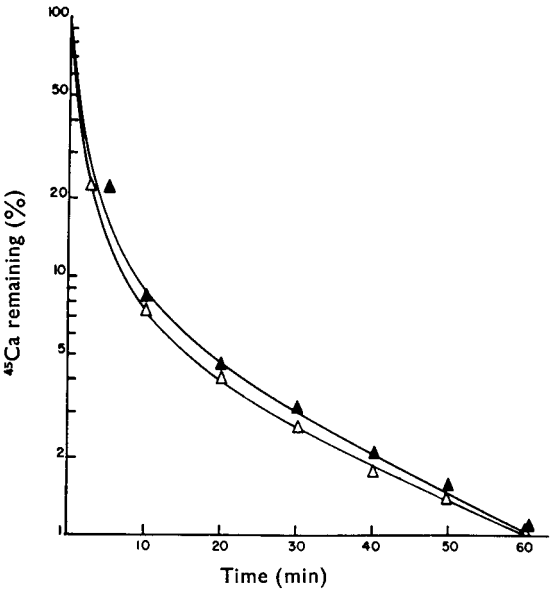

FIG. 2. ^{45}Ca desaturation curves for paired desheathed sciatic frog nerves (treatment according to procedure I). The experimental nerves were exposed first to 5 mM sodium pentobarbitone for 10 min and then soaked in [^{45}Ca] Ringer solution for 5 min. In the mate control nerves the same time schedule was observed; however, the nerves were exposed first to barbiturate-free Ringer solution and then to the [^{45}Ca]Ringer solution. All preparations were washed out in Ringer solution for 60 min. In this kind of procedure the pH of the solution was kept constant at 6·8. (Number of paired preparations = 5.) Open symbols control, closed symbols treated preparations.

All desaturation curves revealed at least two distinctive rates of ^{45}Ca release. After 30 min the curves showed a low rate of decline that appeared linear on a semi-logarithmic plot. The regression lines of this slow phase were calculated for each nerve and its intercept with the y-axis extrapolated. These intercepts correspond to the percentage of ^{45}Ca, taken up in a slowly exchanging compartment of the nerve, presumably of intracellular origin (Bianchi, 1968). On the other hand, the difference between the total ^{45}Ca content and ^{45}Ca uptake in this compartment of slow exchange is the amount of ^{45}Ca taken up in a compartment of the nerve characterized by a fast exchange. It can be assumed that this compartment represents the interstitial fluid, the interstitial connective tissue as well as the surface of the nerve membrane and the myelin sheath.

The treatment of the nerves with pentobarbitone or phenobarbitone had no effect on the shape of the desaturation curves. As shown in Table 2 the barbiturates studied did not interfere with the ^{45}Ca uptake in the two compartments of the nerves as revealed by the desaturation curves.

DISCUSSION

Our results indicate that the local anaesthetic activity of barbiturates is affected by their degree of ionization. In the same concentration the non-ionized forms of both pentobarbitone and phenobarbitone are more potent in blocking the action potential of desheathed nerves than the ionized form. The experimental procedure used in this investigation excludes the possibility that this difference in potency is only a consequence of the drug distribution across the excitable nerve membrane. Since barbiturates are acid, indeed, they penetrate biological membranes more rapidly at a low than at a high pH, it could, therefore, be argued that the higher blockade observed at pH 6·8 than at pH 8·8 is only the result of a higher drug concentration at the interphases of the excitable membrane. However, the sudden occurrence of a decrease in blockade, when the bathing solution is shifted from pH 6·8 to 8·8, demonstrates that the non-ionized form is more potent in affecting the action potential. It has been shown that such a shift in pH affects first the degree of ionization of tertiary amine local anaesthetics at the interphases of the excitable membrane; only thereafter a redistribution of these compounds occurs across the membrane (Bianchi & Strobel,1969). As a matter of fact the time course of the recovery of the action potential, when shifting the pH from 6·8 to 8·8 of the barbiturate-Ringer solution is of two phases. It is, therefore, tempting to assume that the first, fast phase of this recovery curve reflects the change in the degree of ionization, whereas the second, slow phase is the consequence of the redistribution of the barbiturates studied. Thus the initial rapid phase of recovery, when the pH is shifted from 6·8 to 8·8 would be a consequence of the ionization of the barbiturate trapped in the outer surface of the excitable membrane; the second slower phase of recovery would be attributed to the loss of barbiturate from the nerve fibres as the weak acid would tend to accumulate in the alkaline interstitial space.

On the other hand, if the ionized form of the barbiturates would be more potent, a transient increase of the blockage should have occurred immediately upon changing the pH from 6·8 to 8·8 (Bianchi & Strobel, 1968). However, the reverse, a diminution of the blockade, was observed in every experiment. This strongly supports the common concept that in contrast to tertiary amine local anaesthetics, barbiturates are more active in the same form that also diffuses more easy through biological

membranes (Sharpless, 1968). However our experimental data do not permit the exclusion of the possibility that the ionized form of these compounds may also diminish, but to a lesser extent, the excitability of the nerve membrane.

It has recently been shown that the ionized tertiary amine local anaesthetics interfere with the uptake of calcium in desheathed nerves (Suarez-Kurtz, Bianchi & Krupp, 1969). In contrast, the present results demonstrate that in neither form, the non-ionized or the ionized, do barbiturates modify the calcium binding and release in this preparation. These data, therefore, seem to indicate that the local anaesthetic effect of barbiturates is not related to the calcium binding to sites which govern the increased membrane conductance during nerve excitation.

The fact that barbiturates do not appear to interfere with the calcium binding in the nerve and more effectively block the action potential in the free acid form, suggests that these compounds affect the excitable membrane by increasing its surface pressure (Shanes, 1958). The lipid-soluble moiety of barbiturates may, indeed, decrease the conductance to sodium and potassium by dissolving in the lipid bilayer of the excitable membrane (Butler, 1950; Blaustein & Goldman, 1966). That the ionized form of these agents is less potent may reflect a repulsion of the anionic drug molecules by the negative charges in the membrane.

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