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Influence of pH on the inhibitory action of local anaesthetics on smooth muscle contraction

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Summary

1. The guinea-pig isolated ileum kept in a calcium-free depolarizing solution contracts when calcium is added ; the responses are proportional to the concentration of calcium and are reversed by washing.

2. The contractions elicited by calcium are inhibited by three different classes of compounds: local anaesthetics, pentobarbitone and ethanol. The inhibitory action of the local anaesthetics increases with an increase in pH, the inhibitory action of pentobarbitone increases with a decrease in pH and the inhibitory action of ethanol is not affected by variations in Tyrode pH.

3. It is suggested that the antagonists are active when unionized and that the inhibition is obtained when unionized compounds have dissolved in the lipid material of the cell membrane. The possible site of action of the antagonists is discussed.

Introduction

Local anaesthetics are known to interfere with the movement of calcium across biological membranes (Shanes, Freygang, Grundfest & Amatniek, 1959 ; Taylor, 1959 ; Weiss, Coalson & Hurwitz, 1961 ; Hurwitz, Battle & Weiss, 1962 ; Feinstein, 1963). They inhibit the stimulating effect of calcium on catecholamine secretion by the adrenal glands (Rubin, Feinstein, Jaanus & Paimre, 1967 ; Douglas & Kanno, 1967 ; Douglas, 1968) and also the smooth muscle contraction elicited by calcium in depolarized preparations (Feinstein, 1966 ; Feinstein, Paimre & Lee, 1968).

On the other hand, it is an established fact that local anaesthesia is markedly influenced by variations in the pH of the bathing medium (Trevan & Book, 1927 ; Skou, 1954 ; Albert, 1952 ; Ritchie & Greengard, 1966). The present work was carried out to test whether changes in pH influenced the inhibitory action of local anaesthetics on the muscular contractions elicited by calcium in the depolarized ileum of the guinea-pig. The inhibitory effect of local anaesthetics, which are weak bases, was compared with that of a barbituric acid derivative and with ethanol, which does not ionize.

Methods

Smooth muscle preparation and measurement of contraction

Guinea-pigs of either sex, weighing from 200 to 300 g, were killed by a blow on the head. A length of about 30 cm of the terminal ileum was removed, carefully

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washed and kept in conventional Tyrode solution (NaCl, 137 ; KCl, 3 ; $MgCl_2 \cdot 6H_2O$, 1 ; $CaCl_2$, 2 ; $NaHCO_3$, 12 ; $NaH_2PO_4 \cdot H_2O$, 0·4 and glucose 5·5 mM). A segment approximately 4 cm long was cut from the terminal part of the ileum and suspended in a 10 ml muscle chamber kept at 37° C and filled with modified Tyrode solution at the desired pH (see below) continuously bubbled with room air. The intestinal segment was attached to a frontal writing isotonic lever, the load being approximately 1 gramme. The isotonic contractions of the longitudinal muscle, magnified 5 times, were registered on a smoked drum.

Depolarizing Tyrode solution of varying pH

Most of the bicarbonate of the conventional Tyrode solution was replaced by Tris buffer, 70% of NaCl was replaced by KCl to maintain the muscle in a depolarized state and calcium was omitted to keep the muscle in a relaxed state. The composition of this modified Tyrode solution was: NaCl, 42 ; KCl, 98 ; $MgCl_2 \cdot 6H_2O$, 1 ; $NaHCO_3$, 1·2 ; $NaH_2PO_4 \cdot H_2O$, 0·04 ; Tris, 5 and glucose 5·5 mM, the final pH being 8·6–8·7. Adjustments of the pH were achieved immediately before starting the experiments by addition of 0·4 N HCl or 0·4 N NaOH. The pH was determined with a TTT-Radiometer (Copenhagen). Experiments were performed with pH values differing by 0·05 units, but only six main values are represented in the graphs.

The guinea-pig ileum kept in the depolarizing and calcium-free Tyrode solution responded to small amounts of $CaCl_2$ added to the organ bath. The contractions were highly reproducible and proportional to the doses of calcium ; the withdrawal of calcium by washing the preparation led to a rapid relaxation of the longitudinal muscle. Varying doses of calcium were added every 3 min to the muscle chamber ; the inhibitors were added directly to the chamber 30 s before the addition of calcium. The effects of local anaesthetics, pentobarbitone and ethanol were estimated as the percentage inhibition of calcium contraction.

Drugs

The following drugs and reagents were used: acetylcholine chloride (Roche) ; calcium chloride (Allied Chemical Co.) ; cocaine hydrochloride (Merck, Darmstadt) ; dextrose (Baker) ; ethanol, redistilled (National Co.) ; hydrochloric acid (Baker) ; magnesium chloride (Merck, Darmstadt), potassium chloride (Merck, Darmstadt) ; procaine hydrochloride (Hoechst) ; sodium bicarbonate (Baker) ; sodium chloride (Baker) ; sodium hydroxide (Baker) ; sodium phosphate (Allied Chemical Co.) ; sodium pentobarbitone (Abbott). All doses refer to the salts.

Results

Influence of the pH of the Tyrode solution on the isotonic contractions elicited by calcium

The mean values of the isotonic contractions elicited by 0·2 mM $CaCl_2$ for all experiments performed at a pH range of 7·5 to 10·4 are shown in Fig. 1. This range was chosen because values below 7·5 led to a very slow relaxation and at values above 10·4 the responses were not observed for more than 20–30 min. It was found that the height of the isotonic contractions increased with increases in pH.

Inhibitory action of cocaine

Cocaine in a concentration of 0·03 mM inhibited the contraction elicited by calcium in the depolarized guinea-pig ileum. The inhibitory action is most marked at pH values around 9·25 and is reversed by increasing the concentration of calcium (Fig. 2).

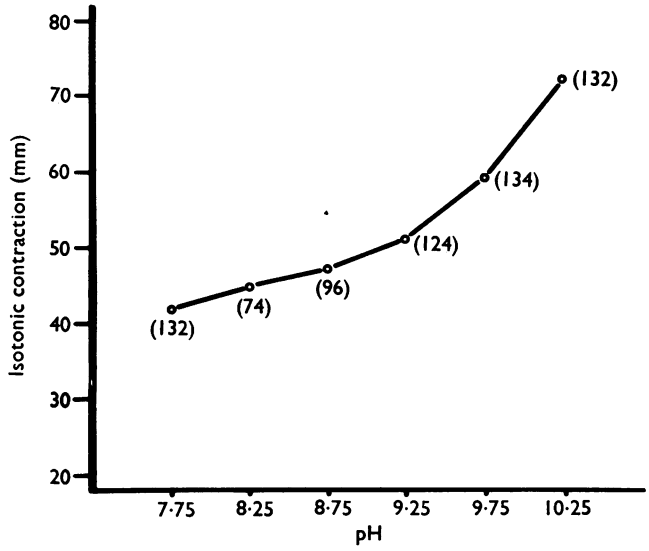

FIG. 1. Influence of the pH of Tyrode solution on the isotonic contractions of the depolarized ileum of the guinea-pig. Contractions elicited by 0·2 mM (final) $CaCl_2$. The individual points represent the mean of all experiments (numbers in parentheses).

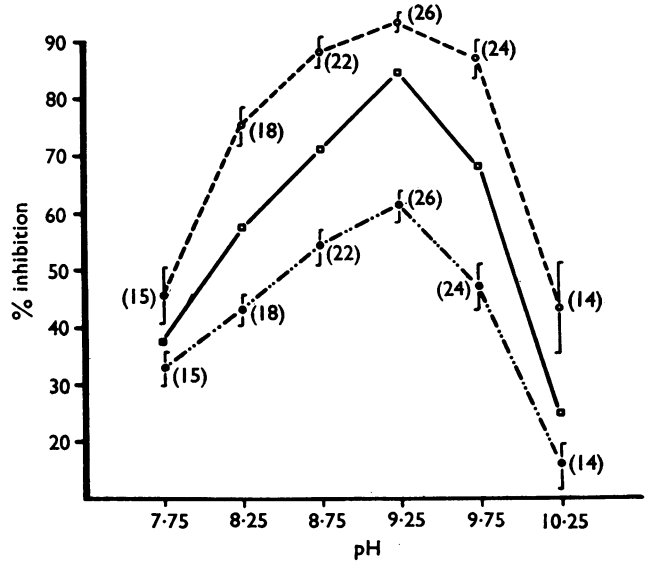

FIG. 2. Influence of the pH of Tyrode solution on the inhibitory action of cocaine (0·03 mM). The effect of cocaine is expressed as the % inhibition of control contractions elicited by: 0·2 (○– – –○), 0·4 (□——□) and 0·8 mM (○·· — ·· — ·· ○) (final) $CaCl_2$. The individual points represent the mean of 14–28 experiments, the vertical bars indicating S.E. of the mean.

Other inhibitors; type of antagonism

The effects of procaine, ethanol and pentobarbitone were compared with the effect of cocaine. When the reciprocals of the concentrations of calcium were plotted against the reciprocals of the effects elicited by calcium in the absence and presence of the inhibitors, it was found that the regression lines had a common intercept with the ordinate; this observation may suggest a competitive nature of the inhibition (Fig. 3).

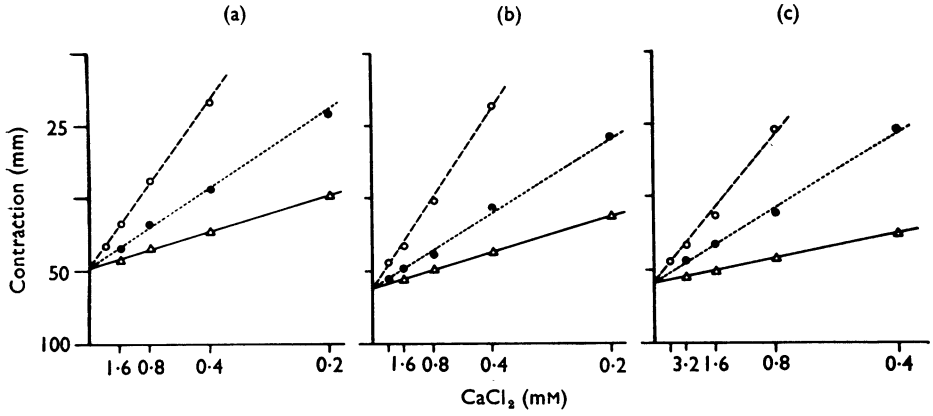

FIG. 3. Double reciprocal plots of the isotonic contraction against calcium concentration in the absence (△——△) and presence of the following antagonists: (a) 0·073 (●– – –●) and 0·146 (○– – –○) mM procaine; (b) 0·169 (●– – –●) and 0·338 (○– – –○) M ethanol; (c) 0·12 (●– – –●) and 0·24 (○– – –○) mM pentobarbitone.

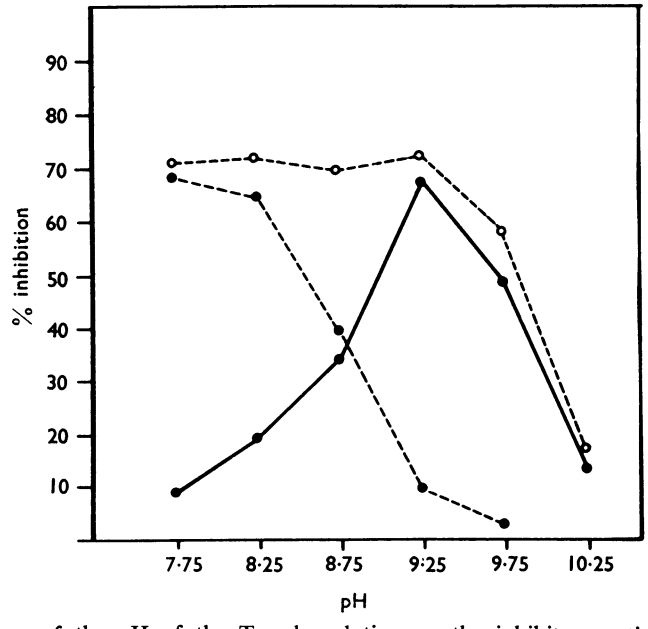

FIG. 4. Influence of the pH of the Tyrode solution on the inhibitory actions of procaine (●——●, 0·073 mM), ethanol (○– – –○, 0·338 M) and pentobarbitone (●– – –●, 0·12 mM). The effects are expressed as % inhibition of control contractions elicited by 0·4 mM (final) $CaCl_2$. The individual points represent the mean of ten to thirty experiments.

Influence of the pH on the inhibitory action of procaine, ethanol and pentobarbitone

Procaine was less potent than cocaine as inhibitor of the responses to calcium, but exhibited the same pH dependence; that is, the maximum inhibition was obtained in pH 9·25 (Fig. 4). In contrast, the effects of ethanol were not influenced by variations of the pH in the range 7·5 to 9·5. Pentobarbitone inhibited the contractions due to calcium at pH values between 7·5 and 8·0; with increasing pH a sharp decline in its inhibitory action was observed. Fig. 5 shows two segments of the same guinea-pig ileum at pH 9·5 and 7·5 respectively: the inhibitory action of procaine is more pronounced at pH 9·5, whereas that of pentobarbitone is more pronounced at pH 7·5.

Discussion

It is well known that the action of local anaesthetics is markedly influenced by variations in pH. When administered in alkaline solutions, lower concentrations of anaesthetics are necessary to block nerve conduction (Trevan & Book, 1927). The experiments by Skou (1954) showed that the blockade of nerve impulse conduction by local anaesthetics is decreased by lowering the pH; in other words, the anaesthetic action decreases with the ionization of the anaesthetic agent. In desheathed nerve fibres the ionized compound is the active form at the receptor level (Ritchie & Greengard, 1966). When taken together, these observations suggest that the unionized form, which is lipid-soluble, penetrates the cell membrane and reaches the site of action where a subsequent ionization seems to be necessary for pharmacological action.

Our experiments were designed to test whether ionization may affect the action of inhibitors on the calcium induced contraction of the depolarized longitudinal muscle of the guinea-pig ileum. The inhibitory action produced by local anaesthe-

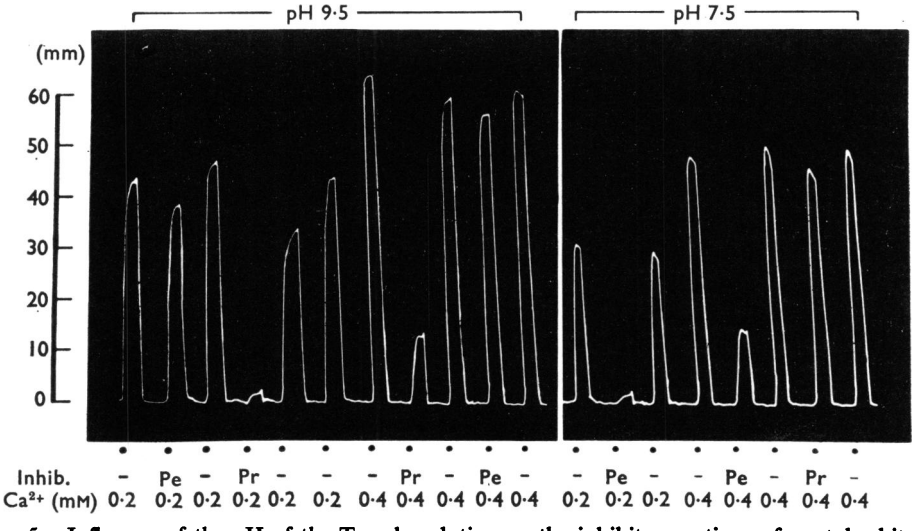

FIG. 5. Influence of the pH of the Tyrode solution on the inhibitory actions of pentobarbitone (Pe, 0·12 mM) and procaine (Pr, 0·073 mM). The isotonic contractions elicited by Ca (0·2 and 0·4 mM), in two fragments of the same guinea-pig ileum, one at pH 9·5 and the other at pH 7·5, are differently inhibited by pentobarbitone and procaine.

tics, which have a pKa of about 9·0, is most pronounced at pH values around 9·25, whereas the inhibitory action of sodium pentobarbitone, with a pKa of about 7·5, is strongest at pH values close to 7·5. These observations suggest that the inhibitory action of local anaesthetics and pentobarbitone decreases with increasing ionization. As would be expected, ethanol had the same inhibitory action over the whole pH range from 7·5 to 9·5. Our results are in agreement with those obtained by Clowes & Keltch (1931) in their studies on the immobilization of *Arenicola* by the same substances.

We have no explanation for the abrupt decline in the inhibitory action of local anaesthetics and ethanol in pH values above 9·5. It could be associated with an alteration of the cell membrane or of the system transporting calcium, because the height of the isotonic contractions is much greater at pH values above 9·5.

As in experiments with depolarized rat uterus (Feinstein, 1966), the antagonism studied in the present work is reversed by increasing the calcium concentration. The representation of the phenomenon by means of double reciprocal plots, in the presence and absence of the inhibitor, may suggest a competitive type of antagonism for the three kinds of compounds. It is quite difficult, however, to imagine that the three compounds, which are structurally different, could act at the same site at the muscle membrane. Moreover, the contraction elicited by calcium seems to be brought about by a direct interaction of the cation with the myofibrils. Calcium seems to act as the intracellular transducer of the excitation-contraction coupling (Sandow, 1965 ; Nayler, 1967). Although most of the work showing the importance of calcium for excitation-contraction coupling has been done on striated and cardiac muscle, the same dependence on calcium exists in smooth muscle (Robertson, 1960 ; Axelsson, 1961 ; Durbin & Jenkinson, 1961 ; Edman & Schild, 1962). Since the contraction elicited by calcium in the guinea-pig ileum is obtained in a calcium-free and depolarized preparation it is reasonable to assume that the calcium added to the bath fluid crosses the cell membrane to act in the interior of the muscle fibre. The exact nature of this transport is not known but it is probably passive (Holland, Klein & Briggs, 1964). Its inhibition by cocaine and procaine favours this possibility.

Our results suggest that local anaesthetics, pentobarbitone and ethanol probably block the membrane movement of calcium ; the effect may be obtained after the inhibitor has been dissolved in the lipid material of the cell membrane as the unionized form of the drug is the most active. It is difficult to draw a conclusion about the active form of the local anaesthetic at the " receptor site ", the pH of which is unknown. Our results with ethanol, which is known to act in a manner similar to that of cocaine (Hurwitz, 1961) and which is probably unionized in the pH range studied, indicate that the unionized form of the inhibitor is the active form.

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