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EFFECTS OF PROCAINE AND LIGNOCAINE ON ELECTRICAL AND MECHANICAL ACTIVITY OF SMOOTH MUSCLE OF SHEEP CAROTID ARTERIES

A. JACOBS & W.R. KEATINGE

Department of Physiology, The London Hospital Medical College, Turner Street, London E1 2AD

1 Procaine in concentrations up to 20 mM facilitated or induced electrical and mechanical activity in arterial smooth muscle, even with tetrodotoxin present.

2 Procaine (up to 20 mM) caused relaxation when electrical activity was blocked by prior potassium depolarization.

3 Procaine (1 mM) reduced mechanical responses to noradrenaline (1 μM) which were not accompanied by action potentials. It generally reduced mechanical responses to noradrenaline (1 mM) while increasing electrical activity induced by this.

4 Lignocaine (1 mM) did not facilitate electrical activity significantly; it relaxed arteries in saline or potassium-rich solution and reduced mechanical responses to noradrenaline.

5 High concentrations of procaine (at least 80 mM) or lignocaine (at least 20 mM) blocked electrical activity and caused contraction followed by relaxation and complete unresponsiveness.

Introduction

Procaine and lignocaine can relax arteries (Astrom, 1964; Hudgins & Weiss, 1968). The present experiments were designed to see whether they do so partly by reducing electrical activity. Vasodilator agents such as nitrite relax arterial smooth muscle partly by stabilizing membrane potential near resting level and partly by non-electrical means (Keatinge, 1966). Procaine and lignocaine stabilize cardiac and striated muscle membranes by blocking action potentials (Weidmann, 1955; Draper & Karzel, 1961; Inoue & Frank, 1962), and procaine relaxes rabbit aorta by non-electrical means (Somlyo, Vinall & Somlyo, 1969). However, it seemed important to record the electrical effects of local anaesthetics on arterial muscle, since action potentials of arteries, though sodium-based like those of striated muscle, are resistant to tetrodotoxin (Keatinge, 1968a, b).

The present experiments showed unexpectedly that procaine facilitated electrical activity in arteries while lignocaine had little effect on it. Studies were also carried out with tetrodotoxin present to block nerve activity.

Methods

Common carotid arteries were removed from sheep within 10 min of the animals being killed, and were cut into helical strips 1 mm wide.

Standard saline used in the initial stages of all experiments contained (mM): NaCl 133.0, $NaHCO_3$ 15.0, KCl 4.7, $CaCl_2$ 1.25 and dextrose 7.5 (pH 7.35). Solutions with increased potassium, used to induce electrical activity, were made by mixing standard saline with solution containing (mM): KCl 137.7, $KHCO_3$ 15.0, $CaCl_2$ 1.25 and dextrose 7.5. 'Potassium-rich solution' used in some mechanical experiments and in the reference side of the sucrose-gap apparatus contained (mM): K_2SO_4 91.9, $KHCO_3$ 16.3, KH_2PO_4 1.38, $CaCl_2$ 1.25 and dextrose 7.5. Sucrose solution contained sucrose 318.6 mM and dextrose 7.5 mM. All were made from Analar chemicals and glass-distilled water, and all except sucrose solution were aerated with 95% O_2 and 5% CO_2 before and during experiments.

Solutions containing local anaesthetics were made by adding solid procaine hydrochloride (Evans) or lignocaine hydrochloride (Xylotox, Pharmaceutical Mnfr Co.), aerating continuously with 95% O_2 and 5% CO_2, and when necessary adding NaOH solution (Volucon, May & Baker) until the pH was restored to 7.3-7.4. Noradrenaline bitartrate (Koch-Light) and tetrodotoxin (Sigma) were dissolved in water and added to perfusion solutions as required.

Electrical and mechanical activity was recorded from the artery strips by the sucrose-gap apparatus

described previously (Keatinge, 1964; 1966). The electrical output was fed into an electrometer amplifier (input resistance 500 MΩ) and displayed with the output of the isotonic tension (2.5 g) mechanical transducer on multi-channel recording galvanometers (response flat to 200 Hz) or (for responses to noradrenaline) on a Devices heat stylus recorder (flat to 50 Hz). For purely mechanical experiments, contractions were recorded simultaneously from 4 artery strips by isotonic (2.5 g) penwriting levers on a kymograph. All experiments were carried out at 36 ± 0.5° C.

Before and between experiments arterial strips were left in standard saline for at least an hour. The order of experiments in a given series was crossed over. Statistical group comparisons were made with the *t* test.

Results

Effects of procaine and lignocaine on electrical behaviour of arteries in physiological saline and during potassium depolarization

Without local anaesthetic. Artery strips in standard saline never showed spontaneous electrical activity. When they were depolarized by progressive increases in potassium concentration after 80 min in the saline, electrical discharges were recorded in seven out of ten experiments. Figure 1a shows one of them. When potassium concentration was increased from 4.7 to 34.4 mM by replacement of sodium, there was initially slow depolarization. This was interrupted by an electrical discharge which was followed by abrupt contraction. Further step increases to potassium 63.9, 93.5 and 152.7 mM produced only smooth depolarizations and contractions without electrical discharges. In the ten experiments, the number of discharges per experiment (mean and s.e.) was 1.1 ± 0.3. No more than two discharges were recorded in any experiment.

With procaine. Figure 1b shows that when an artery had been exposed to procaine 2.0 mM for 20 min, the first of the series of steps of potassium depolarization produced a series of five electrical discharges, each followed by contraction. At least one discharge was observed in each of ten such experiments, and as many as ten discharges were observed in one of the experiments. The number of discharges per experiment (mean and s.e.) was 3.9 ± 1.1, significantly greater ($P < 0.05$) than the number in the absence of procaine. None of these arteries developed electrical activity in procaine (2.0 mM) before potassium depolarization was started; they showed slight mechanical relaxation (up to 0.1 mm/mm) during this time.

In 12 similar experiments with procaine 20.0 mM, spontaneous electrical and mechanical activity appeared in two cases within 20 min of adding the procaine in standard saline. Potassium depolarization induced discharges in nine of the remaining ten experiments. They averaged 2.8 ± 0.8 (mean and s.e. per experiment) in the ten experiments; difference from group without procaine $P < 0.10 > 0.05$. The electrical waves sometimes took the form of slow potential oscillations (see later discharges in Figure 1c). When procaine 20.0 mM was first added to the arteries in standard saline, it caused slight mechanical relaxation (up to 0.1 mm/mm).

Further experiments were done to see whether procaine facilitated electrical activity even when tetrodotoxin was present to block nerve action potentials in both procaine and control runs. After 20 min exposure to standard saline containing tetrodotoxin (3.0 μM), K depolarization induced 1.1 ± 0.2 discharges (mean and s.e., ten experiments). After 20 min exposure to standard saline with procaine (2.0 mM) as well as tetrodotoxin (3.0 μM), potassium depolarization induced 2.5 ± 0.4 discharges (mean and s.e., ten experiments). The difference between the experiments with and without procaine was significant ($P < 0.01$), showing that procaine still facilitated electrical activity in the presence of tetrodotoxin.

Very high concentrations of procaine depressed electrical activity after initial facilitation. Procaine 80.0 mM induced immediate electrical activity in one of ten experiments, but the activity ceased within 30 s; potassium depolarization 20 min after addition of the procaine failed to induce a clear electrical discharge or contraction in this or any other of ten similar experiments. An irregularity that might represent an abortive spike was present in one trace.

It can be seen in Fig. 1c that with procaine 20.0 mM present, the first step increase in potassium concentration induced an initial hyperpolarization of more than 1.0 mV. Comparable hyperpolarization at this step was seen in three of the ten such experiments with procaine 20.0 mM, and in one of the ten with procaine 2.0 mM. Little hyperpolarization (<1.0 mV) was produced during the initial increase of potassium concentration in experiments without procaine, or in those with procaine 80.0 mM.

With lignocaine. Lignocaine failed to produce clear facilitation of electrical activity in any concentration. It blocked activity in lower concentration than procaine did.

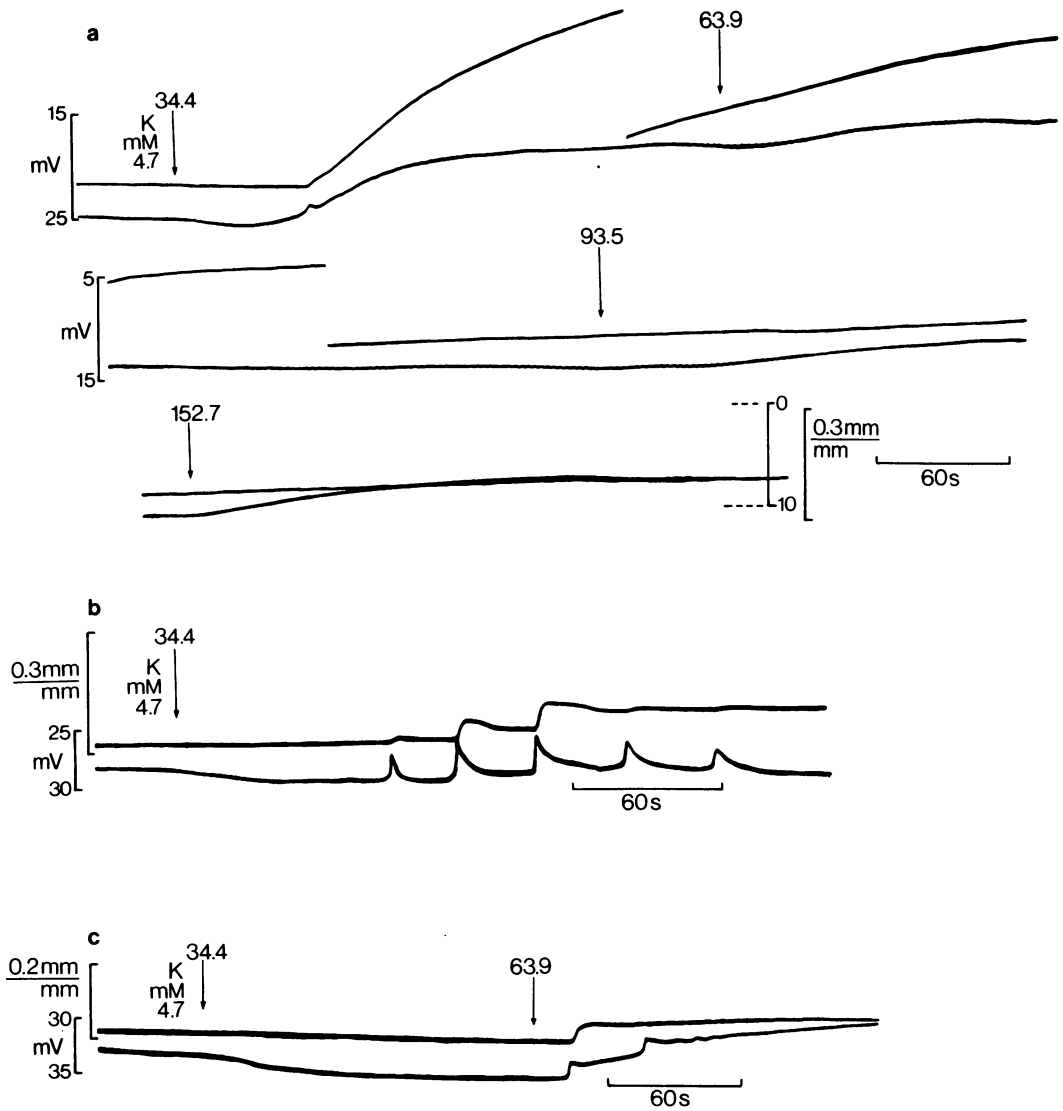

Fig. 1 Electrical and mechanical response of sheep carotid arteries to depolarization by external K: (a) without local anaesthetic; (b) in the presence of procaine 2.0 mM; (c) with procaine 20.0 mM. At start of each record, lower trace is electrical, upper trace is mechanical. Mechanical scale gives mm shortening per mm strip length.

Lignocaine (0.2 or 2.0 mM) had no clear effect on the electrical activity of the arteries. Potassium depolarization after 20 min in lignocaine 0.2 mM induced discharges in nine out of ten experiments (Fig. 2a); the number of discharges for the group (mean and s.e.) was 1.2 ± 0.3, not significantly different from the number without any local anaesthetic. Potassium depolarization after 20 min in lignocaine 2.0 mM produced discharges in eight out of ten experiments; the number for the group (mean and s.e.) was 1.1 ± 0.3, the same as without local anaesthetic.

Potassium depolarization after 20 min in lignocaine 20.0 mM failed to induce electrical discharges in any of ten experiments (Fig. 2b), although irregularities that might represent abortive spikes were present in two of them.

The addition of lignocaine was followed by some mechanical relaxation (up to 0.1 mm/mm) in all these experiments during the 20 min before the

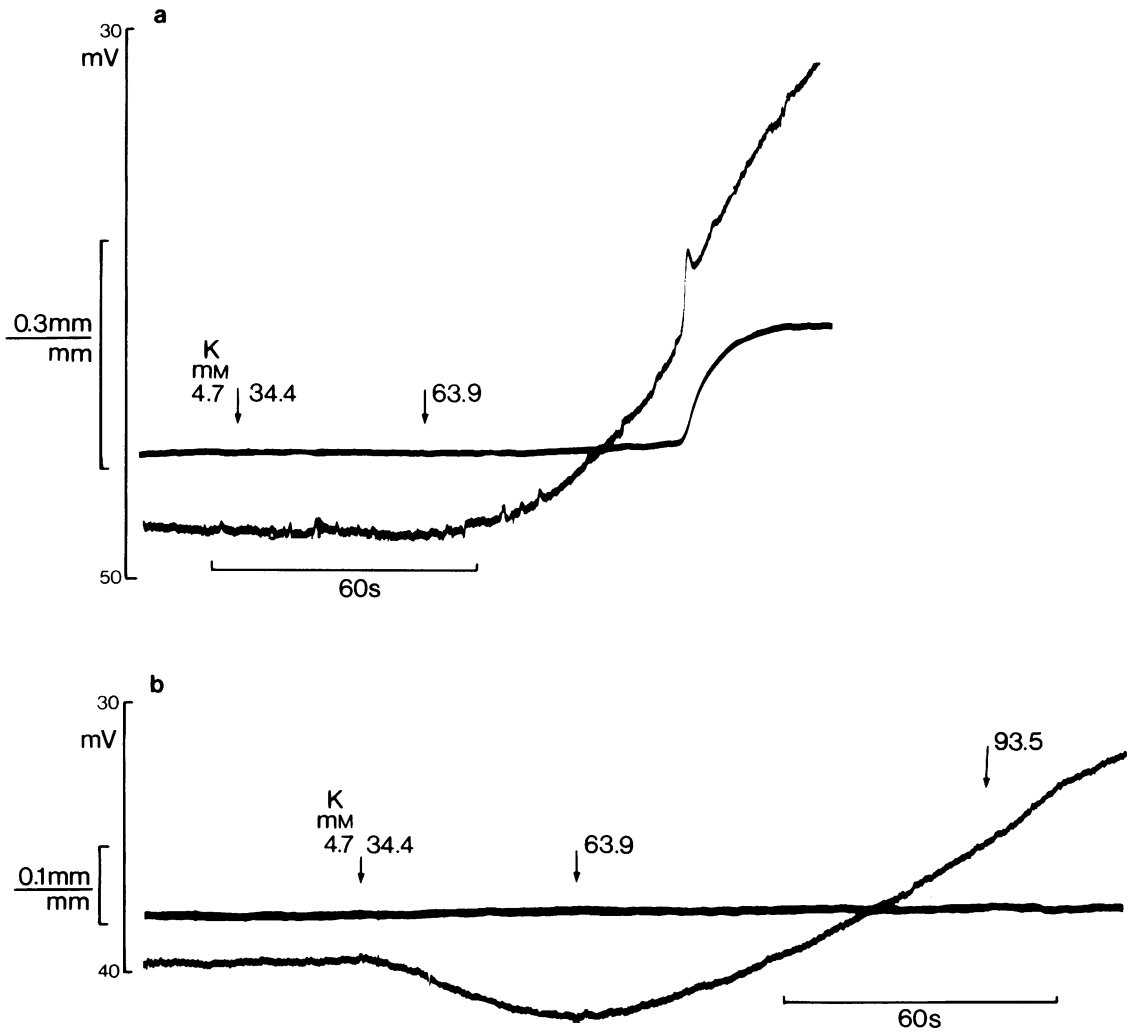

Fig. 2 Electrical and mechanical response of arteries to external K in the presence of lignocaine (a) 2.0 mM and (b) 20.0 mM. At start of each record, lower trace is electrical, upper is mechanical. Mechanical scale gives mm shortening per mm strip length.

step increases in potassium were started; with lignocaine 20.0 mM the relaxation was followed by contraction.

Hyperpolarization of more than 1.0 mV was produced by the first step increase of potassium concentration in three of the ten experiments with lignocaine 20.0 mM (see Fig. 2b) and in two of the ten experiments with lignocaine 2.0 mM, but in none of the ten with lignocaine 0.2 mM.

Mechanical actions of local anaesthetics not mediated by electrical activity

The mechanical responses of the arteries to increasing concentrations of the local anaesthetics were determined in standard saline and in potassium-rich solution.

Figure 3a shows the effect of increasing concentrations of procaine on the length of an artery strip in standard saline. The lowest concentration 1.0 mM had little effect while 10.0 mM produced rhythmical contractions, after a 10 min delay. Procaine 100.0 mM stopped the rhythmical activity but produced further smooth contraction; procaine 500.0 mM caused further contraction followed after 30 min by relaxation.

Figure 3b shows that lignocaine produced relaxation in 0.1 mM concentration and caused no rhythmical contractions in any concentration. It was more potent than procaine in producing final

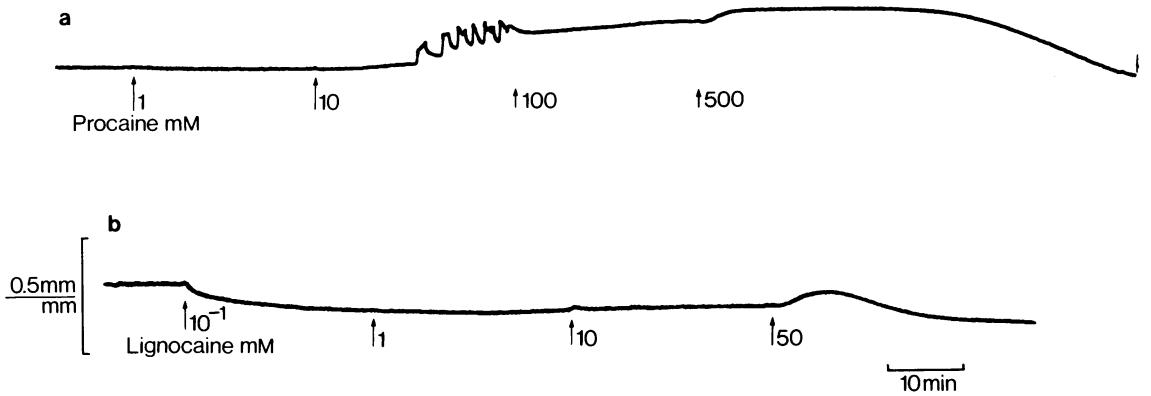

Fig. 3 Mechanical responses to increasing concentrations of (a) procaine and (b) lignocaine by arteries in physiological saline. Vertical scale gives mm shortening per mm strip length.

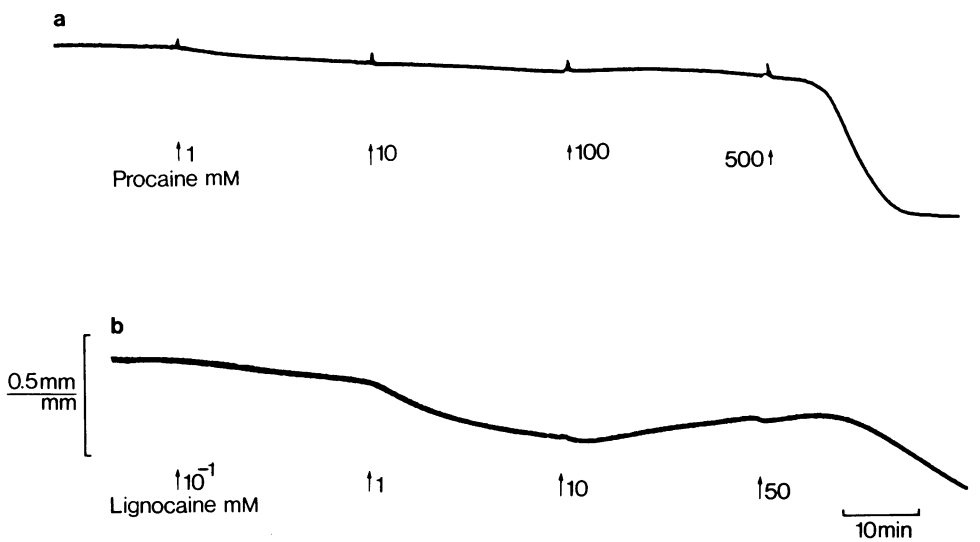

Fig. 4 Mechanical responses to increasing concentrations of (a) procaine and (b) lignocaine by arteries in K-rich solution. Vertical scale gives mm shortening per mm strip length.

contraction and relaxation, doing this in 50.0 mM concentration.

Similar experiments were performed in potassium-rich solution with a second set of strips cut from the same arteries. The potassium-rich solution initially caused contraction, and the local anaesthetic was added 20 min later when the contraction had reached a maximum. Figure 4 shows that procaine 1.0 mM produced definite relaxation and procaine 10.0 mM induced no rhythmical contractions. The highest concentrations of both local anaesthetics caused contraction followed by relaxation as in standard saline, although the contraction was smaller and the relaxation larger, no doubt because of the higher initial tone in potassium-rich solution.

Similar results to those illustrated in Figs. 3 and 4 were obtained from three similar additional experiments. They therefore support the other evidence that low concentrations of both local anaesthetics could cause relaxation by non-electrical means while procaine could induce electrical and mechanical activity. The additional finding that very high concentrations of the local anaesthetics in either sodium- or potassium-based solution caused contraction followed by relaxation may be explained by their direct action on endoplasmic reticulum; high concentrations of

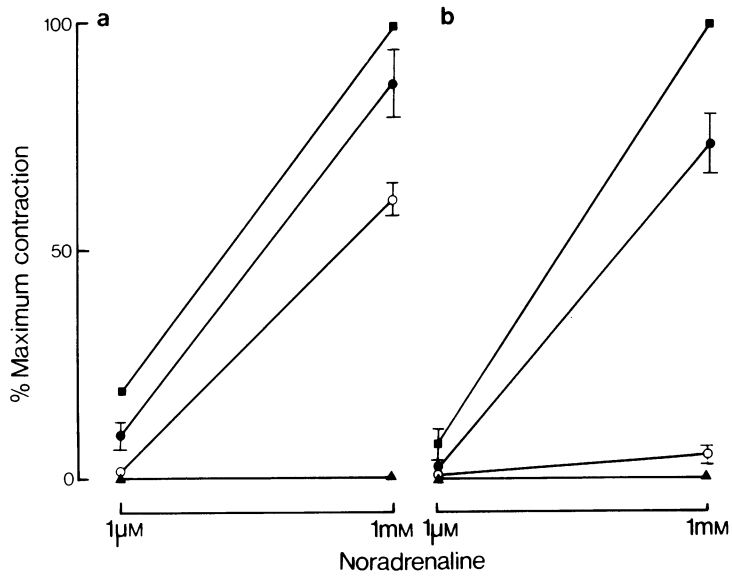

Fig. 5 Effect of different concentrations (mM) of (a) procaine and (b) lignocaine on mechanical responses of arteries to noradrenaline. (Means and s.e. of 8 experiments). (■) 0 mM; (●) 1 mM; (○) 10 mM; (▲) 50 mM.

procaine release calcium and then block calcium movement in the reticulum of striated muscle (Wilcox & Fuchs, 1969; Thorpe & Seeman, 1971).

Effect of local anaesthetics on the electrical and mechanical response to noradrenaline

The action of each local anaesthetic on the mechanical response to noradrenaline was studied in eight experiments. Preliminary experiments showed that noradrenaline 1.0 mM produced maximal contraction and noradrenaline 1 μM none. Figure 5 shows that procaine or lignocaine 1.0 mM reduced responses to noradrenaline 1.0 mM by 13% and 27% respectively, and reduced responses to noradrenaline 1 μM by more than 50%. Procaine or lignocaine 50.0 mM abolished all response to noradrenaline.

Sucrose-gap experiments showed that procaine facilitated the electrical response to the higher concentrations of noradrenaline. Noradrenaline was first added in steps of 0.001, 0.01, 0.1 and 1.0 mM to arteries in standard saline, without local anaesthetic present. No action potentials were seen in four out of six experiments; in the other two a single discharge was recorded when the noradrenaline concentration reached 0.1 mM; repeated action potentials were never seen. In nine experiments with procaine 1.0 mM present, noradrenaline 0.01 mM caused a single action potential in one case; noradrenaline 0.1 mM

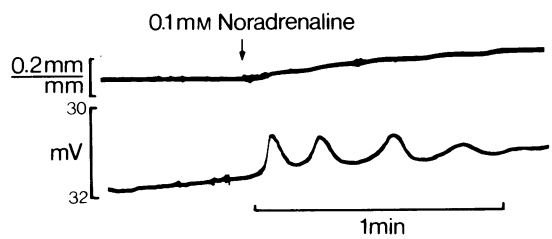

Fig. 6 Repetitive electrical and mechanical activity induced by noradrenaline in the presence of procaine (1 mM). Lower trace is electrical, upper mechanical. Mechanical scale gives mm shortening per mm strip length.

induced repeated electrical discharges in this artery and two or more discharges in each of another five experiments (Fig. 6); two of the remaining three arteries developed electrical activity when the noradrenaline concentration was increased to 1.0 mM and only one failed to show electrical activity throughout the experiment.

In seven similar experiments with lignocaine 1.0 mM present instead of procaine, noradrenaline did not produce electrical activity in any case.

Discussion

The most striking finding was that procaine facilitated, and lignocaine failed to block, action

potentials in concentrations that block the sodium-based action potentials of mammalian nerve and striated muscle. Procaine does not block action potentials of crustacean muscle, which are calcium-based (Fatt & Katz, 1953; Ozeki, Freeman & Grundfest, 1966; Hagiwara & Nakajima, 1966; Hagiwara, Hayashi & Takahashi, 1969). However, the arterial action potential is largely sodium-based but is anomalous in being resistant to tetrodotoxin (Keatinge 1968a, b). The present results suggest that its sodium channels are also insensitive to procaine and lignocaine. Procaine possibly facilitated electrical activity in the arteries by blocking potassium channels normally opened by depolarization; it is known to facilitate crustacean muscle action potentials in this way.

The other important effect of procaine and lignocaine in moderate concentration was mechanical relaxation. It was obtained consistently when electrical activity was blocked by potassium-rich solution, and was clearly mediated by non-electrical means. It may have been brought about by block of a channel for calcium entry since procaine is known to inhibit calcium exchange in uterine smooth muscle (Feinstein, 1966). It greatly depressed the response of the arteries to low concentrations of noradrenaline, which were never accompanied by action potentials, although with high concentrations of noradrenaline the depression was partly offset by procaine's facilitation of the electrical response that these concentrations of noradrenaline produced. The concentration of noradrenaline at which procaine facilitation was clearly seen (0.1 mM) is approximately the concentration produced locally in the artery wall by nerve release (Bell & Vogt, 1971).

Constrictor agents generally produce part of their effect on these arteries by inducing electrical activity, and dilator agents produce part of their effect by inhibiting electrical activity (Keatinge, 1964; 1966). The action of procaine in facilitating electrical activity while depressing mechanical responses mediated by non-electrical means is unusual.

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References

ASTROM, A. (1964). Influence of some local anaesthetics upon the adrenaline contraction of isolated strips of rabbit aorta. *Act physiol. scand.*, **60**, 30-38.

BELL, C. & VOGT, M. (1971). Release of endogenous noradrenaline from an isolated muscular artery. *J. Physiol., Lond.*, **215**, 509-520.

DRAPER, M.H. & KARZEL, K. (1961). The action of local anaesthetics on the action potential of frog sartorius muscle fibres. *J. Physiol., Lond.*, **155**, 27-28P.

FATT, P. & KATZ, B. (1953). The electrical properties of crustacean muscle fibres. *J. Physiol., Lond.*, **120**, 171-204.

FEINSTEIN, M.B. (1966). Inhibition of contraction and calcium exchangeability in rat uterus by local anaesthetics. *J. Pharmac. exp. Ther.*, **152**, 516-524.

HUDGINS, P.M. & WEISS, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.*, **159**, 91-97.

HAGIWARA, S., HAYASHI, H. & TAKAHASHI, K. (1969). Calcium and potassium currents of the membrane of a barnacle muscle fibre in relation to the calcium spike. *J. Physiol., Lond.*, **205**, 115-129.

HAGIWARA S. & NAKAJIMA, S. (1966). Differences in Na and Ca spikes as examined by application of tetrodotoxin, procaine and manganese ions. *J. gen. Physiol.*, **49**, 793-806.

INOUE, F. & FRANK, G.B. (1962). Action of procaine on frog skeletal muscle. *J. Pharmac. exp. Ther.*, **136**, 190-196.

KEATINGE, W.R. (1964). Mechanism of adrenergic stimulation of mammalian arteries and its failure at low temperatures. *J. Physiol., Lond.*, **174**, 184-205.

KEATINGE, W.R. (1966). Electrical and mechanical responses of vascular smooth muscle to vasodilator agents and vaso-active polypeptides. *Circulation Res.*, **18**, 641-649.

KEATINGE, W.R. (1968a). Ionic requirements for arterial action potential. *J. Physiol., Lond.*, **194**, 169-182.

KEATINGE, W.R. (1968b). Sodium flux and electrical activity of arterial smooth muscle. *J. Physiol., Lond.*, **194**, 183-200.

OZEKI, M., FREEMAN, A.R. & GRUNDFEST, M. (1966). The membrane components of crustacean neuromuscular systems. I. Immunity of different electrogenic components to tetrodotoxin and saxitoxin. *J. gen. Physiol.*, **49**, 1319-1334.

SOMLYO, A.V., VINALL, P. & SOMLYO, A.P. (1969). Excitation-contraction coupling and electrical events in two types of vascular smooth muscle. *Microvascular Res.*, **1**, 354-373.

THORPE, W.R. & SEEMAN, P. (1971). The site of action and caffeine and procaine in skeletal muscle. *J. Pharmac. exp. Ther.*, **179**, 324-330.

WEIDMANN, S. (1955). Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. *J. Physiol., Lond.*, **129**, 568-582.

WILCOX, W.D. & FUCHS, F. (1969). The effect of some local anaesthetic compounds on sarcotubular calcium transport. *Biochim. Biophys. Acta*, **180**, 210-212.

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