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Modification by ouabain of the electrical and mechanical effects of acetylcholine in isolated rabbit atria

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Summary

1. Left atrial preparations isolated from rabbits were stimulated electrically at frequencies between 6 and 240/min. Tension-frequency curves were obtained from control preparations and preparations treated with ouabain and acetylcholine. Transmembrane potentials were recorded from single cells of the left atrium stimulated at different frequencies.

2. The tension-frequency curve was moved downwards by acetylcholine (10^{-6} g/ml). Ouabain (10^{-6} g/ml) caused characteristic alterations in the tension-frequency relationship, enhancing the contractile tension at low but not high frequencies. The negative inotropic effect of acetylcholine was reduced by treatment with ouabain.

3. Action potential durations were significantly influenced by alterations in frequency of contraction. The 10% duration increased with frequency within the range between 6 and 60/min but decreased at frequencies higher than 120/min. The 50% duration increased with frequency between 6 and 120/min but decreased at frequencies higher than 180/min. The dependence of the 50% duration upon frequency paralleled that of contractile tension. The 90% duration, the overshoot and the resting potential were not affected by frequency of contraction.

4. Acetylcholine (10^{-6} g/ml) shifted the 10%, 50% and 90% duration-frequency curves downwards, but did not significantly alter the overshoot and the resting potential. Ouabain (10^{-6} g/ml) shifted the duration-frequency curves downwards and also reduced the size of the overshoot and the resting potential. Treatment of atrial preparations with 10^{-6} g/ml ouabain potentiated the membrane effects of acetylcholine.

5. The inhibition by ouabain of the negative inotropic effect of acetylcholine did not appear to be due to antagonism at the receptor level, but to interference with the mechanisms responsible for the mechanical events.

Introduction

In an earlier report (Toda & West, 1966) it was shown that the negative chronotropic response of isolated rabbit atria to acetylcholine was potentiated by ouabain, whereas the negative inotropic response was reduced or abolished. Slowing of the heart induced by acetylcholine may be primarily attributed to the increase in the permeability of the S-A nodal membrane to K^+ (Burgen & Terroux, 1953), which

accounts for a reduction in the slope of diastolic depolarization and for hyperpolarization in pacemaker fibres (Toda & West, 1967). However, alterations in contractile strength are not obviously related to alterations in the membrane potential (Edman, 1965).

Contractile force of the atrial and ventricular myocardium is dependent on the rate of contraction (Blinks & Koch-Weser, 1961). This frequency dependence is altered by ouabain (Koch-Weser & Blinks, 1962) and by acetylcholine (Friedman, Buccino, Sonnenblick & Braunwald, 1967), both of which are known to affect the properties of cardiac membranes. When ouabain (Sleator, Furchgott, De Gubareff & Krespi, 1964) or acetylcholine (Hoffman & Suckling, 1953) is applied, the early repolarization of atrial and ventricular action potentials is accelerated, as it is when the diastolic interval is prolonged (Hoffman & Suckling, 1954; Edmands, Greenspan & Fisch, 1966).

In the study by Toda & West (1966) alterations in strength of contraction induced by acetylcholine were observed in spontaneously-beating atria in which the rate was markedly reduced by the drug. In the present study the rate of contraction of rabbit left atria was maintained constant by electrical stimulation. The aims of this investigation were to examine (*a*) modification by ouabain of the inotropic and the membrane effects of acetylcholine and (*b*) alteration in the frequency-dependence of electrical and mechanical activity in the presence of acetylcholine and ouabain.

Methods

Forty-one albino rabbits of either sex, weighing 1·8 to 2·2 kg, were used. Under ether anaesthesia the animals were killed by bleeding. The whole heart was removed and ventricles were discarded. In warm, oxygenated nutrient solution the left atrium was separated from the right along the interatrial septum (Toda, 1969a, b). Pacemaker tissue was excluded from the left atrial preparation. The isolated specimen was fixed horizontally between two pairs of hooks (endocardial surface uppermost) under a resting tension of 300 to 400 mg in a muscle bath of 60 ml capacity. The nutrient solution was maintained at 30° ± 0·5° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Hooks anchoring an appendage of the left atrium were connected to a force-displacement transducer (Nihonkoden Kogyo Co.). The preparation was stimulated electrically through a pair of hooks attached to the cut end of the preparation. The composition of the nutrient solution was as follows (mM): Na^+, 162·1; K^+, 5·4; Ca^{++}, 2·2; Cl^-, 157·0; HCO_3^-, 14.9; dextrose, 5·6. The preparation was driven at a frequency of 60/min for 60–90 min before measurements were taken.

Left atrial preparations were driven electrically by a train of 3 ms rectangular pulses of supramaximal intensity (about twice threshold intensity) at a frequency of 60/min, except when the tension-frequency curve was determined. Stimulus pulses were provided by a Sanei type ES-103-Z pulse generator. The tension of the left atrium was displayed on a two channel penwriter (Sanei Sokki Co.). The tension-frequency curve was obtained by raising the driving frequency in steps from 6/min to 240/min or higher. Stimulation at a fixed frequency was maintained until the tension became steady. The tension-frequency curve took about 15 min to complete. The tension-frequency curves were normally obtained before application of drugs and after 45 min exposure to ouabain. The curves were also

obtained after 10 min exposure to acetylcholine in control preparations and in preparations treated with ouabain for 60 min.

Transmembrane potentials were recorded from single cells of the left atrium by means of a floating microelectrode, and recorded on film moving at a speed of 10 cm/s. Parameters of the membrane potential measured were as follows: (1) resting potential; (2) overshoot; and (3) durations of the action potential at the level of the 10%, 50% and 90% repolarization, which will be termed 10%, 50% and 90% durations, respectively. The membrane potential was recorded from preparations in which contractions were stabilized at controlled frequencies. Recordings of action potentials were obtained from preparations under control conditions and from preparations exposed to 10^{-6} g/ml of acetylcholine for 10 to 20 min or to 10^{-6} g/ml of ouabain for 45 to 90 min.

Results presented in figures and the text are mean values $\pm$ standard errors of the means. Comparisons of the results were made using the Student's *t* test.

Ouabain, U.S.P. (Nutritional Biochemicals Corp.) in concentrations of 2×10^{-7} and 10^{-6} g/ml, acetylcholine chloride (10^{-6} g/ml) and atropine sulphate (10^{-6} g/ml) were used. The drugs were applied directly to the solution in the muscle bath. Concentrations of the drugs are expressed in terms of g/ml of the salts.

Results

Contractile tension

The tension developed varied with frequency of stimulation in control preparations and in preparations exposed to ouabain 2×10^{-7} g/ml. The results are shown in Fig. 1. In control preparations the tension increased with increasing frequency between 6 and 120/min, but it decreased at frequencies higher than 120/min.

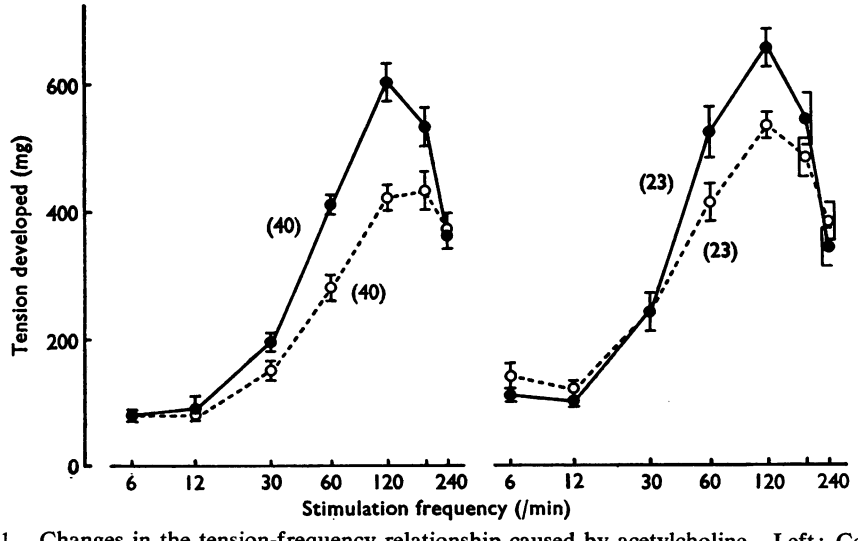

FIG. 1. Changes in the tension-frequency relationship caused by acetylcholine. Left: Control preparations; ●——●, before acetylcholine; ○——○, after 10 min exposure to acetylcholine 10^{-6} g/ml. Right: Ouabain-treated preparations; ●——●, after 45 min exposure to ouabain 2×10^{-7} g/ml; ○- -○, after 10 min exposure to acetylcholine in preparations treated with ouabain for 60 min. Figures in parentheses indicate the number of experiments. Vertical bars represent standard errors of the means.

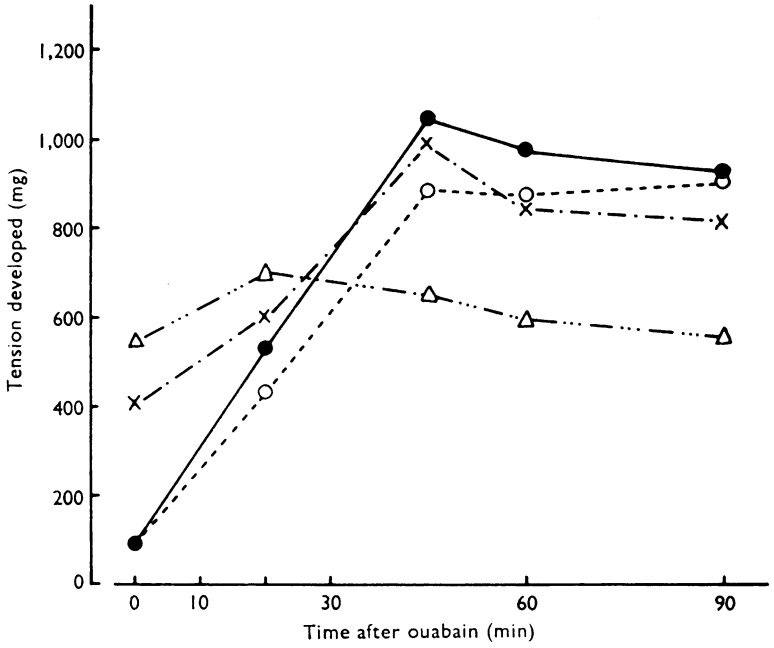

FIG. 2. Time course of the inotropic effect of ouabain (10^{-6} g/ml). Tension developed at various stimulation frequencies (●——●, 6/min ; ○- -○, 12/min ; ×-·-×, 60/min ; △-···-△, 120/min) was determined before and after 20, 45, 60, and 90 min exposures to ouabain. Except when these tension frequency curves were determined preparations were stimulated constantly at a frequency of 60/min. Each point represents the mean values from five experiments.

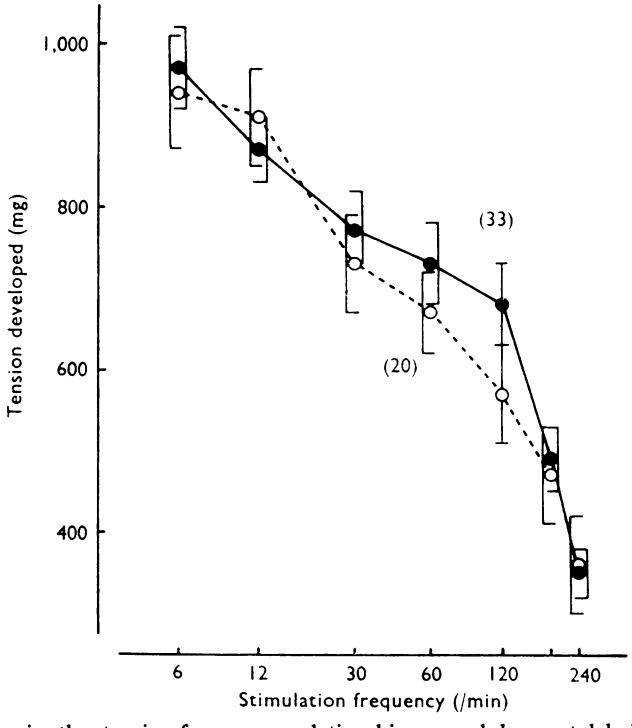

FIG. 3. Changes in the tension-frequency relationship caused by acetylcholine. ●——●, After 45 min exposure to ouabain 10^{-6} g/ml ; ○- -○, after 10 min exposure to acetylcholine 10^{-6} g/ml in preparations treated with ouabain for 60 min. The number of experiments are indicated in parentheses.

Acetylcholine (10^{-6} g/ml) shifted the tension-frequency curve downwards, the reduction in tension produced at frequencies between 30 and 180/min being significant ($P<0.01$ for 60 and 120/min; $P<0.02$ for 30 and 180/min) (Fig. 1, left). Following treatment of preparations with ouabain 2×10^{-7} g/ml the tension-frequency curve was moved upwards. At this concentration the negative inotropic effect of acetylcholine (10^{-6} g/ml) was slightly reduced, although the decrease in tension at frequencies of 60 and 120/min induced by acetylcholine was still significant ($P<0.02$) (Fig. 1, right).

Ouabain (10^{-6} g/ml) increased the force of contraction. This effect was greatest at the lowest frequency (6/min), and was not seen at a frequency of 120/min (Fig. 2). The effect took about 45 min to develop fully (Fig. 2), and did not change between

TABLE 1. *Paired analyses of the mechanical effect of acetylcholine before and after ouabain (10^{-6} g/ml)*

		Frequency of stimulation			
		30/min		60/min	
	N	Tension (mg) before ACh	% decrease	Tension (mg) before ACh	% decrease
Control	14	181±27	31±6	384±38	35±4
Ouabain	14	858±61*	2±2*	868±69*	9±3*

Preparations were exposed to acetylcholine (10^{-6} g/ml) for 10 min before measurements were taken. *N*, Number of preparations. * Significant difference from control, $P<0.01$.

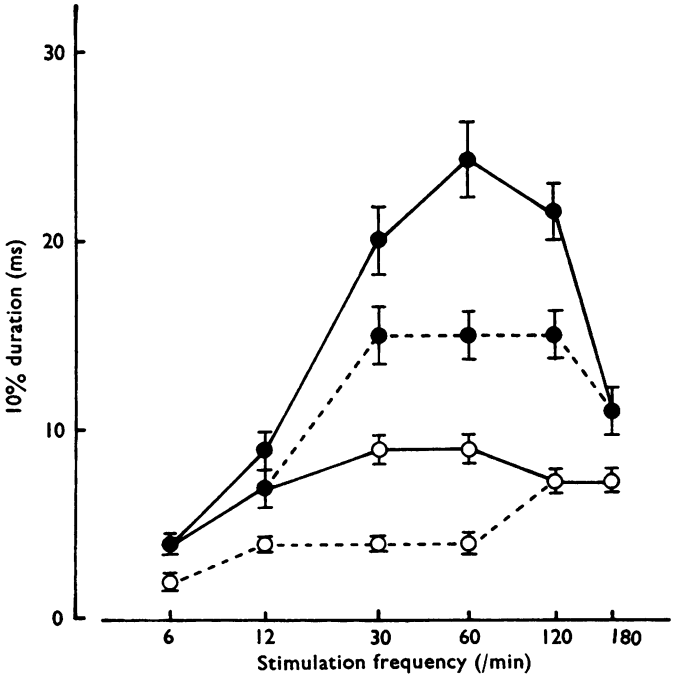

FIG. 4. Dependence of the 10% duration on frequency of contraction. Solid lines, before acetylcholine; broken lines, after 10 to 20 min exposure to acetylcholine 10^{-6} g/ml; solid circles, control preparations; open circles, preparations treated with ouabain 10^{-6} g/ml for 45 to 90 min. Each point represents the mean value of sixteen to fifty-four separate atrial fibres obtained from eight to nineteen preparations.

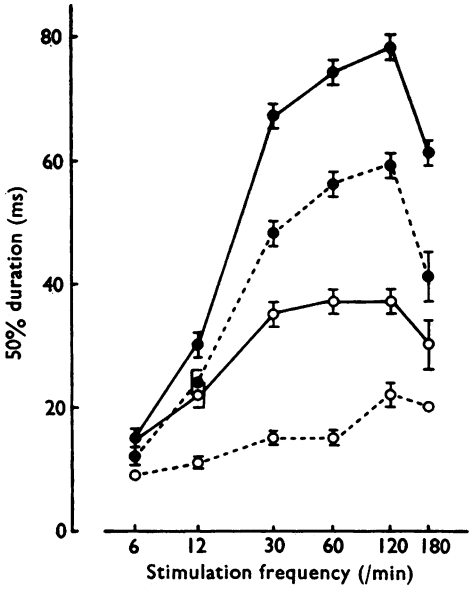

FIG. 5. Dependence of the 50% duration on frequency of contraction. Solid lines, before acetylcholine; broken lines, after 10 to 20 min exposure to acetylcholine 10^{-6} g/ml; solid circles, control preparations; open circles, preparations treated with ouabain 10^{-6} g/ml for 45 to 90 min. Each point represents the mean value of sixteen to fifty-four separate atrial fibres obtained from eight to nineteen preparations.

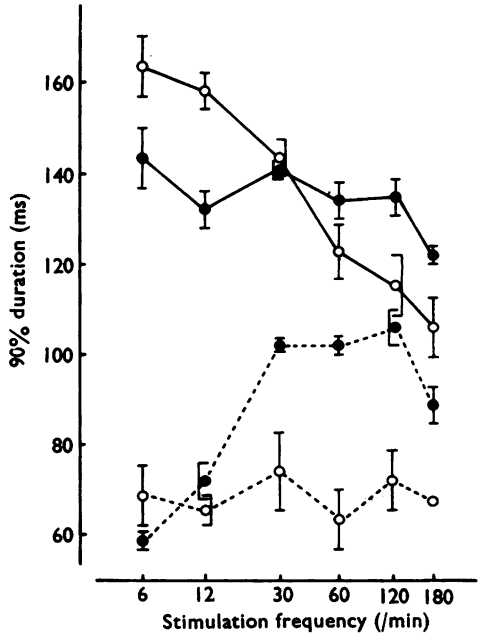

FIG. 6. Dependence of the 90% duration on frequency of contraction. Solid lines, before acetylcholine; broken lines, after 10 to 20 min exposure to acetylcholine 10^{-6} g/ml; solid circles, control preparations; open circles, preparations treated with ouabain 10^{-6} g/ml for 45 to 90 min.

45 and 90 min exposure to ouabain. The tension developed after 45 min exposure to ouabain was therefore taken as control for evaluating the inotropic effect of acetylcholine. As illustrated in Fig. 3, treatment with a higher concentration of ouabain (10^{-6} g/ml) altered markedly the tension-frequency relationship; the tension decreased progressively as the frequency was increased. Acetylcholine (10^{-6}

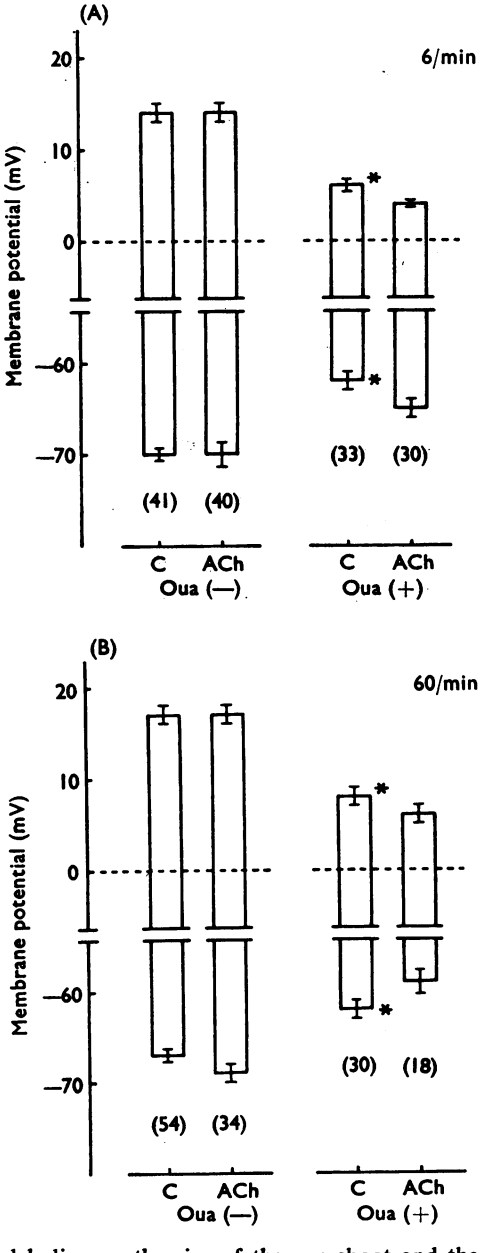

FIG. 7. Effects of acetylcholine on the size of the overshoot and the resting potential in the presence or absence of ouabain. A, Preparations driven electrically at 6/min. B, Preparations driven at 60/min. C, Control; ACh, acetylcholine 10^{-6} g/ml; Oua, ouabain, 10^{-6} g/ml. Figures in parentheses indicate the number of atrial fibres. *Significant difference from ouabain-free control, $P<0{\cdot}01$.

g/ml) was added to preparations treated with ouabain after the tension-frequency relationship was obtained (which took about 15 min). In ouabain-treated atria driven at 60/min acetylcholine (10^{-6} g/ml, 1 min exposure) reduced the force of contraction by $16 \pm 4\%$ ($N=5$); this was smaller than the effect of acetylcholine ($37 \pm 9\%$, $N=6$) in control preparations. The tension frequency curve was obtained after 10 min exposure to acetylcholine. In ouabain-treated preparations the tension-frequency curve was not significantly affected by acetylcholine (Fig. 3). Results of paired analyses of the negative inotropic response to acetylcholine before and after adding ouabain are illustrated in Table 1. The percentage decrease in tension produced by acetylcholine was significantly reduced by the ouabain-treatment. In ten of fourteen preparations treated with ouabain absolute values of the decrease in tension were smaller than those in the control preparations, whereas in the remaining four the values did not differ.

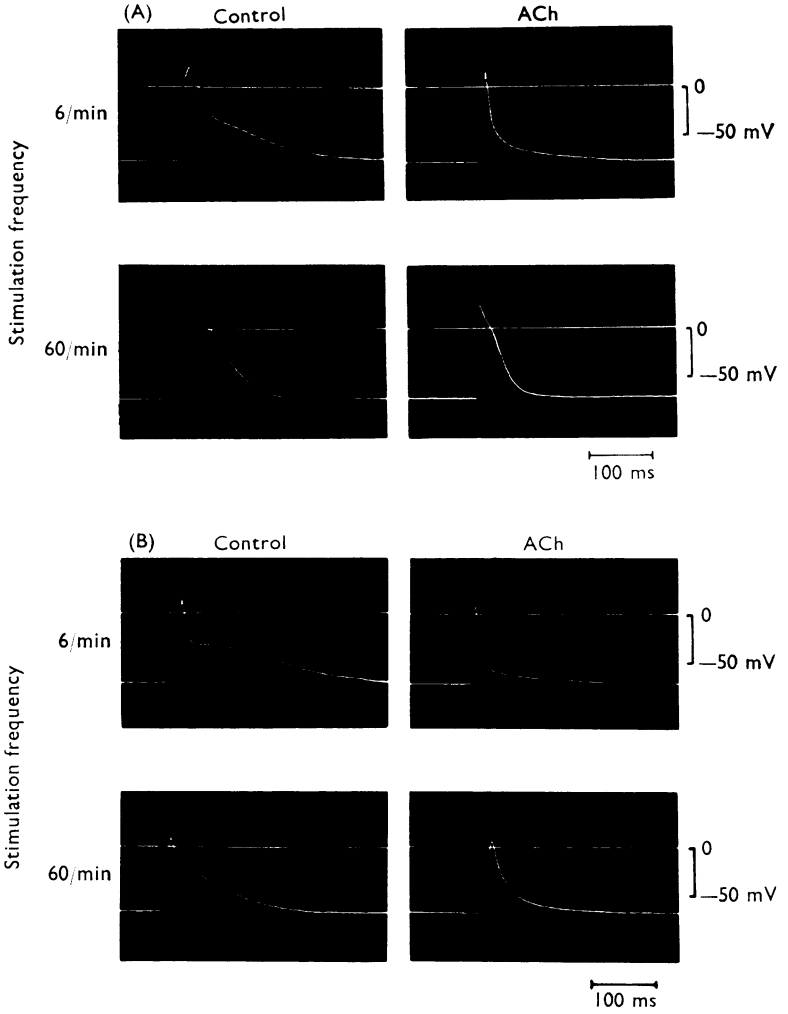

FIG. 8. Alterations in the configuration of atrial action potentials caused by acetylcholine 10^{-6} g/ml. A, Control preparations. B, Preparations treated with ouabain 10^{-6} g/ml for 50 min.

Membrane potential

The configuraton of the action potential was markedly dependent on frequency of stimulation. The 10% duration increased with increasing frequency within the range 6–60/min, but decreased at higher frequencies (Fig. 4). The 50% duration increased with increasing frequency up to 120/min, but was reduced at higher frequencies (Fig. 5). The dependence of the 50% duration on frequency of contraction paralleled that of contractile strength (compare Fig. 1). The 90% duration was independent of frequency up to 120/min (Fig. 6). The resting potential and the size of the overshoot were not significantly affected by alterations in frequency.

Acetylcholine (10^{-6} g/ml) shifted the duration-frequency curves downwards (Figs. 4, 5 and 6). Shortening of the 10% and 50% durations was most marked when preparations were driven at high frequencies (30 to 120/min), whereas reduction of the 90% duration was most marked at low frequencies (6 and 12/min). Acetylcholine had no significant effect on the resting potential and the overshoot at any frequency (Fig. 7). Typical changes in the configuration of action potentials induced by acetylcholine are shown in Fig. 8A. Both the electrical and the mechanical effects of acetylcholine were abolished by atropine (10^{-6} g/ml).

Shortening of the action potential was more marked in atrial preparations exposed to 10^{-6} g/ml of ouabain for 45 to 90 min than in those exposed to 10^{-6} g/ml of acetylcholine for 10 to 20 min (Figs. 4 and 5). The decrease in these durations

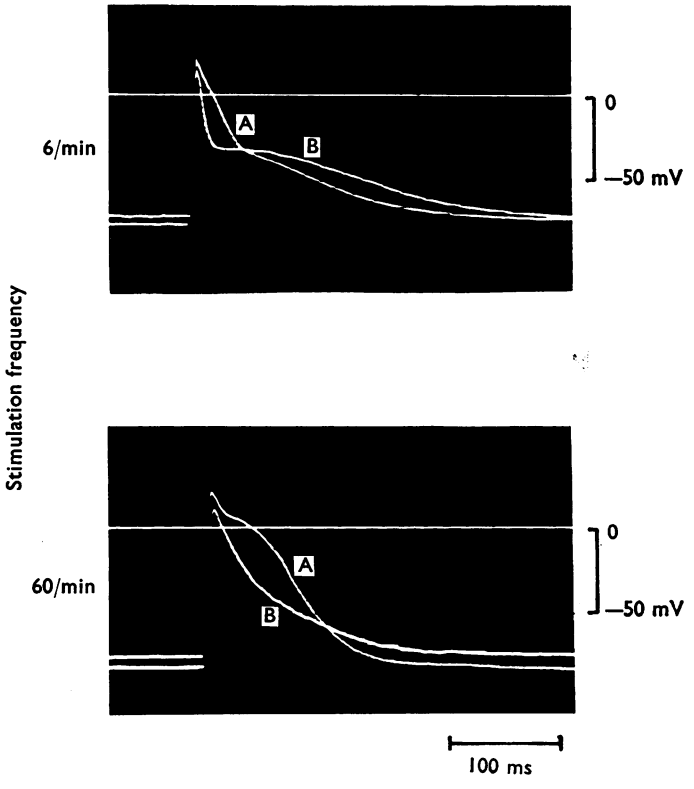

FIG. 9. Alterations in the configuration of atrial action potentials caused by ouabain. A, Control; B, after 60 min exposure to ouabain 10^{-6} g/ml.

might be due partly to the reduction in action potential amplitude induced by ouabain (Fig. 7). The effect of ouabain on the 90% duration was different from that of acetylcholine. Following treatment with ouabain the 90% duration was appreciably increased at frequencies of 6 and 12/min, but was decreased at higher frequencies (Fig. 6). In the presence of ouabain the decrease in the 90% duration paralleled the decrease in force of contraction as the frequency was increased (compare Fig. 3). These results indicate that ouabain accelerates early repolarization but slows late repolarization. As illustrated in Fig. 7, ouabain caused a significant reduction in the magnitude of the resting potential and of the overshoot. Differences in the configuration of action potentials recorded from the preparation exposed to ouabain and from the control preparation are shown in Fig. 9.

In ouabain-treated preparations a further shortening of the 10% and 50% durations was produced by acetylcholine. Shortening of the action potential was marked at frequencies of 30 and 60/min, resulting in flattening of the duration-frequency curve. Acetylcholine applied to preparations treated with ouabain 10^{-6} g/ml markedly shortened the 90% duration and also caused flattening of the duration-frequency curve. The results are shown in Figs. 4, 5, 6 and 8B. In Table 2 the membrane effect of acetylcholine before and after adding ouabain are compared. Shortening of the action potential by acetylcholine was significantly enhanced by the ouabain-treatment. In preparations treated with ouabain the resting potential and the size of the overshoot were not significantly affected by acetylcholine (Fig. 7).

Discussion

Data obtained in the present study are in agreement with the results of other investigators (Koch-Weser & Blinks, 1963; Toda, 1969a, b), indicating that the force of contraction of isolated atria varies with the frequency of contraction, being maximal at a frequency of 120/min. The 10% and 50% durations of atrial action potentials showed a similar variation with frequency of contraction, whereas the 90% duration was independent of frequencies. These results support the view that the action potential duration correlates directly with force of contraction (Vaughan Williams, 1959; Antoni, Engstfeld, Fleckenstein & Klein, 1962; Kaufmann & Fleckenstein, 1965).

TABLE 2. *Paired analyses of the effect of acetylcholine on the duration of action potentials before and after ouabain* (10^{-6} *g/ml*)

			Frequency of stimulation			
			30/min		60/min	
		N	Duration (ms) before ACh	% decrease	Duration (ms) before ACh	% decrease
10% duration	Control	10	21±2	22±3	24±2	32±4
	Ouabain	10	9±1*	56±7*	10±1*	51±6†
50% duration	Control	10	65±7	27±3	73±7	25±3
	Ouabain	10	37±4*	57±8*	39±3*	54±6*
90% duration	Control	10	138±12	20±3	140±15	21±2
	Ouabain	10	142±14	41±7†	125±10	52±8*

N, Number of preparations. Mean values from two to six penetrations at each condition of the preparations were used for calculation. * Significant difference from control, $P<0.01$; †$P<0.02$.

The 10% and 50% durations of atrial action potentials were reduced by ouabain, as well as by acetylcholine and by an increase in the diastolic interval. Ouabain, however, increased the force of contraction, whereas the other procedures reduced it. Thus, with ouabain, the correlation between the 10% or 50% duration and force of contraction was lost. The similar loss of the correlation between the two phenomena has been demonstrated in the case of post-extrasystolic potentiation (Hoffman, Bindler & Suckling, 1956) and following alterations in extracellular concentrations of Ca^{2+} or Na^{+} (Stanley & Reiter, 1965).

Whereas atropine blocked both the electrical and mechanical effects of acetylcholine, ouabain potentiated the membrane effect but reduced the mechanical effect. Potentiation by ouabain of the effects of acetylcholine on the S-A nodal membrane and the spontaneous rate was previously demonstrated by Toda & West (1966). It is therefore unlikely that the inhibitory action of ouabain on the mechanical response to acetylcholine is due to antagonism at the receptor level. Ouabain probably affects excitation-contraction coupling in heart muscle, and further studies are required to define the interactions of cardiac glycosides and acetylcholine on the Ca^{2+} movement, the adenyl cyclase and the other mechanisms involved (Grossman & Furchgott, 1964; Robison, Butcher & Sutherland, 1967).

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