Br. J. Pharmac. (1969), **36**, 393–408.

Interactions of ouabain and noradrenaline in isolated rabbit's atria

N. TODA

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto, Japan

1. The present study was made using sympathetic nerve-atria preparations, conventional atrial preparations and left atria isolated from rabbits in order that interactions of ouabain and noradrenaline on the transmembrane potential of S-A nodal pacemaker fibres, the pacemaker rate and the contractile force of the left atrium could be investigated.

2. In pacemaker action potentials, the maximal diastolic potential was decreased, the early repolarization was accelerated and the late repolarization was slowed by toxic concentrations of ouabain. Oscillating potentials of the diastolic pacemaker membrane were produced.

3. Stimulation of the postganglionic sympathetic nerve caused an increase in the slope of diastolic depolarization, resulting in an acceleration of the pacemaker rate. In the presence of ouabain, the pacemaker action potential configuration was significantly altered by nerve stimulation in preparations which showed ouabain-induced bradycardia, but the potential configuration was not changed by nerve stimulation in preparations which showed ouabain-induced tachycardia. Cardiac noradrenaline altered the ion permeability of the cardiac pacemaker membrane during diastole, but it had no effect during systole. Sympathetic nerve stimulation transiently corrected the overshoot and the repolarization of atrial action potentials altered by toxic concentrations of ouabain.

4. The positive chronotropic effect of sympathetic nerve stimulation was not affected by either therapeutic or toxic concentrations of ouabain. Ouabain shifted the concentration-chronotropic response curve for exogenous noradrenaline to the left, but did not shift the curve for tyramine.

5. Relationship between the developed tension and the rate of contraction was altered differently by ouabain and by noradrenaline. The positive inotropic effect of noradrenaline was not significantly altered by therapeutic concentrations of ouabain but it was inhibited by toxic concentrations.

In earlier reports (McEwen, 1956; Toda & West, 1966) it has been shown that ouabain causes a potentiation of the negative chronotropic response of isolated mammalian hearts to vagal stimulation and to acetylcholine and an alteration in the membrane effect of cardiac acetylcholine. However, little quantitative data are available on the interaction of ouabain and noradrenaline on the pacemaker membrane, on the S-A nodal rate and on the contractile force of isolated hearts.

Ouabain in toxic concentrations causes measurable changes in the transmembrane potential of S-A nodal pacemaker fibres (Toda & West, 1966), which are postulated

to result from an inhibition of the active membrane process (Skou, 1957) and, as a result, from a decrease in the ratio of $[K^+]i$ (intracellular concentrations of K^+)/ $[K^+]o$ (extracellular concentrations of K^+) and of $[Na^+]o/[Na^+]i$. Cardiac noradrenaline causes alterations in the ion permeability of the pacemaker membrane during diastole but not during systole (Toda & Shimamoto, 1968).

Dengler, Michaelson, Spiegel & Titus (1962) indicated that slices of the brain and the heart took up labelled noradrenaline from incubation media by a mechanism having the characteristics of an active transport system. The uptake of noradrenaline by sympathetic nerve terminals is considered to be a physiologically important mechanism by which the amine's action is terminated. An application of desmethylimipramine and a reduction of $[Na^+]o$ cause an inhibition of activities of the membrane ATPase (Tarve & Brechtlova, 1967 ; Lee & Yu, 1963), a reduction of the uptake of noradrenaline by the isolated heart (Titus, Matussek, Spiegel & Brodie, 1966 ; Iversen & Kravitz, 1966) and a potentiation of the positive chronotropic effect of sympathetic nerve stimulation (Matsuo & Toda, 1968 ; Toda, 1968). If the uptake process were active as suggested by Dengler *et al.* (1962), cardiac effects of endogenous and exogenous noradrenaline would be potentiated by ouabain.

The contractile force of the heart is stimulated by both ouabain and noradrenaline. However, a dependency of the force on frequency of contraction is differently affected by ouabain (Koch-Weser & Blinks, 1962) and by noradrenaline. Also, ouabain produces a net loss of K^+ from cardiac muscles, whereas noradrenaline produces a net gain of K^+ (Sarnoff, Gilmore & Wallace, 1965). Changes in $[K^+]i$ have been considered to correlate with changes in contractility of the heart.

These differences in the action of ouabain and noradrenaline on the pacemaker membrane, on the heart rate and on the contractile force, as well as the frequent implication of noradrenaline in ouabain-induced inotropy and toxicity, prompted me to quantify the interactions of ouabain and noradrenaline. Various different atrial preparations were used in order that experimental conditions could be more readily evaluated.

Methods

Fifty-six rabbits of either sex, weighing 1·8–2·2 kg, were used. The animals were killed by exsanguination from the common carotid arteries under ether anaesthesia. In the present studies three different preparations were used. For studies on the atrial effect of endogenous noradrenaline, the isolated sympathetic nerve-atria preparation, previously described (Toda & Shimamoto, 1968), was used. For studies of exogenous noradrenaline and tyramine the S-A node-right atrium preparation was used, and for studies on changes in contractile force, when the rate was maintained constant by artificial electrical stimulation, the left atrial preparation was used. These isolated preparations were fixed horizontally (endocardial surface uppermost) between hooks under a resting tension of 300–450 mg in a muscle bath of 60 ml. capacity. The specimens were immersed in a nutrient solution maintained at 30° ±0·5° C and gassed by a mixture of 95% oxygen–5% carbon dioxide. Constituents of the solution were as follows (mM): Na^+, 162·1 ; K^+, 5·4 ; Ca^{++}, 2·2 ; Cl^-, 157·0 ; HCO_3^-, 14·9 ; glucose, 5·6. Changes in the contractile force were recorded by means of a force-displacement transducer connected to the atrium by a lever containing hooks which anchored the atrial appendage. Hooks anchoring

the cut end of the left atrium were connected to a pulse-generator for driving the preparations. After mounting the preparations, 60 to 90 min were allowed for equilibration.

The right sympathetic postganglionic nerve was placed on a bipolar silver electrode which was lifted above the surface of the solution. The sympathetic nerve was stimulated for periods of 3 sec by trains of rectangular pulses of 1·0 msec duration with supramaximal intensity, applied at a frequency of 20 c/s, unless otherwise indicated. The stimulation interval was usually 5 min. The left atrium was driven by rectangular pulses of 3 msec duration with supramaximal intensity, at frequencies of 6, 12, 30, 60, 120 and 240 beats/min. Unless recordings for the developed tension-frequency relationships were performed, the preparation was always driven at a constant frequency of 60 beats/min. Electrical stimuli were provided by a Sanei type ES-103-Z pulse generator.

Transmembrane potentials were recorded from single pacemaker fibres of the S-A node by the use of a floating microelectrode. The action potential was displayed on a two channel pen-writer simultaneously with contractions of the right atrium. Recordings of pacemaker action potentials were obtained from preparations exposed to 10^{-6} g/ml. of ouabain for 40 to 60 min. Membrane potentials were recorded from a VC-7 oscilloscope (Nihonkoden Kogyo Co.) on film moving at a rate of 5 cm/sec. The following parameters of the membrane potential were measured: cycle length between pacemaker action potentials; maximal diastolic potential; threshold potential; the size of overshoot; 10% and 90% duration; and depolarization time. These parameters have been defined in earlier reports (Toda & Shimamoto, 1968; Toda, 1968). The parameters of the same pacemaker fibres were compared before and after sympathetic nerve stimulation. The S-A nodal rate was taken as the mean value of ten measurements of the cycle length between contractions before nerve stimulation and when the maximum response to the stimulation was obtained.

For studies on the interaction of ouabain and noradrenaline sympathetic nerve stimulation was applied after 20 min of exposure to various concentrations of ouabain. For the concentration-response curve of noradrenaline and tyramine, drugs were added in cumulative concentrations directly to the muscle bath. Only one concentration-response curve for tyramine was obtained from each preparation. The amines were applied after 20 min exposure to ouabain. For making the developed tension-rate curve artificial electrical stimulation of the left atrium was increased stepwise from 6 to 240 beats/min. The tension-rate curve took about 15 min to complete. The curves were obtained after 20 and 45 min of exposure to ouabain in control experiments. In experiments on the effect of noradrenaline in solutions containing ouabain, noradrenaline was applied immediately after the 20 min curve was obtained. The tension-rate curve was obtained 10 min after noradrenaline. Thus, the curves were compared for tissues exposed to ouabain for 45 min, with or without exposure to noradrenaline. The results were expressed as mean values $\pm$ standard errors of the means. Comparisons of results were made using the Student *t* test.

Ouabain, U.S.P. (Nutritional Biochemical Corp.) in concentrations of 5×10^{-8}, 2×10^{-7} and 10^{-6} g/ml., which correspond to $6{\cdot}85 \times 10^{-8}$, $2{\cdot}75 \times 10^{-7}$ and $1{\cdot}37 \times 10^{-6}$M, respectively, (−)-noradrenaline hydrochloride and tyramine hydrochloride were used. Concentrations of the drugs are expressed in terms of g/ml. of the salt.

Results

Effects of ouabain on the membrane potential of pacemaker fibres

The spontaneous atrial rate was markedly accelerated after 20 to 40 min of exposure to ouabain 10^{-6} g/ml. in ten of eleven preparations. The tachycardia was followed by arrhythmic bradycardia in eight of these ten preparations. Measurements in the present study were made in atria showing tachycardia with subsequent bradycardia. Mean values of transmembrane potential changes were recorded from preparations in which tachycardia was produced and from preparations in which bradycardia was produced. These changes are illustrated as O_2 and O_1, respectively, in Figs. 1 and 2. Control values were obtained from previous experiments in which the same experimental procedures were used (Toda & Shimamoto, 1968). In preparations which showed either tachycardia or bradycardia the maximal diastolic potential and the threshold potential were reduced but the size of the overshoot was not significantly affected (Fig. 1). The 10% duration and the depolarization time

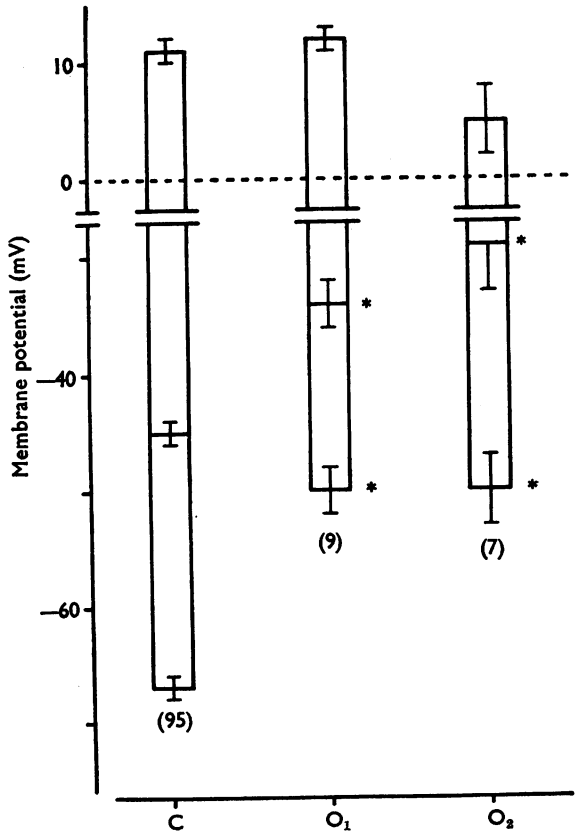

FIG. 1. Changes in the membrane potential of pacemaker fibres induced by ouabain 10^{-6} g/ml. Columns above the 0 mV level represent the overshoot and those under the level represent the threshold potential and the maximal diastolic potential. Vertical bars on the columns represent standard errors of the means. The number of pacemaker fibres from which the membrane potential was recorded is shown in parentheses. The number of preparations were twenty-five, seven and six in control, O_1 and O_2, respectively. C, Control; O_1, preparations in which bradycardia was produced by ouabain 10^{-6} g/ml.; O_2, preparations in which tachycardia was produced. * Significant difference from control, $P<0{\cdot}01$.

were decreased (Fig. 2). Typical changes in the pacemaker action potential induced by ouabain 10^{-6} g/ml. are illustrated in Fig. 3. The magnitude of membrane potential changes from preparations showing tachycardia and from preparations showing bradycardia differed. The size of the overshoot was decreased and the 10% duration was prolonged by an acceleration of the atrial rate in the presence of the toxic concentration of ouabain. The other parameters measured were not affected by alterations in rate. These differences would indicate some dependence of

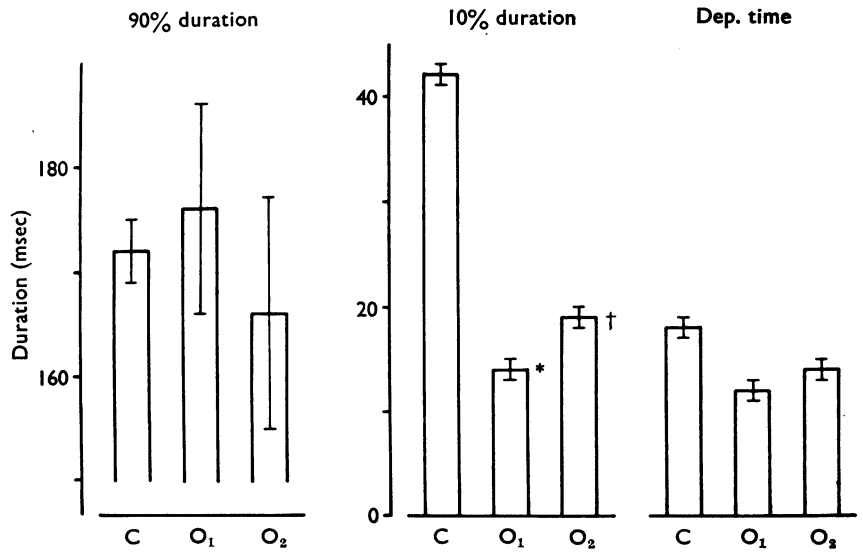

FIG. 2. Changes in the action potential duration induced by ouabain 10^{-6} g/ml. C, O_1 and O_2, see legend to Fig. 1. * Significant difference from control, $P<0.01$. † Significant difference from control, $P<0.01$, and from O_1, $P<0.05$.

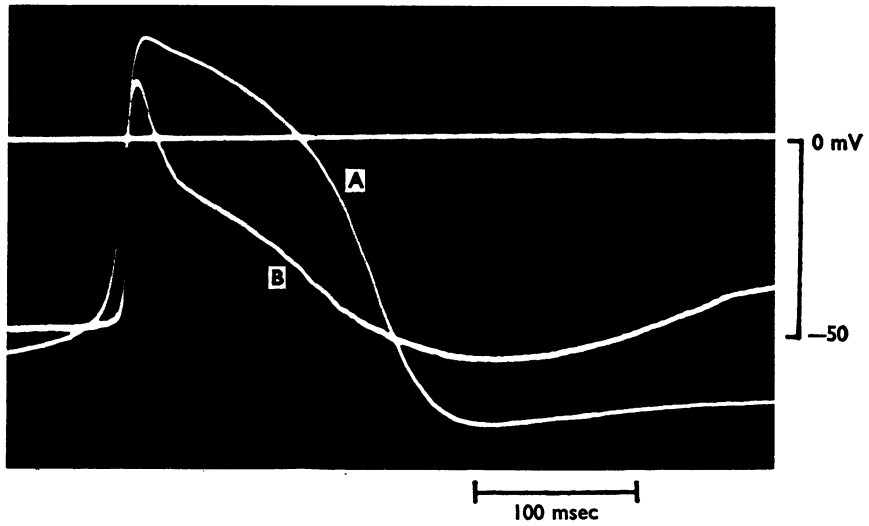

FIG. 3. Membrane potential recordings from S-A nodal pacemaker fibres. A, Recording in the control solution; B, recording from the preparation in which bradycardia was produced by 40 min exposure to ouabain 10^{-6} g/ml.

the action potential configuration on the atrial rate. In preparations in which bradycardia and arrhythmia were produced by ouabain, sub-threshold oscillations were observed in fully-repolarized pacemaker membranes.

Interaction of sympathetic nerve stimulation with ouabain

Stimulation of postganglionic sympathetic nerves caused a frequency-dependent increase in the S-A nodal rate and the contractile force of the right atrium. The prestimulation rate was not altered by ouabain 5×10^{-8} and 2×10^{-7} g/ml. Effects of sympathetic nerve stimulation on the rate of the S-A node exposed to various concentrations of ouabain are summarized in Table 1. Although the magnitude

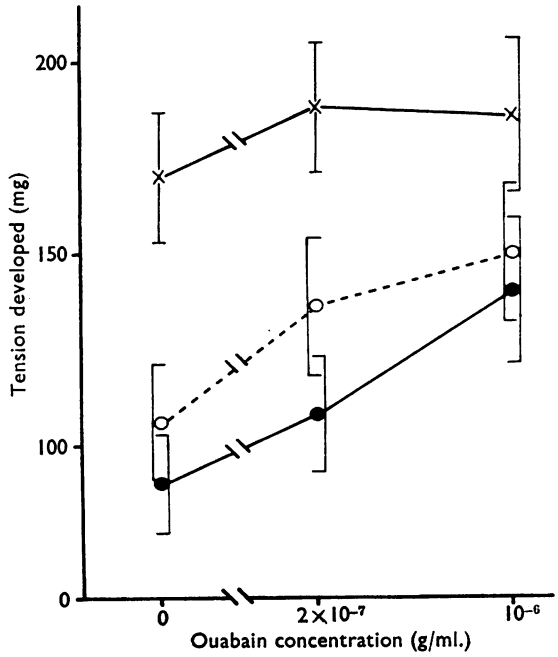

FIG. 4. Modification by ouabain of the positive inotropic response of spontaneously-beating atria to sympathetic nerve stimulation. Each point is the mean of five to nine experiments. Vertical bars represent standard errors of the means. Results were obtained from preparations exposed to 2×10^{-7} g/ml. for 50–65 min, and to 10^{-6} g/ml. for 40–60 min. ●——●, Control; ○- - - -○, 5/sec; ×——×, 20/sec.

TABLE 1. *Positive chronotropic effect of sympathetic nerve stimulation applied at a frequency of 20 c/s*

Procedure	*N*	Atrial rate (beats/min) Pre-stimulation	Post-stimulation	Increase	Duration of response (min)
Control	30	86±4	138±3	51±5	5·0±0·4
Ouabain 5×10^{-8} g/ml.	6	88±8	146±6	58±9	5·8±0·8
Ouabain 2×10^{-7} g/ml.	12	83±4	138±4	54±7	6·4±1·0
Ouabain 10^{-6} g/ml.*	10	78±5	137±5	59±6	
Ouabain 10^{-6} g/ml.†	7	141±8‡	161±10‡	20±4‡	
After wash	10	76±5	135±5	59±6	5·3±0·8

* Preparations in which the prestimulation rate was slightly slowed.
† Preparations in which tachycardia was induced.
‡ Significant difference from control, $P<0.01$.
N, Number of experiments.

of the positive chronotropic effect of nerve stimulation was not significantly affected by ouabain at the two lower concentrations, these concentrations of ouabain did increase the duration of the effect. In preparations in which bradycardia developed after 20 min of exposure to ouabain 10^{-6} g/ml., the positive chronotropic response to nerve stimulation applied at 20 c/s was slightly potentiated. Similarly, in preparations in which tachycardia developed after ouabain 10^{-6} g/ml., the maximum rate induced by sympathetic nerve stimulation at 20 c/s was increased, but the increase in rate was significantly reduced. The positive chronotropic effect of nerve stimulation at frequencies of 1 and 5 c/s was not affected by ouabain. In five of twelve preparations exposed to ouabain 10^{-6} g/ml., tachycardia induced by sympathetic stimulation persisted for longer than 10 min and was converted to arrhythmia. In order to test whether the provocation of the ouabain toxicity was due to actions of cardiac noradrenaline or to an acceleration of the atrial rate, three atria were

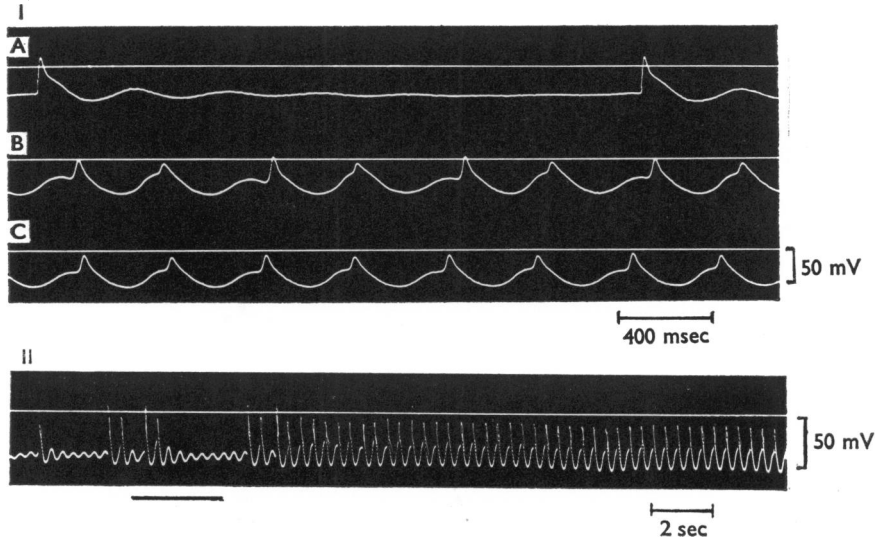

FIG. 5. Effects of sympathetic nerve stimulation on the transmembrane potential recorded from an S-A nodal pacemaker fibre exposed to ouabain 10^{-6} g/ml. for 45 min. I: High speed recording. A, Prestimulation ; B, 5 sec after and C, 30 sec after sympathetic nerve stimulation ; II: Low speed recording. Horizontal bar just under the tracing represents sympathetic nerve stimulation.

TABLE 2. *Modification by sympathetic nerve stimulation of parameters of the membrane potential of pacemaker fibres exposed to ouabain* (10^{-6} *g/ml.*)

	O_1 (n=9)			O_2 (n=7)		
Parameters measured	Control	Stimulation	% Change	Control	Stimulation	% Change
Cycle length (msec)	>1,500	388±12		402±18	372±15	
Maximal diastolic potential (mV)	50·4±1·8	48·8±2·3	−3±1	49·7±3·2	46·9±3·2	−1±2
Threshold potential (mV)	34·2±2·4	21·6±1·7*	−40±4	29·4±4·2	23·8±5·3	−3±11
Overshoot (mV)	11·8±1·4	0·7±0·8*		5·2±2·8	5·0±2·7	
90% Duration (msec)	176±10	149±5‡	−11±2	166±11	156±15	−4±2
10% Duration (msec)	14·4±1·3	21·0±1·2*	+64±15	18·6±1·2	18·0±1·5	−1±8
Depolarization time (msec)	11·7±1·3	16·0±1·3†	+55±13	14·3±0·9	14·2±1·2	+5±11

O_1, Preparations which showed ouabain-induced bradycardia; O_2, preparations which showed ouabain-induced tachycardia. n, Number of pacemaker fibres from which the membrane potential was recorded. Significant difference from control: * $P<0.01$; † $P<0.02$; ‡ $P<0.05$.

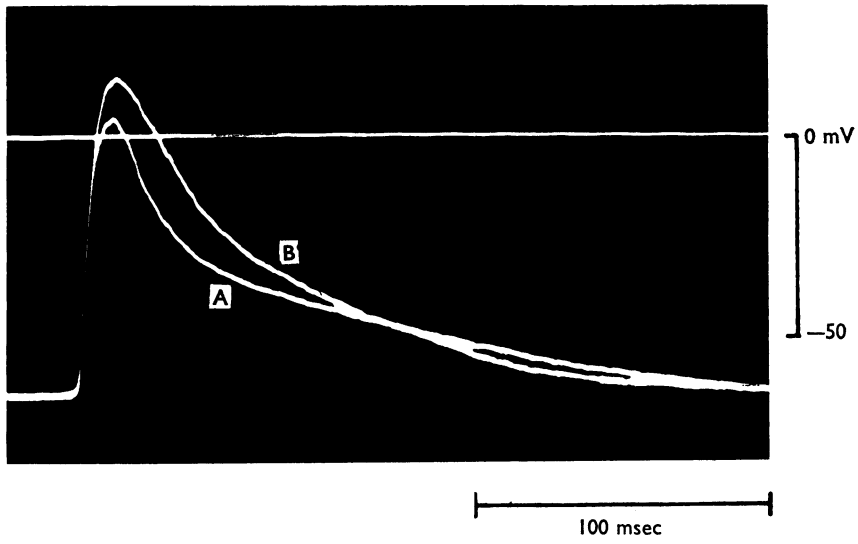

FIG. 6. Effects of sympathetic nerve stimulation on the membrane potential of a fibre of the right atrium exposed to ouabain 10^{-6} g/ml. for 35 min. A, Prestimulation; B, 20 sec after nerve stimulation.

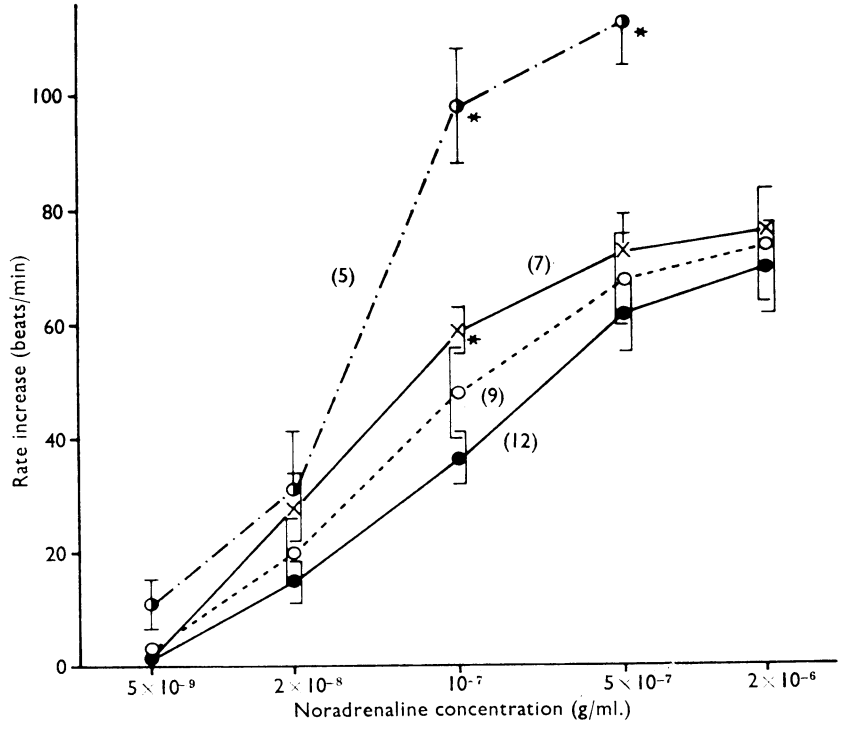

FIG. 7. Modification by ouabain of the positive chronotropic response to noradrenaline. ●——●, Control; ○- - -○, ouabain 5×10^{-8} g/ml.; ×——×, ouabain 2×10^{-7} g/ml.; ◑——◑, ouabain 10^{-6} g/ml. Figures in parentheses indicate the number of experiments on which the mean was based. * Significant difference from control, $P<0.01$.

driven by electrical stimulation at a frequency of 2 c/s applied for 3 min after 20 min exposure to ouabain 10^{-6} g/ml. In all these preparations tachycardia persisted after the drive stimulation was terminated. At the frequency and duration of the drive, measurable tachycardia, which would be expected to result from noradrenaline released from intracardiac adrenergic nerves, was not produced after its termination in three of three control preparations (pre-drive rate, 87 ± 2 beats/min; post-drive rate, 88 ± 2; $n=6$).

The contractile force of the right atrium was increased after 20 min exposure to ouabain 2×10^{-7} and 10^{-6} g/ml. The maximum force of the atrial contraction induced by sympathetic nerve stimulation at a frequency of 20 c/s did not differ in ouabain-free solutions and in solutions containing ouabain 10^{-6} g/ml. (Fig. 4).

Effects of endogenously released noradrenaline on the membrane potential in the presence of ouabain (10^{-6} g/ml.) are shown in Table 2. Sympathetic nerve stimulation caused additional changes in the membrane potential of pacemaker fibres showing bradycardia after ouabain 10^{-6} g/ml. (O_1 in Table 2). The slope of diastolic depolarization was increased. The amplitude of subthreshold oscillations, which appeared during diastole, was increased to a level where conducted action potentials were generated. The threshold potential, the size of the overshoot and the 90% duration were significantly decreased, whereas the 10% duration and the depolarization time were increased. Figure 5 (I and II) shows the effects of cardiac noradrenaline on the pacemaker membrane potential. On the other hand, sympathetic nerve stimulation did not significantly affect the membrane potential of pacemaker fibres showing tachycardia after ouabain 10^{-6} g/ml. (O_2 in Table 2). Values

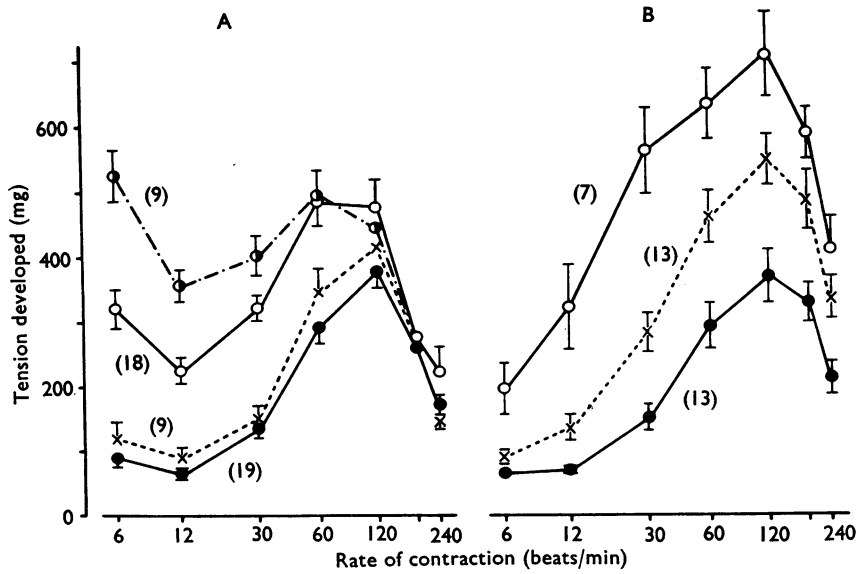

FIG. 8. Changes in tension-rate curves of left atria induced by ouabain (A, ×---×, 2×10^{-7} g/ml., 45 min; ○——○, 10^{-6} g/ml., 20 min; ◑——◑, 10^{-6}, 45 min) and noradrenaline (B, ×---×, 10^{-7} g/ml.; ○——○, 5×10^{-7} g/ml.). ●——●, Control. Figures in parentheses indicate the number of experiments.

presented in the column " Control " in O_2 did not significantly differ from those in the column " Stimulation " in O_1. These results indicate that changes in the action potential configuration induced by sympathetic nerve stimulation were primarily due to a decrease in the cycle length and not to direct actions of cardiac noradrenaline.

In fibres of the right atrium exposed to ouabain 10^{-6} g/ml. the configuration of action potentials was considerably altered: the resting potential and the size of the overshoot were decreased, early repolarization was accelerated and the late repolarization was slowed. These changes were characteristic of toxic actions of ouabain. In three of six preparations sympathetic nerve stimulation appreciably corrected the overshoot and the repolarization without affecting the resting membrane potential. The typical effect is shown in Fig. 6.

Interaction of exogenous noradrenaline or tyramine with ouabain

An increase in rate of isolated S-A nodes induced by exogenous noradrenaline was not affected by ouabain 5×10^{-8} g/ml., but was slightly potentiated by ouabain 2×10^{-7} g/ml. (Fig. 7). The S-A nodal rate before the application of noradrenaline was 97 ± 6 ($n=12$), 95 ± 9 ($n=9$) and 86 ± 10 beats/min ($n=7$) in control solutions and solutions containing ouabain in concentrations of 5×10^{-8} and 2×10^{-7} g/ml., respectively. In preparations showing a slight bradycardia (72 ± 11 beats/min, $n=5$) after exposure for 20 min to ouabain 10^{-6} g/ml., noradrenaline caused a marked increase in the rate (Fig. 7). The maximum rate increase induced by noradrenaline 10^{-7} and 5×10^{-7} g/ml. was greater in the presence of ouabain 10^{-6} g/ml. than in the presence of the two lower concentrations and in ouabain-free solutions. The increase in rate at 10^{-6} g/ml. would be regarded as a sum of noradrenaline- and ouabain-induced tachycardia, because the ouabain toxicity was frequently provoked by sympathetic nerve stimulation, as indicated in the previous section.

The positive chronotropic effect of tyramine (10^{-6} to 10^{-4} g/ml.) was not modified by prior application of ouabain in a concentration of 2×10^{-7} g/ml.

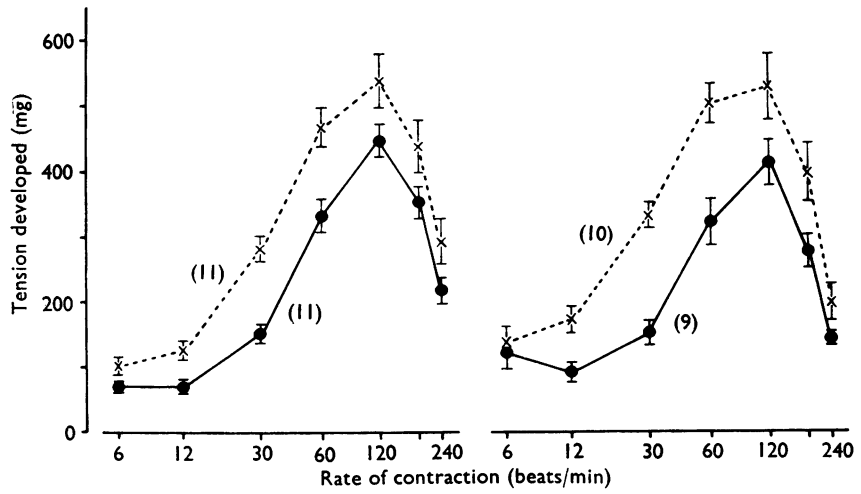

FIG. 9. Modification by ouabain of the positive inotropic response to noradrenaline. ●——●, 45 min exposure to ouabain 5×10^{-8} g/ml. (left) and 2×10^{-7} g/ml. (right); ×---×, 10 min after noradrenaline 10^{-7} g/ml. which was applied to preparations exposed to ouabain for 35 min.

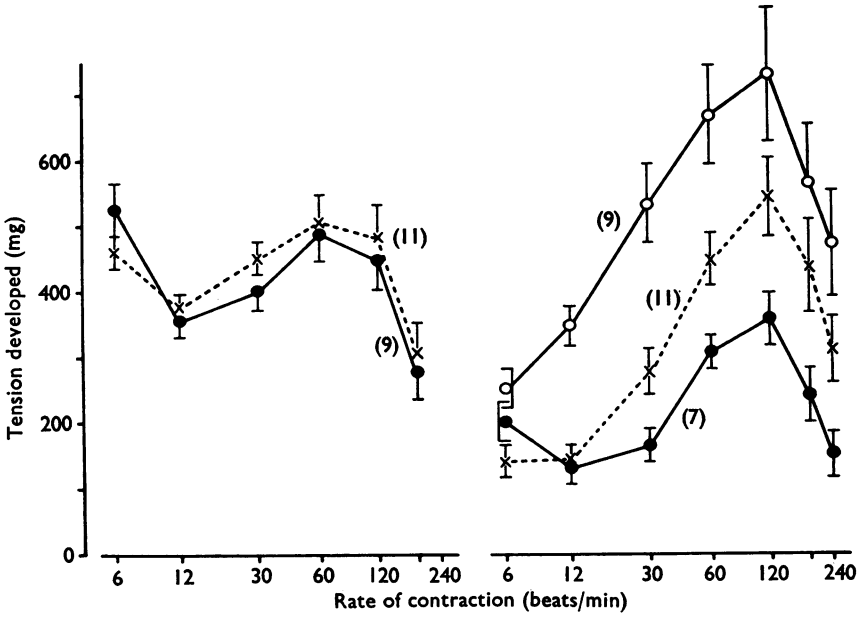

FIG. 10. Modification by ouabain of the positive inotropic response to noradrenaline. Left, ●——●, 45 min exposure to ouabain 10^{-6} g/ml.; ×---×, 10 min after noradrenaline 10^{-7} g/ml. which was applied to preparations exposed to ouabain for 35 min. Right, ●——●, 60–90 min after washout of ouabain 10^{-6} g/ml.; ×---×, noradrenaline 10^{-7} g/ml.; ○——○, noradrenaline 5×10^{-7} g/ml.

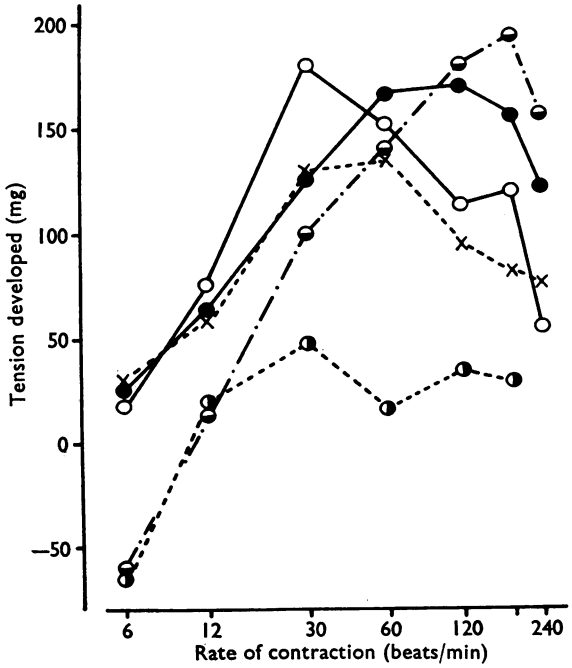

FIG. 11. Comparison of the positive inotropic effect of noradrenaline (10^{-7} g/ml.) in the presence and absence of ouabain. ●——●, Control; ×---×, ouabain 5×10^{-8} g/ml.; ○——○, ouabain 2×10^{-7} g/ml.; ◑——◑, ouabain 10^{-6} g/ml.; ◒——◒, after wash. Ordinate represents difference in tension in noradrenaline-free solutions from that in solutions with noradrenaline added.

The developed tension of the atrium is known to be a function of the rate of contraction (Blinks & Koch-Weser, 1961). The dependency of the tension on the rate was measurably altered by ouabain as illustrated in Fig. 8. Ouabain in a concentration of 10^{-6} g/ml. caused a marked increase in the tension of preparations driven at slow rate (6–60 beats/min) but a slight increase or no change at fast rate (120–240 beats/min). On the other hand, the curve of the developed tension was shifted upward at all frequencies of stimulation by noradrenaline. The shift varied directly with concentrations of the amine (Fig. 8). The positive inotropic response of the left atrium to noradrenaline, 10^{-7} g/ml., driven at various rates was not significantly affected by prior application of ouabain in concentrations of 5×10^{-8} and 2×10^{-7} g/ml. but was considerably reduced by ouabain 10^{-6} g/ml. After washout of ouabain, the tension-rate curve was corrected by noradrenaline. The results are illustrated in Figs. 9 and 10. Figure 11 shows changes in the developed tension induced by noradrenaline. Ouabain in the two lower concentrations reduced the positive inotropic response of atria driven at a fast rate (60–240 beats/min) to noradrenaline. In the presence of ouabain 10^{-6} g/ml. and after wash, noradrenaline caused a reduction in the tension at a rate of 6 beats/min.

Discussion

The membrane potential

In an earlier report (Toda & West, 1966) it has been shown that the maximal diastolic potential and the size of the overshoot were reduced in pacemaker fibres of rabbit S-A nodes exposed to ouabain 10^{-6} g/ml. The present experiments have provided more detailed information concerning the membrane effect of ouabain in toxic concentrations: marked changes are observed in the maximal diastolic potential and in repolarization. It is well known that ouabain inhibits the active membrane transport process by which K^+ is taken up and Na^+ is extruded, resulting in an accumulation of Na^+ and a deprivation of K^+ in cardiac fibres. The resultant decrease in the ratio of $[K^+]i/[K^+]o$ and a decrease in the K^+-permeability of the pacemaker membrane during diastole (Dudel & Trautwein, 1958) would account for a decrease in the maximal diastolic potential. The configuration of pacemaker action potentials recorded from the S-A node exposed to toxic concentrations of ouabain simulates that in Na^+-poor (Toda, 1968) and Ca^{++}-rich solutions (Toda, unpublished data): the early repolarization is accelerated and the late repolarization is slowed. The change induced by ouabain and by low $[Na^+]o$ in the repolarization would be due to a decrease in the ratio of $[Na^+]o/[Na^+]i$, whereas the Ca^{++}-induced change would be due to an antagonism of Ca^{++} to Na^+ during repolarization. Effective inactivation of the Na^+-carrying system at the end of depolarization is suggested to account for the change in the early repolarization in Na^+-poor solutions (Toda, 1968). An acceleration of repolarization induced by ouabain is also observed in atrial (Sleator, Furchgott, Gubareff & Krespi, 1964), ventricular (Stutz, Feigelson, Emerson & Bing, 1954; Vassalle, Karis & Hoffman, 1962) and Purkinje fibres (Kassebaum, 1963). This change is suggested to result from the increased K^+ conductance. However, in S-A nodal pacemaker fibres the repolarization is not appreciably affected by an elevation of $[K^+]o$ (Toda, unpublished data) which causes an increase in the K^+ conductance (Carmeliet, 1961). An increase in the cycle length between pacemaker action potentials would result in accelerating the early repolarization and in increasing the size of the overshoot in ouabain-

containing solutions. A similar dependency of repolarization on the cycle length is shown in action potentials of canine atria (Greenspan, Edmands & Fisch, 1967). On the other hand, the depolarization time and the rate of rise which was estimated from the action potential amplitude and the depolarization time were not decreased by ouabain or by an elevation of $[Ca^{++}]o$ (Toda, unpublished data) but by a reduction of $[Na^{+}]o$ (Toda, 1968). The influx of labelled Ca^{++} through the cardiac membrane is increased by ouabain (Govier & Holland, 1965). Whether or not Ca^{++} can enter into pacemaker fibres when the membrane is depolarized is not known. However, the possibility is suggested in guinea-pig papillary muscles (Winegrad & Shanes, 1962).

Sympathetic nerve stimulation caused an increase in the slope of diastolic depolarization and an increase in the amplitude of subthreshold oscillations during diastole until the pacemaker membrane was depolarized to a level of firing conducted action potentials, resulting in an acceleration of the S-A nodal rate. The membrane potential of pacemaker fibres showing bradycardia after ouabain was significantly affected by sympathetic nerve stimulation. However, the membrane potential of fibres in which the cycle length was markedly decreased by sympathetic stimulation (" Stimulation " of O_1 in Table 2) were not considerably different from that obtained from preparations in which the prestimulation cycle length was short (" Control " of O_2 in Table 2). Cardiac noradrenaline did not affect the membrane potential of pacemaker fibres which showed tachycardia after ouabain 10^{-6} g/ml. These results indicate that the ion permeability of the pacemaker membrane is altered by cardiac noradrenaline in the direction of accelerating the depolarization during diastole, but is not considerably affected during systole, and that electrophysiological properties of the activated membrane depend on the cycle length under the influence of ouabain.

In fibres of the right atrium the resting potential and the size of the overshoot were decreased and the repolarization was accelerated by toxic concentrations of ouabain. Sympathetic nerve stimulation corrected the overshoot and the repolarization, but it did not reverse the changes in the resting potential. The S-A nodal-atrial conduction block produced by 16·2 mM $[K^{+}]o$ (3 times normal $[K^{+}]o$) is reversed by sympathetic nerve stimulation (Toda, unpublished data). It is possible that an increased permeability to Na^{+} on depolarization could lead to a transient correction of the conduction block induced by K^{+} or ouabain. Thus cardiac noradrenaline might act on the Na^{+}-permeability of the atrial membrane to reverse ouabain toxicity. However, in pacemaker fibres there is no evidence to support an increase in Na^{+}-permeability by noradrenaline.

S-A nodal rate

The uptake of labelled noradrenaline by the isolated brain and peripheral sympathetically-innervated tissues is suggested to be due to an active transport mechanism, because the tissues are able to take up the amine until the concentration in them is several times that in the incubation media. Furthermore, ouabain and dinitrophenol inhibit the uptake (Dengler *et al.*, 1962). However, according to Muscholl & Weber (1966), the uptake of noradrenaline by the isolated perfused heart was not affected by therapeutic or toxic concentrations of ouabain. Drugs inhibiting the uptake of noradrenaline such as desmethylimipramine and cocaine are known to potentiate actions of endogenous (Matsuo & Toda, 1968) and exogenous noradrenaline, but to

inhibit actions of indirectly acting sympathomimetic amines (Trendelenburg, 1963).

In the present study, although a slight potentiation of the positive chronotropic response to exogenous noradrenaline was produced by therapeutic concentrations of ouabain, no potentiation of the response to sympathetic nerve stimulation and no inhibition of the response to tyramine were produced. Ouabain in therapeutic and toxic concentrations used in the present study does not seem to inhibit the uptake of noradrenaline and tyramine effectively, in spite of the fact that the electrophysiological study showed measurable changes in the transmembrane potential of pacemaker fibres which possibly result from a considerable inhibition of the ATPase activity of the pacemaker membrane. Different susceptibility to ouabain of the ATPase activity of the pacemaker membrane and of the membrane of sympathetic nerve terminals is not known.

On the other hand, Méndez, Acheves & Méndez (1961) demonstrated the anti-adrenergic effect of ouabain on the chronotropy and the A-V conduction in anaesthetized dogs. In isolated atrial preparations, however, no inhibition in the positive chronotropic effect of endogenous and exogenous noradrenaline was obtained even by toxic concentrations of ouabain.

Cardiac noradrenaline is indicated by some investigators (Tanz, 1964 ; Tanz & Marcus, 1966) to play an important role in producing ouabain inotropy and toxicity. The present experiments showed that toxic actions of ouabain were frequently provoked by sympathetic nerve stimulation. However, the occurrence of ouabain toxicity would not be due to a direct action of cardiac noradrenaline but resulted secondarily from an increase in the S-A nodal rate.

Developed tension

The positive inotropic response of the left atrium to ouabain was more marked at the slow rate of contraction than at the fast rate, whereas the response to noradrenaline was more marked at the fast rate than at the slow rate. The similar dependency of the developed tension on the rate of contraction obtained in solutions containing ouabain was seen in Na^+-poor and K^+-free solutions (Toda, unpublished data). A reduction of $[Na^+]o$ and $[K^+]o$ inhibits activities of the membrane ATPase of cardiac muscles (Lee & Yu, 1963) as does ouabain. A decrease in $[K^+]i$ is produced in papillary muscles exposed to ouabain, Na^+-free or K^+-free solutions (Page, Goerke & Storm, 1964). Müller (1965) suggested a close relationship between the K^+ loss and the positive inotropic effect of ouabain, because of a striking parallelism between the two phenomena. Results indicating the lack of direct relationship between the two phenomena have also been presented by some investigators (Regan, Frank, Lehan & Hellems, 1963 ; Grupp & Charles, 1964). In contrast to ouabain, noradrenaline causes a net gain of K^+ (Sarnoff *et al.*, 1965) in spite of producing the positive inotropic effect.

The uptake of labelled Ca^{++} by isolated guinea-pig auricles is a function of frequencies of driving stimulation (Grossman & Furchgott, 1964). The curve for the net uptake of Ca^{++} per beat versus frequencies does not parallel the curve for the developed tension versus the rate: the maximum uptake is obtained at a frequency of 30 beats/min and the maximum tension is obtained at 120 beats/min. Ouabain increases the net flux of Ca^{++} (Sekul & Holland, 1960 ; Lüllmann & Holland, 1962): the maximum flux is observed at a rate of 10 beats/min (Govier &

Holland, 1965). The Ca^{++} flux per beat varies invensely with the rate of contraction in the presence of toxic concentrations of ouabain. Under the influence of ouabain the increase in the Ca^{++} flux might correlate with an increase in the tension at the slow rate.

The positive inotropic effect of noradrenaline was not significantly affected by therapeutic concentrations of ouabain but was inhibited by its toxic concentrations. However, noradrenaline antagonized the ouabain action on the tension-rate curve, which did not seem to be corrected by washout of ouabain. It is unlikely that ouabain blocks adrenoceptive receptors of both cardiac specialized and contractile tissues, since the chronotropic response to noradrenaline is not reduced by ouabain. Antagonism of ouabain to noradrenaline might appear in mechanisms relating to contraction.

REFERENCES

BLINKS, J. R. & KOCH-WESER, J. (1961). Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *J. Pharmac. exp. Ther.*, **134**, 373–389.

CARMELIET, E. E. (1961). The potassium and chloride permeability in Purkinje fibers. In *The Specialized Tissues of the Heart*, ed. DeCarvalho, A. P., DeMello, W. C. & Hoffman, B. F., pp. 108–114. New York: Elsevier.

DENGLER, H. J., MICHAELSON, I. A., SPIEGEL, H. E. & TITUS, E. (1962). The uptake of labeled norepinephrine by isolated brain and other tissues of the cat. *Int. J. Neuropharmac.*, **1**, 23–38.

DUDEL, J. & TRAUTWEIN, W. (1958). Elektrophysiologische Messungen zur Strophantinwirkung am Herzmuskel. *Arch. exp. Path. Pharmak.*, **232**, 393–407.

GOVIER, W. C. & HOLLAND, W. C. (1965). The relationship between atrial contractions and the effect of ouabain on contractile strength and calcium exchange in rabbit atria. *J. Pharmac. exp. Ther.*, **148**, 284–289.

GREENSPAN, K., EDMANDS, R. E. & FISCH, C. (1967). Effects of cycle-length alteration on canine cardiac action potentials. *Am. J. Physiol.*, **212**, 1416–1420.

GROSSMAN, A. & FURCHGOTT, R. F. (1964). The effects of frequency of stimulation and calcium concentration on Ca^{45} exchange and contractility on the isolated guinea-pig auricle. *J. Pharmac. exp. Ther.*, **143**, 120–130.

GRUPP, G. & CHARLES, A. (1964). Effect of ouabain and 3-acetyl strophanthidin on potassium exchange in the dog heart *in situ*. *J. Pharmac. exp. Ther.*, **143**, 356–365.

IVERSEN, L. L. & KRAVITZ, E. A. (1966). Sodium dependence of transmitter uptake at adrenergic nerve terminals. *Mol. Pharmac.*, **2**, 360–362.

KASSEBAUM, D. G. (1963). Electrophysiological effects of strophanthin in the heart. *J. Pharmac. exp. Ther.*, **140**, 329–338.

KOCH-WESER, J. & BLINKS, J. R. (1962). Analysis of the relation of the positive inotropic action of cardiac glycosides to the frequency of contraction of heart muscle. *J. Pharmac. exp. Ther.*, **136**, 305–317.

LEE, K. S. & YU, D. H. (1963). A study of the sodium- and potassium-activated adenosine-tri-phosphatase activity of heart microsomal fraction. *Biochem. Pharmac.*, **12**, 1253–1264.

LÜLLMANN, H. & HOLLAND, W. (1962). Influence of ouabain on an exchangeable calcium fraction, contractile force, and resting tension of guinea-pig atria. *J. Pharmac. exp. Ther.*, **137**, 186–192.

MATSUO, S. & TODA, N. (1968). The influence of desmethylimipramine on the chronotropic response to endogenous and exogenous noradrenaline in the isolated atria. *Br. J. Pharmac. Chemother.*, **32**, 473–482.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MÉNDEZ, C., ACHEVES, J. & MÉNDEZ, R. (1961). Inhibition of adrenergic cardiac acceleration by cardiac glycosides. *J. Pharmac. exp. Ther.*, **131**, 191–198.

MÜLLER, P. (1965). Ouabain effects on cardiac contraction, action potential, and cellular potassium. *Circulation Res.*, **17**, 46–56.

MUSCHOLL, E. & WEBER, E. (1966). Die Wirkung von Ouabain auf die Elimination von Noradrenalin aus der Perfusionsfluss igkeit des isolierten Kaninchenherzens. *Arch. Pharmak. exp. Path.*, **255**, 309–316.

PAGE, E., GOERKE, R. J. & STORM, S. R. (1964). Cat heart muscle *in vitro*. IV. Inhibition of transport in quiescent muscles. *J. gen. Physiol.*, **47**, 531–543.

REGAN, T. J., FRANK, M. J., LEHAN, P. H. & HELLEMS, H. K. (1963). Relationship of insulin and strophanthidin to myocardial metabolism and function. *Am. J. Physiol.*, **205**, 790–794.

SARNOFF, S. J., GILMORE, J. P. & WALLACE, A. G. (1965). The influence of autonomic nerve activity on adaptive mechanisms in the heart. In *Nervous Control of the Heart*, ed. Randall, W. C., pp. 54–128. Baltimore: Williams & Wilkins.

SEKUL, A. A. & HOLLAND, W. C. (1960). Effects of ouabain on Ca^{45} entry in quiescent and electrically driven rabbit atria. *Am. J. Physiol.*, **199**, 457–459.

SKOU, J. C. (1957). The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. biophys. Acta*, **23**, 394–401.

SLEATOR, W., JR., FURCHGOTT, R. F., DE GUBAREFF, T. & KRESPI, V. (1964). Action potentials of guinea pig atria under conditions which alter contraction. *Am. J. Physiol.*, **206**, 270–282.

STUTZ, H., FEIGELSON, E., EMERSON, J. & BING, R. D. (1954). Effect of digitalis (" Cedelanid ") on the mechanical and electrical activity of extracted and nonextracted heart muscle preparations. *Circulation Res.*, **2**, 555–564.

TANZ, R. D. (1964). The action of ouabain on cardiac muscle treated with reserpine and dichloroisoproterenol. *J. Pharmac. exp. Ther.*, **144**, 205–213.

TANZ, R. D. & MARCUS, S. M. (1966). Influence of endogenous cardiac catecholamines and their response to ouabain. *J. Pharmac. exp. Ther.*, **151**, 38–45.

TARVE, U. & BRECHTLOVA, M. (1967). Effects of psychopharmacological agents on brain metabolism—III. Effects of imipramine and ouabain on the $(Na^+ + K^+)$-activated ATPase from brain microsomes and cooperative interactions with the enzyme. *J. Neurochem.*, **14**, 283–290.

TITUS, E. O., MATUSSEK, N., SPIEGEL, H. E. & BRODIE, B. B. (1966). The effects of desmethylimipramine on uptake of dl-norepinephrine-7-H^3 in the heart. *J. Pharmac. exp. Ther.*, **152**, 469–477.

TODA, N. (1968). Influence of sodium ions on the membrane potential of the sino-atrial node in response to sympathetic nerve stimulation. *J. Physiol., Lond.*, **196**, 677–691.

TODA, N. & SHIMAMOTO, K. (1968). The influence of sympathetic stimulation on transmembrane potentials in the S–A node. *J. Pharmac. exp. Ther.*, **159**, 298–305.

TODA, N. & WEST, T. C. (1966). The influence of ouabain on cholinergic responses in the sinoatrial node. *J. Pharmac. exp. Ther.*, **153**, 104–113.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225–276.

VASSALLE, M., KARIS, J. & HOFFMAN, B. F. (1962). Toxic effects of ouabain on Purkinje fibers and ventricular muscle fibers. *Am. J. Physiol.*, **203**, 433–439.

WINEGRAD, S. & SHANES, A. M. (1962). Calcium flux and contractility in guinea pig atria. *J. gen. Physiol.*, **45**, 371–394.

(*Received January* 14, 1969)