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Effects of calcium, sodium and potassium ions on contractility of isolated atria and their responses to noradrenaline

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1. Rabbit left atrial preparations driven electrically at different rates were used for studies on inotropic effects of cations, drugs and coupled pacing. Sino-atrial node-atrial preparations were used for investigating the chronotropic effect of noradrenaline.

2. The contractile tension-driving rate relationship was moved upwards by an elevation of $[Ca^{++}]o$, coupled pacing and noradrenaline. In preparations exposed to Na^+-poor and K^+-free solutions the contractile strength at low driving rates (6 to 30 c/min) was markedly enhanced, but at high rates (120 to 240 c/min) it was not influenced. The contractile strength was reduced at low $[Ca^{++}]o$ and at high $[K^+]o$.

3. The positive inotropic effect of noradrenaline was markedly inhibited by a reduction of $[Ca^{++}]o$ and to some extent by a reduction of $[K^+]o$. The noradrenaline-inotropy was not appreciably affected by an elevation of $[Ca^{++}]o$ and a reduction of $[Na^+]o$.

4. Cardiac excitability studied in preparations driven at high rates was enhanced by noradrenaline, a reduction of $[K^+]o$ and an elevation of $[Ca^{++}]o$, but was reduced at low $[Ca^{++}]o$, low $[Na^+]o$ and high $[K^+]o$.

5. The positive chronotropic response to noradrenaline was enhanced at high $[Ca^{++}]o$, low $[Na^+]o$ and low $[K^+]o$, but was reduced in solutions deficient in Ca^{++} or rich in K^+.

6. Inotropic effects of the ions and of coupled pacing were compared with those of ouabain. It is suggested that characteristic changes in the tension-rate curve seen in Na^+-poor, K^+-free and ouabain-containing solutions are correlated with an inhibition of active processes in the cardiac cell membrane, which affect ionic movements across it. It seems likely that mechanisms mediating adrenergic responses of the contractile tissue and the S–A node are associated with $[Ca^{++}]o$.

It is widely known that a number of inotropic interventions share a common mechanism that governs the availability of Ca^{++} at some site critical for cardiac contraction. Changes in the movement of Ca^{++} across the cell membrane of cardiac contractile tissue and from intracellular storage sites have been demonstrated to arise when extracellular concentrations of cations are altered (Niedergerke &

Harris, 1957 ; Niedergerke, 1963 ; Grossman & Furchgott, 1964a), when cardioactive drugs such as adrenaline (Reuter, 1964), noradrenaline (Grossman & Furchgott, 1964b) and cardiac glycosides (Klaus & Kuschinsky, 1962 ; Lüllmann & Holland, 1962 ; Grossman & Furchgott, 1964b) are administered, or when augmentation of cardiac contraction is produced by coupled electrical stimulation (Kavaler, Fisher & Stuckey, 1965). When contractility is increased either with drugs or in other ways, there are a net loss of K^+ from heart cells (Sarnoff, Gilmore & Wallace, 1965 ; Glynn, 1964) and an increase in oxygen consumption (Mansfield & McDonald, 1965 ; Ross, Sonnenblick, Kaiser, Frommer & Braunwald, 1965 ; Gousios, Felts & Havel, 1967). The present study was undertaken to investigate alterations in the contractile tension-driving rate relationship caused by varying extracellular concentrations of cations, by noradrenaline and by coupled pacing, as compared with those induced by ouabain (Toda, 1969a).

It has been demonstrated that the positive inotropic effect of sympathomimetic amines is reduced by metabolic inhibitors (Dhalla & Braxton, 1968) and ouabain (Toda, 1969a). It is suggested by the former investigators that some energy-dependent process is involved in an increase in intracellular concentrations of Ca^{++} available for contraction following stimulation of adrenergic receptors. On the other hand, the uptake of noradrenaline by sympathetic nerve terminals which is a physiologically important mechanism for terminating the actions of the amine has been demonstrated to be energy-dependent (Dengler, Michaelson, Spiegel & Titus, 1962). In the present study modification of the inotropic and chronotropic actions of noradrenaline by alterations in $[Ca^{++}]o$, $[Na^+]o$ and $[K^+]o$, which would be expected to influence the active process in cardiac cell membrane (Lee & Yu, 1963 ; Repke, 1965), was also investigated.

Methods

Forty-seven albino rabbits of either sex, weighing 1·8–2·2 kg, were used. Under ether anaesthesia the animals were killed by exsanguination from both common carotid arteries and the whole heart was removed. In the oxygenated, warmed nutrient solution ventricles were discarded. Atria were separated right and left along the interatrial septum. From the left atrium specialized tissues were excluded. The left atrial preparation was used for investigating inotropic effects of variations in extracellular concentrations of cation, of noradrenaline and of coupled pacing, when the rate was constantly maintained by artificial electrical stimulation. The isolated specimen was fixed horizontally between hooks with a resting tension of 300–450 mg in a muscle bath containing 60 ml. of the nutrient solution, which was maintained at 30° ±0·5° C and was gassed with a mixture of 95% oxygen and 5% carbon dioxide. A pair of hooks fixing the cut end of the left atrium was connected to an electronic stimulator. An appendage of the atrium was anchored by the other pair of hooks through which the mechanical contraction was conducted to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co.). For studies on the chronotropic action of noradrenaline, the S-A node-right atrium preparation was used. The preparation was fixed by two pairs of hooks in the similar way to the left atrial preparation, although no electrical stimulation was applied. Constituents of the nutrient solution were as follows (mM): Na^+, 162·1 ; K^+, 5·4 ; Ca^{++}, 2·2 ; Cl^-, 157·0 ; HCO_3^-, 14·9 ; dextrose, 5·6. When $[Ca^{++}]o$ and $[K^+]o$ were altered, no osmotic adjustment was made. Extracellular concentrations of Na^+

were reduced by replacing NaCl with isotonic sucrose. The $[Ca^{++}]o$ used in the present study were 0·22, 2·2, 6·6 and 11·0 mM, the $[K^+]o$ used were 0, 1·7, 5·5 and 10·8 mM, and the $[Na^+]o$ used were 73·1, 102·6 and 162·1 mM. Preparations were equilibrated for 60–90 min in control solutions and for 20–30 min in test solutions.

The left atrial preparation was driven electrically by a train of rectangular pulses of supramaximal intensity (about twice threshold intensity) with 3·0 msec duration, at 60 c/min, unless otherwise mentioned. The contractile tension-driving rate relationship was obtained by raising the rate stepwise from 6 to 240 c/min or higher until the preparation failed to respond to all the stimuli. Constant rates of stimulation were maintained until the steady state contractile tension was attained. The tension-rate relationship was obtained before and 10 min after noradrenaline in control and test solutions. In some preparations coupled electrical stimuli at different intervals of 150, 220 and 320 msec were applied. The preparations were driven at a constant frequency of 60 pairs of stimuli/min, unless otherwise described. Electrical stimuli were provided by a Sanei type ES-103-Z pulse generator.

In spontaneously beating atrial preparations noradrenaline was applied directly to the muscle bath in cumulative concentrations. The dose-chronotropic response curve was obtained in control solutions and 20–30 min after control solutions had been replaced with test solutions.

The contractions of the left or right atrium were displayed on a two-channel pen-writer. The S-A nodal rate was taken as the mean value of ten measurements of the cycle length between contractions. Transmembrane potentials were recorded from single fibres of the left atrium by the use of a floating microelectrode, when required. The membrane potential and the contraction of the left atrium were recorded simultaneously from a VC-7 oscilloscope (Nihonkoden Kogyo Co.) on moving film at a speed of 5 cm/sec. Absolute values of the contractile tension and the S-A nodal rate were compared in control and test solutions, and before and after noradrenaline. The results were expressed as mean values $\pm$ standard errors of the means. Comparisons of results were made using the Student's t test.

l-Noradrenaline hydrochloride was used. Concentrations of the amine were expressed in terms of g/ml. of the salt.

Results

Contractile tension

In left atrial preparations driven electrically, the contractile tension was a direct function of driving rates within the range between 6 and 120 c/min, but was an inverse function of rates at higher than 120 c/min. A fall in $[Ca^{++}]o$ to 0·22 mM reduced the contractile tension at all driving rates applied. The reduction of the tension was marked rates of 30–120 c/min, resulting in a diminution of the rate-dependency of the atrial contraction. A rise in $[Ca^{++}]o$ to 6·6 and 11·0 mM shifted the tension-rate curve upward, the magnitude of the shift being proportional to the increase in $[Ca^{++}]o$. At 11·0 mM $[Ca^{++}]o$ the enhancement of the contractile tension was significantly more marked at a rate of 6 c/min than at 12 c/min. The dependency of the contractile tension on the rates was exaggerated by increasing $[Ca^{++}]o$. The results are summarized in Fig. 1.

A reduction of $[Na^+]o$ produced a marked enhancement of atrial contractions within the rate range between 6 and 60 c/min (Fig. 2). The enhancement of contrac-

tions was an inverse function of $[Na^+]o$. At the higher driving rates (120–240 c/min) the contractile tension was somewhat reduced in preparations exposed to solutions deficient of Na^+ (Fig. 2). A Na^+-deficiency shifted the tension-rate curve upwards and to the left.

According to Wilbrandt & Koller (1948) and Niedergerke & Lüttgau (1957), the strength of contractions of the frog heart is a function of the ratio of $[Ca^{++}]o/[Na^+]o^2$. The relationship between the ratio and the contractile tension at driving rates of 6 and 60 c/min is presented in Fig. 3. It was found that there was a relation between the $[Ca^{++}]o/[Na^+]o^2$ ratio and the tension when atria were driven at a frequency of 6 c/min, whether the ratio was altered by changing $[Ca^{++}]o$ or $[Na^+]o$. However, this was less true when the stimulus frequency was 60 c/min.

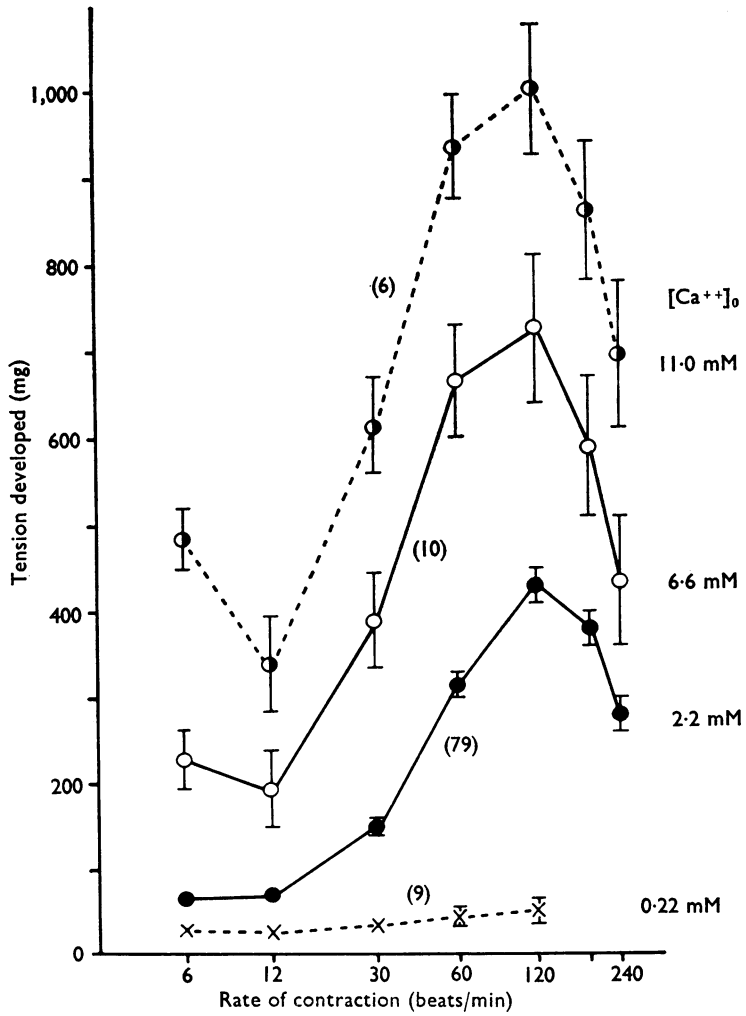

FIG. 1. Modification by alterations in $[Ca^{++}]o$ of the contractile tension-driving rate relationship. Vertical bars represent standard errors of the means. The number of experiments is indicated in parentheses.

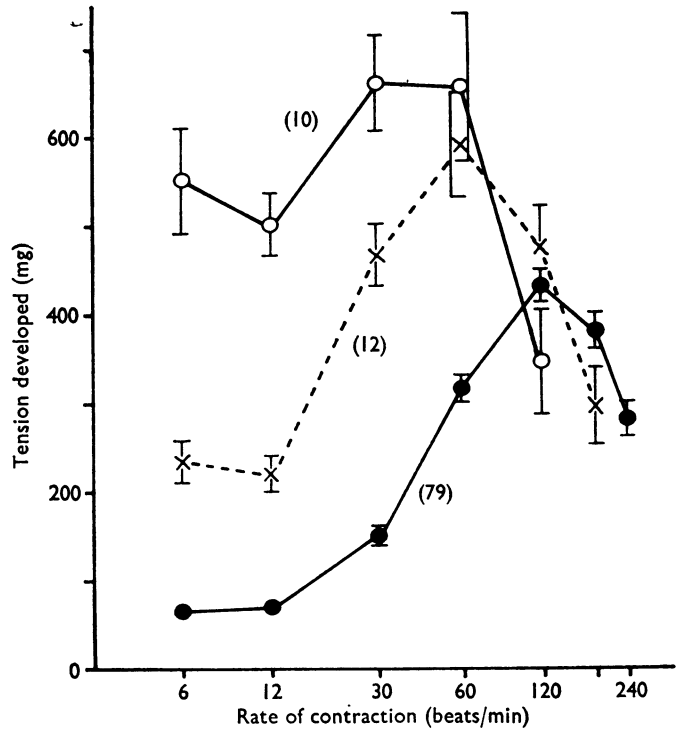

FIG. 2. Modification by a reduction of $[Na^+]o$ (●, 162·1 mM ; ×, 103·2 mM ; ○, 73.8 mM) of the tension-rate relationship. Vertical bars represent standard errors of the means. Figures in parentheses indicate the number of experiments.

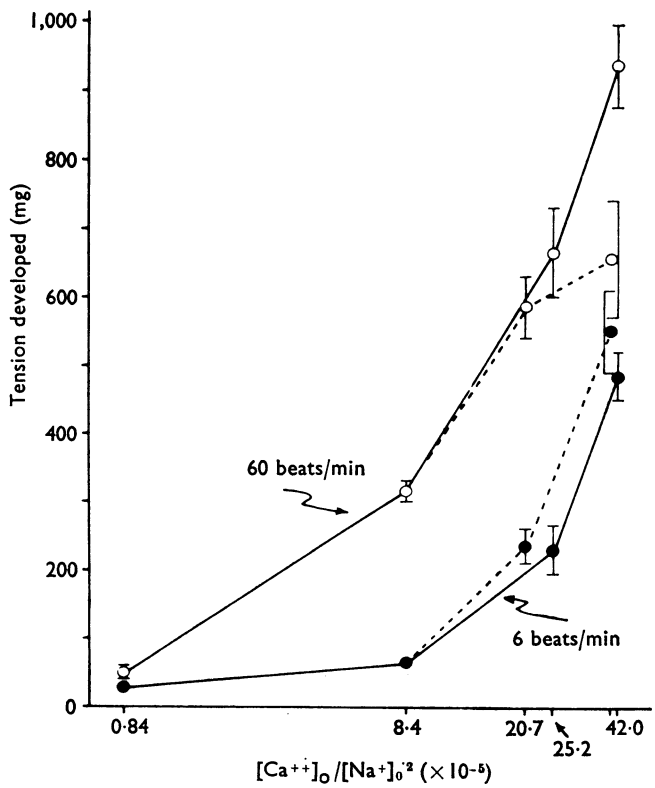

FIG. 3. Relationship between the ratio of $[Ca^{++}]o/[Na^+]o^2$ and the contractile tension of left atria driven at rates of 6 and 60 beats/min. Solid lines, values obtained when $[Ca^{++}]o$ was altered ; broken lines, values obtained when $[Na^+]o$ was reduced.

Lowering of $[K^+]o$ caused an increase in the contractile tension developed, especially at low driving rates (6 to 30 c/min), whereas elevating of $[K^+]o$ caused a decrease in the tension developed at high rates (60 to 180 c/min). The increase in the tension developed at low rates was an inverse function of $[K^+]o$ within the range from 0 to 10·8 mM (Fig. 4).

The technique of coupled pacing of the heart has been shown to be capable of enhancing its force of contraction (Braunwald, Gay, Morrow & Braunwald, 1964; Braunwald, Ross, Frommer, Williams, Sonnenblick & Gault, 1964; Cranefield, Scherlag, Yeh & Hoffman, 1964; Hoffman, Bartelstone, Scherlag & Cranefield, 1965). The magnitude of the enhancement was dependent on the stimulus interval of coupled stimuli, as indicated in Fig. 5. The maximum enhancement was obtained at 220 msec in nine of twelve preparations driven at 60 pairs of stimuli/min. Figure 6 illustrates the contractile tension-rate relationship obtained from preparations driven by a train of single pulses and by a train of coupled pulses. In the latter preparations a stimulus interval of 220 msec was used. The coupled pacing shifted the tension-rate curve upwards.

Noradrenaline shifted the contractile tension-rate curve upwards, the shift being dependent upon its dose (Fig. 7). Similar shifts of the tension rate curves were produced by noradrenaline in preparations exposed to 6·6 mM $[Ca^{++}]o$ (Fig. 8), to 103·2 mM $[Na^+]o$ (Fig. 9) or to 10·8 mM $[K^+]o$ (Fig. 10) and in preparations driven by a train of coupled pulses (Fig. 6). However, a percentage increase in the tension induced by noradrenaline was reduced by an elevation of $[Ca^{++}]o$, a reduction of $[Na^+]o$ and coupled pacing, because the tension was significantly enhanced by these

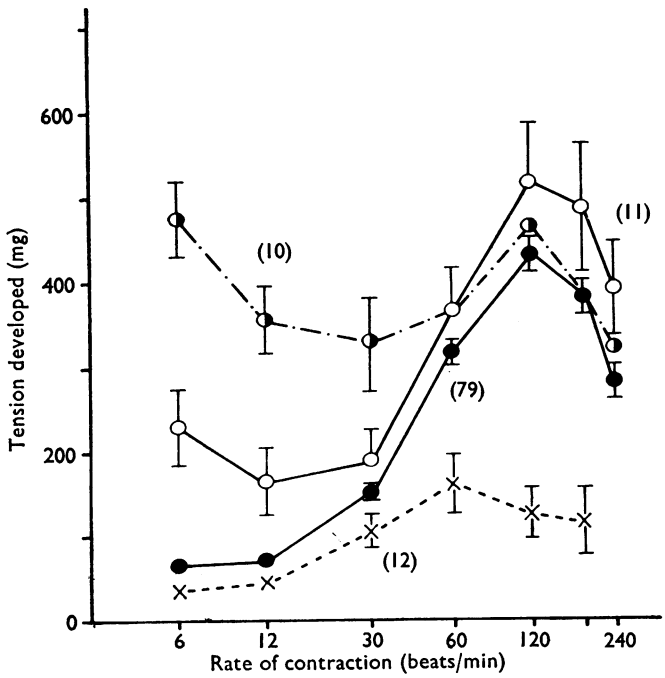

FIG. 4. Modification by alterations in $[K^+]o$ (◑, 0 mM; ○, 1·4 mM; ●, 5·4 mM; ×, 10·8 mM) of the tension-rate relationship. The number of experiments is indicated in parentheses.

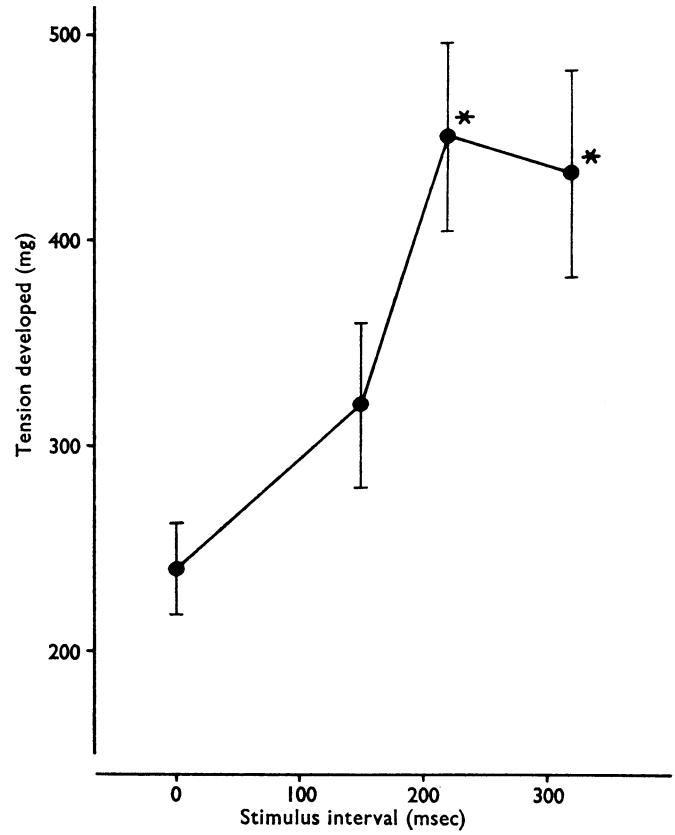

FIG. 5. Effects of stimulus interval of coupled stimuli on the contractile tension. *Significant difference from control, $P<0{\cdot}01$.

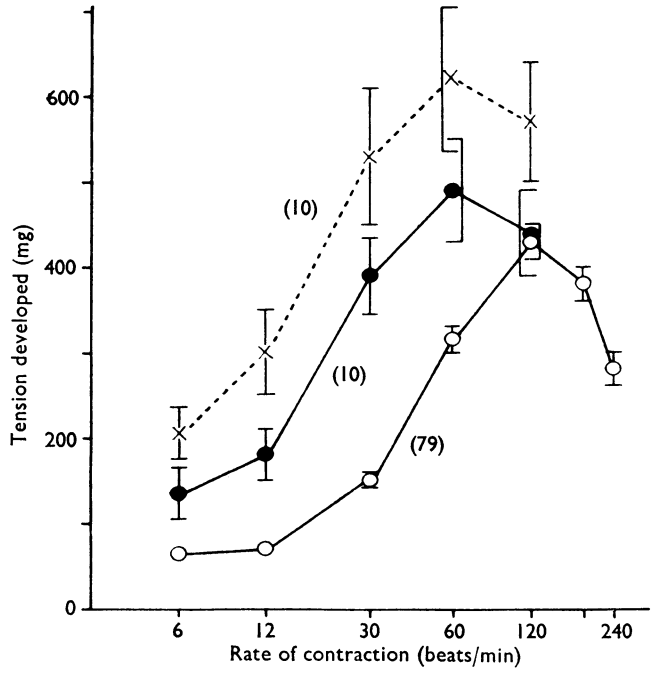

FIG. 6. Alterations in the tension-rate relationship (○, single pacing) induced by coupled pacing (●) and by coupled pacing plus noradrenaline (×). Figures in parentheses indicate the number of experiments.

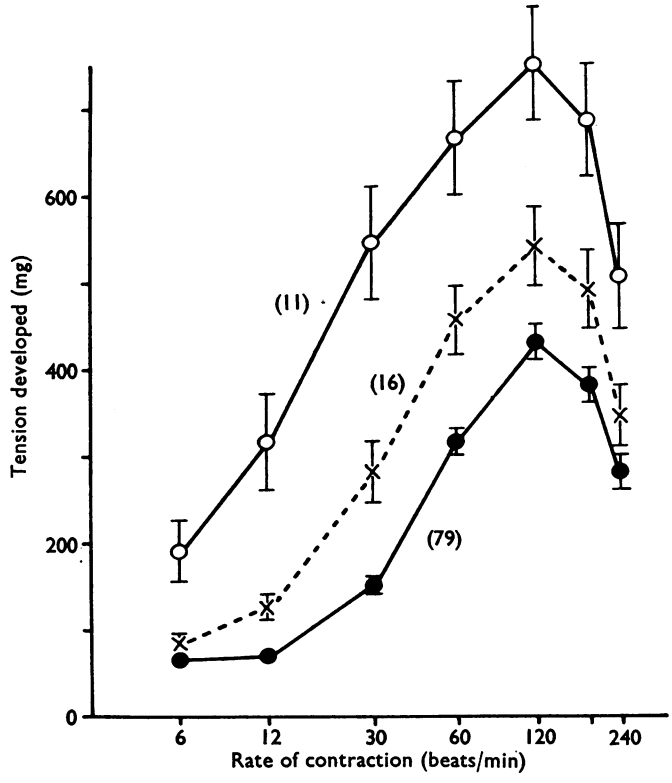

FIG. 7. Effects of noradrenaline on the tension-rate relationship. The number of experiments is indicated in parentheses. ●, Control; ×, noradrenaline 10^{-7} g/ml.; ○, noradrenaline 5×10^{-7} g/ml.

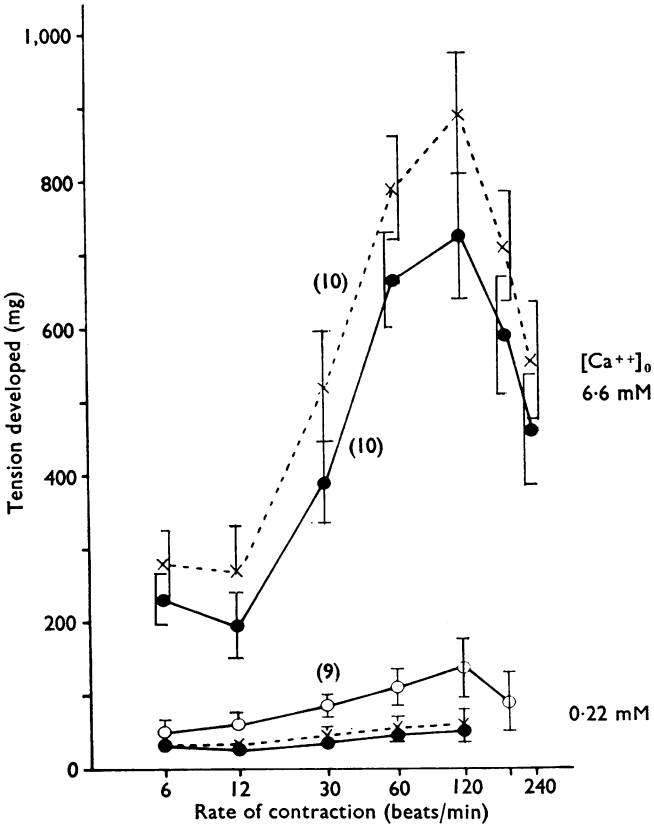

FIG. 8. Effects of noradrenaline on the tension-rate relationship obtained from preparations exposed to 0·22 and 6·6 mM [Ca++]o. ●, Control; ×, noradrenaline 10^{-7} g/ml.; ○, noradrenaline 5×10^{-7} g/ml.

procedures. Conversely, the percentage increase was enhanced by an elevation of $[K^+]o$. The positive inotropic effect of noradrenaline was markedly reduced in solutions deficient in Ca^{++} (0·22 mM) (Fig. 8). Noradrenaline did not correct the tension-rate relationship previously modified by a reduction of $[Na^+]o$ to 73·8 mM, but shifted the curve upwards (Fig. 9). In preparations driven at high rates (60–240 c/min) at reduced $[K^+]o$ (1·4 mM) the inotropic effect of noradrenaline was reduced (Fig. 10). At low $[K^+]o$ noradrenaline frequently provoked automaticity in left

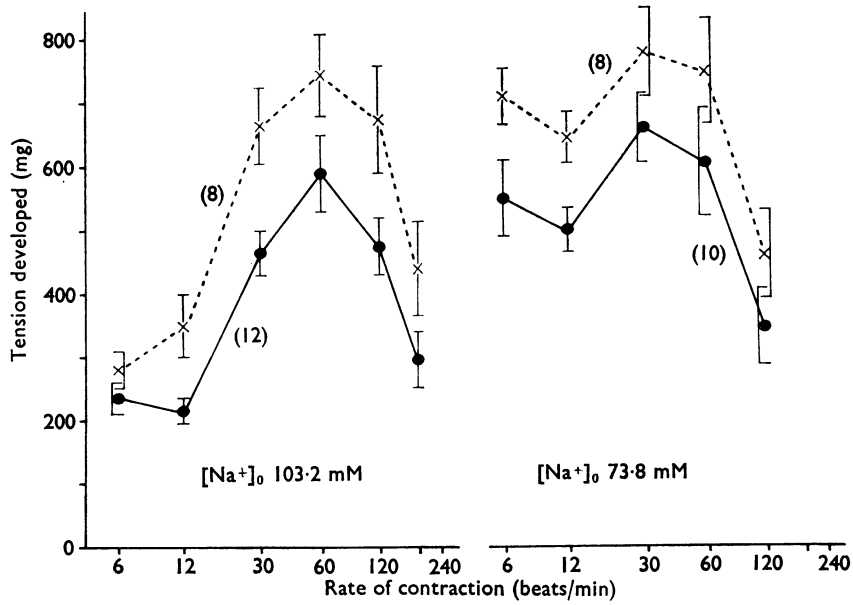

FIG. 9. Effects of noradrenaline on the tension-rate relationship obtained from preparations exposed to reduced $[Na^+]o$. ●, Control; ×, noradrenaline 10^{-7} g/ml.

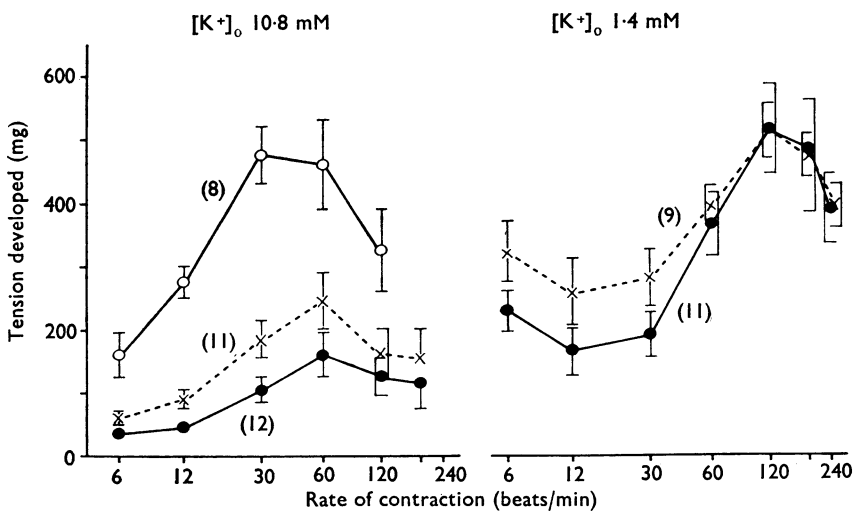

FIG. 10. Effects of noradrenaline on the tension-rate relationship obtained from preparations exposed to 1·4 and 10·8 mM $[K^+]o$. ●, Control; ×, noradrenaline 10^{-7} g/ml.; ○, noradrenaline 5×10^{-7} g/ml.

atrial preparations driven at 60 c/min. Other experimental solutions did not increase an incidence of automaticity induced by noradrenaline. The incidence of automaticity in normal and K^+-deficient solutions is tabulated in Table 1. In six of fourteen preparations exposed to K^+-free solutions automaticity was induced following electrical driving stimulation. Automaticity induced in K^+-free solutions stopped spontaneously within 5 min of removal of the driving stimulation, whereas that induced by noradrenaline persisted longer than 10 min.

TABLE 1. *Relationship between the incidence of automaticity and* $[K^+]o$

$[K^+]o$ (mM)	Incidence of automaticity		
	NA-free	NA 10^{-7} g/ml.	NA 5×10^{-7} g/ml.
10·8	0*/12†	0/11	1/9
5·4	0/16	0/16	4/15
1·7	0/11	2/11	5/10
0	6/16	3/3	

NA, Noradrenaline. * Number of preparations in which automaticity was produced.
† Number of preparations tested.

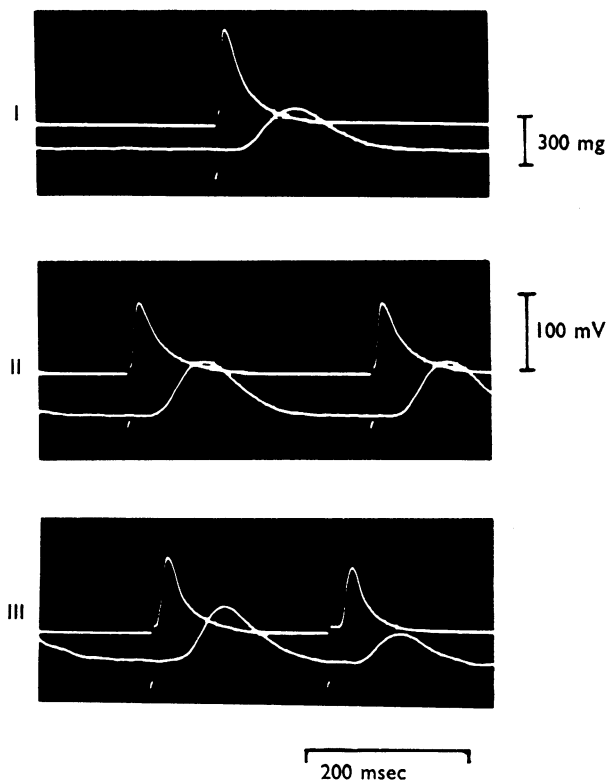

FIG. 11. Electrical and mechanical responses to driving with stimuli at different frequencies. I, Drive at 60 c/min ; II, at 180 c/min (R_1 in this preparation) ; III, at 300 c/min (R_2 in this preparation). Upper tracing, Transmembrane potentials recorded from a single cell of the left atrium ; lower, contractions of the left atrium.

TABLE 2. *Mean values of threshold driving rates for alternating contractions, R_1 and R_2, in various test solutions.*

Procedure	*n*	R_1	R_2
Control	79	170	260
NA 10^{-7} g/ml.	16	170	300
NA 5×10^{-7} g/ml.	11	210	280
Ca^{++}, 0·22 mM	9	120	140
+NA 10^{-7} g/ml.	9	120	140
+NA 5×10^{-7} g/ml.	9	120	200
Ca^{++}, 6·6 mM	10	180	280
+NA 10^{-7} g/ml.	10	190	280
Ca^{++}, 11·0 mM	6	190	330
Na^{+}, 102·6 mM	12	120	150
+NA 10^{-7} g/ml.	8	140	190
Na^{+}, 73·1 mM	10	70	100
+NA 10^{-7} g/ml.	8	90	140
K^{+}, 0 mM	10	190	320
K^{+}, 1·7 mM	11	190	350
+NA 10^{-7} g/ml.	9	190	350
K^{+}, 10·8 mM	12	110	190
+NA 10^{-7} g/ml.	11	100	180
+NA 5×10^{-7} g/ml.	8	110	130
Ouabain, 2×10^{-7} g/ml.	19	160	240
Ouabain, 10^{-6} g/ml.	21	140	200
+NA 10^{-7} g/ml.	12	160	190

n, Number of experiments.

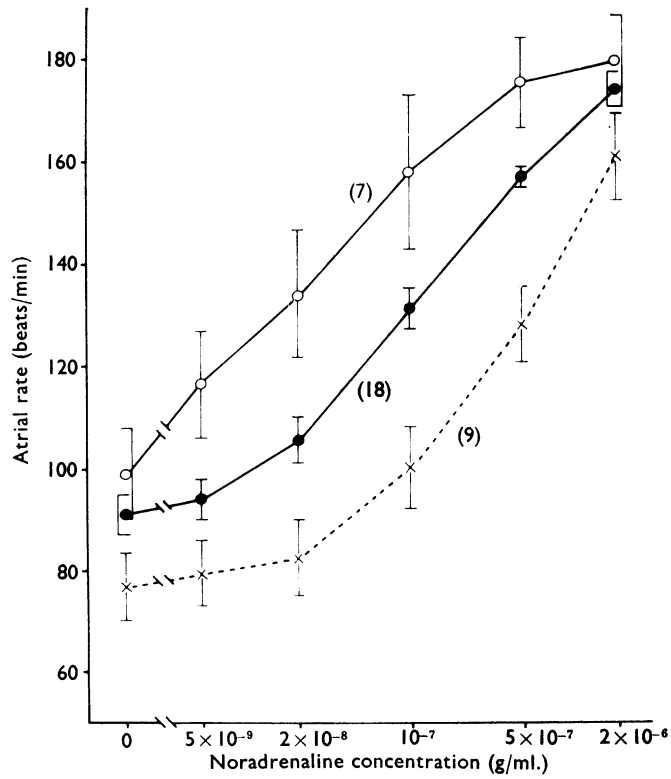

FIG. 12. Modification by alterations in $[Ca^{++}]o$ (×, 0·22 mM; ●, 2·2 mM; ○, 6·6 mM) of the positive chronotropic response to noradrenaline.

Excitability of left atria driven at high rates

Isolated left atrial preparations were driven by electrical pulses of supra-threshold intensity (about twice threshold). Driving rates were raised stepwise until the contraction did not follow each stimulus. The maximum driving rate at which the amplitude of two successive contractions was the same will be termed "R_1", and the maximum rate at which alternating contractions were produced in which the amplitude of the smaller contractions were not less than one-third the amplitude of the larger contractions will be termed "R_2" in this report. At driving rates producing slight alternating contractions no measurable change in latency from stimulation artefact to electrical excitation of atrial fibres were observed, although the amplitude and the duration of action potentials were frequently reduced. On the other hand, in preparations driven by the higher rates producing marked alternating contractions (like those described above as R_2) a prolongation of the latency and a reduction of depolarization velocity were observed (Fig. 11). According to Hoffman & Cranefield (1960), the period during which excitation can be evoked by a strong stimulus and after a long latency represents the "relative refractory period". The moment when the stimulus-response latency reaches the value characteristic of phase 4 (diastole) is called the "full-recovery time". Thus it may be considered that the

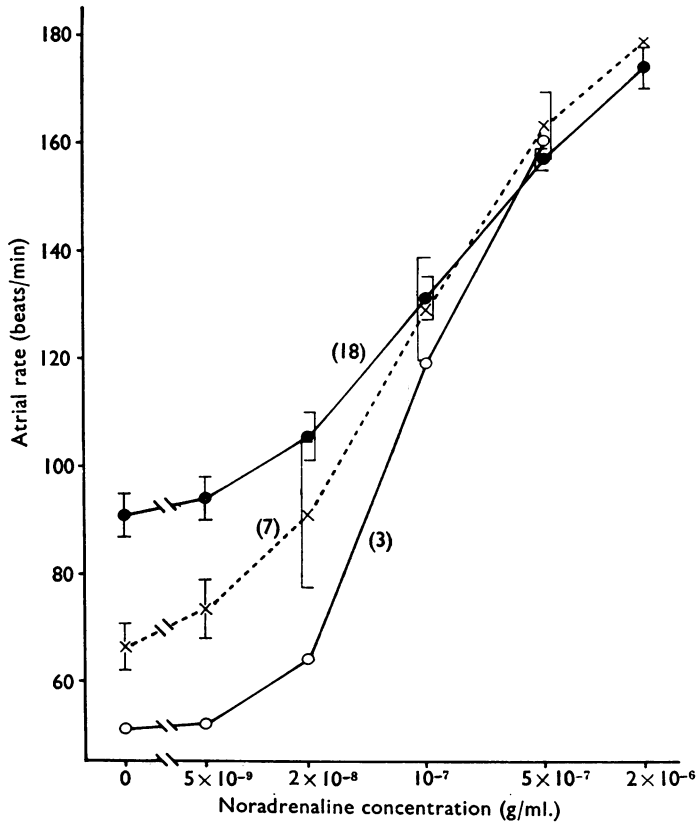

FIG. 13. Modification by a reduction of [Na+]o (●, 162·1 mM ; ×, 103·2 mM ; ○, 73·8 mM) of the positive chronotropic response to noradrenaline.

R_1 and R_2 represent the recovery time and the relative refractory period, respectively. In fact, quinidine in concentrations of 10^{-5} to 5×10^{-5} g/ml. produced a measurable decrease in both of R_1 and R_2 (Toda, unpublished data).

Changes in R_1 and R_2 induced by noradrenaline, ouabain and variations in $[Ca^{++}]o$, $[Na^{+}]o$ or $[K^{+}]o$ are summarized in Table 2. Data relating to ouabain were obtained from previous experiments (Toda, 1969a), in which the same experimental conditions were used. Trends of increasing R_1 and R_2 were observed in preparations exposed to noradrenaline, low $[K^{+}]o$ and high $[Ca^{++}]o$, whereas those of decreasing R_1 and R_2 were observed in preparations exposed to toxic concentrations of ouabain, low $[Na^{+}]o$, high $[K^{+}]o$ and low $[Ca^{++}]o$. The decrease in R_1 and R_2 induced by ouabain and by a reduction of $[Na^{+}]o$ or of $[Ca^{++}]o$ was partly corrected by noradrenaline.

Sino-atrial pacemaker rate

The pacemaker rate under steady state conditions was decreased by a reduction of $[Ca^{++}]o$ to 0·22 mM, but not significantly affected by its elevation to 6·6 mM.

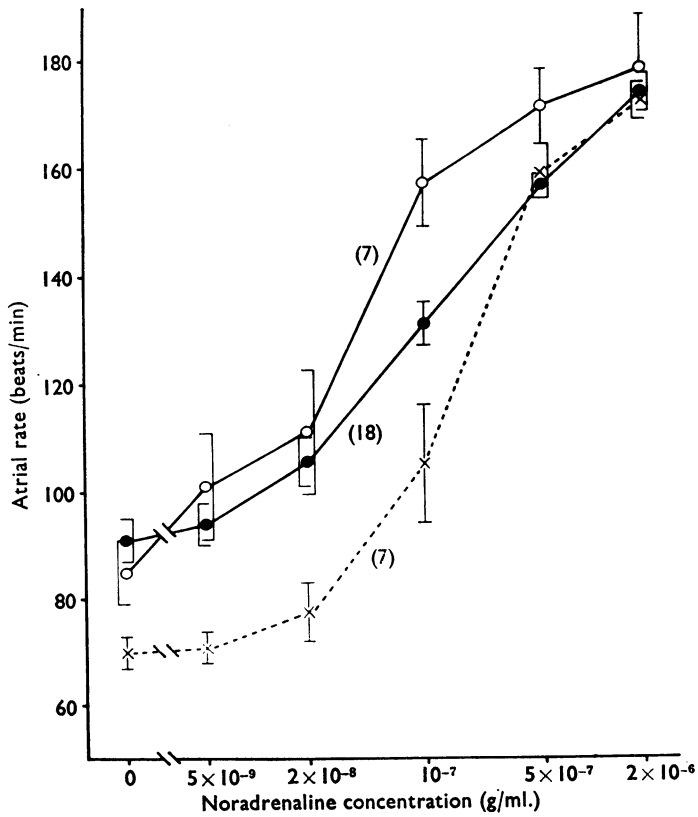

FIG. 14. Modification by alterations in $[K^{+}]o$ (○, 1·4 mM ; ●, 5·4 mM ; ×, 10·8 mM) of the positive chronotropic response to noradrenaline.

The noradrenaline concentration-chronotropic response curve in normal solutions shifted to the right at low [Ca^{++}]o and to the left at high [Ca^{++}]o (Fig. 12).

The pacemaker rate under steady state conditions varied directly with [Na^{+}]o. The positive chronotropic effect of noradrenaline was slightly potentiated by a fall in [Na^{+}]o (Fig. 13).

The steady state pacemaker rate was not affected by a reduction of [K^{+}]o to 1·4 mM, but significantly slowed by its elevation to 10·8 mM. The positive chronotropic response to noradrenaline was potentiated at low [K^{+}]o and somewhat reduced at high [K^{+}]o (Fig. 14).

Discussion

Pharmacological and other methods for increasing atrial contractile tension can be divided into two groups: (*a*) an elevation in [Ca^{++}]o, noradrenaline and coupled pacing which shift the tension-rate curves upward, and (*b*) a reduction of [Na^{+}]o, a reduction of [K^{+}]o and toxic concentrations of ouabain (Toda, 1969a) which produce an enhancement of the contractile tension only at low driving rates. Baker & Baker (1955) demonstrated similarities between the actions of adrenaline and Ca^{++} on the electrical and mechanical events of frog hearts. The present study suggested a dependency of noradrenaline-inotropy on [Ca^{++}]o. Both a rise in [Ca^{++}]o and noradrenaline have been demonstrated to increase the exchange of $^{45}Ca^{++}$ in guinea-pig atria (Winegrad & Shanes, 1962 ; Grossman & Furchgott, 1964a, b) and also to produce a net gain of K^{+} by the heart (Sarnoff, Gilmore & Wallace, 1965). In contrast, coupled pacing produces a net loss of K^{+} from the heart (Sarnoff, Gilmore & Wallace, 1965), which is comparable with that seen after a subtoxic dose of acetylstrophanthidin. Therefore, coupled pacing is thought of as a technique of electrodigitalization (Sarnoff, Gilmore, Daggett, Mansfield, McDonald & Weisfeldt, 1965). However, alterations in the tension-rate curve induced by coupled pacing and ouabain (Toda, 1969a) differed.

Characteristic changes in the tension-rate curve induced by a fall in [Na^{+}]o or in [K^{+}]o and ouabain would be considered to result either from an enhancement of the contractile tension at low driving rates or from a reduction of the tension of preparations driven at high rates in which the tension-rate curve shifted upward. Activities of the membrane ATPase in the heart are known to be inhibited by these procedures (Glynn, 1964). Lowering of temperature which would be expected to inhibit metabolic processes in the heart caused similar changes in the tension-rate curve (Toda, unpublished data) and an increase in the uptake of $^{45}Ca^{++}$ (Shelburne, Serena & Langer, 1967). Calcium ions move down a large electrochemical gradient into heart cells during excitation (Niedergerke & Orkand, 1966 ; Reuter, 1967). Recently, an ATP-dependent active outward transport of Ca^{++} has been demonstrated in erythrocyte ghosts (Schatzmann, 1966). If active processes are involved in an extrusion of Ca^{++} as suggested by Niedergerke (1963) and Orkand (1968), procedures that inhibit energy-dependent mechanisms in cardiac membrane are capable of decreasing the amount of Ca^{++} efflux, resulting in an accumulation of Ca^{++} in heart cells. On the other hand, the contractile tension of left atria driven at high rates is reduced by N-ethylmaleimide, an SH-inhibitor, but at low rates is not reduced (Toda & Konishi, 1969). The reduction of contractions at high rates may result from an inhibition of metabolic processes, if these are involved in a staircase

enhancement of contractions with increasing driving rates. This may also be the case for preparations in which atrial contractility is enhanced by a fall in $[Na^+]o$ or in $[K^+]o$ and ouabain, in association with an increase in the uptake of Ca^{++}.

A Na^+-deficiency in bathing solutions is shown to cause an increase in the Ca^{++} influx (Niedergerke & Harris, 1957 ; Niedergerke, 1963) and a decrease in the Ca^{++} efflux (Reuter & Seitz, 1968) in the isolated heart. The strength of cardiac contractions is found to be a function of the ratio of $[Ca^{++}]o/[Na^+]o^2$ (Wilbrandt & Koller, 1948). This observation has been supported by studies on frog ventricles driven at 2 and 5 c/min (Niedergerke & Lüttgau, 1957 ; Lüttgau & Niedergerke, 1958) and guinea-pig papillary muscles driven at 30–60 c/min (Reiter, 1966). Results obtained in the present study using rabbit atria are consistent with their observations when preparations were driven at a rate of 6 c/min but inconsistent with them when driven at 60 c/min. Antagonism of Na^+ to the Ca^{++} flux as supported by Lüttgau & Niedergerke (1958) would be a significant explanation for ionic intervention in contractions when the heart is beating slowly.

Cardiac glycosides are known to increase the Ca^{++} uptake by the heart (Sekul & Holland, 1960 ; Govier & Holland, 1964) and the exchangeable Ca^{++} (Klaus & Kuschinsky, 1962 ; Lüllmann & Holland, 1962 ; Grossman & Furchgott, 1964b). Lowering of $[Na^+]o$ and of $[K^+]o$ causes a loss of K^+ from isolated papillary muscles (Page, Goerke & Storm, 1964), as do cardiac glycosides from isolated and *in situ* hearts (Hajdu & Leonard, 1959 ; Glynn, 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. However, numbers of studies do not support the simple relationship (Koch-Weser & Blinks, 1963). Findings obtained here and in an early study (Toda, 1969a) indicate that the actions of ouabain on the contractile strength are similar to those of a fall in $[Na^+]o$ or in $[K^+]o$ but different from those of a rise in $[Ca^{++}]o$. The ouabain-inotropy would not be only correlated with Ca^{++} fluxes but a loss of K^+ and a gain of Na^+. Quite a few similarities between the effects of ouabain and a fall in $[Na^+]o$, both of which cause an increase in the ratio of $[Na^+]i/[Na^+]o$, are observed in electrical and mechanical events of isolated atria: changes in configuration of action potentials of pacemaker fibres ; occurrence of sub-threshold oscillations in diastolic pacemaker membrane (Toda, 1968, 1969a) ; supersensitivity of pacemaker rate to acetylcholine ; subsensitivity of atrial contractions to acetylcholine (Toda & West, 1966a, b) ; alterations in the tension-rate relationship ; and a reduction of atrial excitability, observed as R_1 and R_2. Most of these changes are associated with neither an increase in Ca^{++} fluxes nor a decrease in the ratio of $[K^+]i/[K^+]o$.

Actions of noradrenaline on the contractile strength seemed to be additive to the actions of a rise in $[Ca^{++}]o$ and a fall in $[Na^+]o$ or in $[K^+]o$, although a percentage increase in contractions was reduced by these procedures. Similar results have been presented recently for rat ventricular strips by Dhalla & Braxton (1968). A marked reduction of noradrenaline actions was produced by a fall in $[Ca^{++}]o$, which suggested a close relationship between the noradrenaline-inotropy and the Ca^{++} flux. On the other hand, findings that sensitivity of S-A nodes to noradrenaline varied directly with $[Ca^{++}]o$ may suggest an involvement of Ca^{++} in adrenoceptive receptor mechanisms. A reduction of inotropic actions of adrenaline in rat perfused hearts is observed when fluoroacetate or iodoacetate is present in the perfusion fluid (Horn, Aronson, Hess & Haugaard, 1967). It has also been shown that mechanical

responses of isolated rat ventricles to adrenaline is markedly reduced by metabolic inhibitors such as fluorodinitrobenzene, an inhibitor of ATP and creatine phosphotransferase (Infante & Davies, 1965), and sodium fluoride, a well known inhibitor of the Embden-Meyerhof pathway (Dhalla & Braxton, 1968). These authors assumed that some of the events initiated by stimulation of adrenoceptive receptors by sympathomimetic amines leading to an increase in $[Ca^{++}]i$ in free form are energy-dependent. A reduction of noradrenaline-inotropy due to toxic concentrations of ouabain (Toda, 1969a) would support their assumption. However, a fall in $[Na^+]o$ and in $[K^+]o$ which is shown to inhibit activities of the cardiac membrane ATPase (Lee & Yu, 1963; Repke, 1965) did not measurably reduce the noradrenaline action.

The positive chronotropic effect of noradrenaline was enhanced in solutions containing high $[Ca^{++}]o$, low $[Na^+]o$ or low $[K^+]o$, as was the effect of sympathetic nerve stimulation (Toda, 1968, 1969b). When $[Na^+]o$ is reduced, an inhibition of the uptake of noradrenaline by sympathetic nerve terminals (Iversen & Kravitz, 1966) which terminates the actions of the amine may be involved in the supersensitivity of the S-A node. A possibility of alteration in susceptibility of the pacemaker membrane to noradrenaline in association with variations in $[K^+]o$ is discussed in an earlier report (Toda, 1969b). Further, an inhibition of the noradrenaline uptake by the nerve terminals is suggested when $[K^+]o$ is reduced.

Values of R_1 and R_2 were influenced by varying extracellular concentrations of cations, noradrenaline and ouabain. Similar results regarding threshold driving rates for pulsus alternans have been demonstrated in conjunction with adrenaline, $[Ca^{++}]o$ and $[K^+]o$ (Badeer, Rye, Gassner, Kass, Cavaluzzi, Gilbert & Brooks, 1967). They postulated an involvement of the refractory period or electrical alternans in the mechanical alternans. In the present study no electrical alternans was produced at high driving rates inducing slight alternating contractions, whereas considerable electrical alternans was produced by higher rates at which alternating contractions were marked as described for definition of R_2, probably due to an alternating prolongation of latency from stimulation artefact to electrical excitation (Fig. 11). Values of R_1 and R_2 do not represent directly the refractory period of isolated atria, but may provide parameters indicating changes in the cardiac excitability. The results obtained here are consistent with those reported by Teiger, Scheider & Farah (1967) as changes in the effective refractory period.

In conclusion, atrial contractions developed only at low driving frequencies are markedly enhanced in Na^+-deficient and K^+-free solutions as in the presence of toxic concentrations of ouabain, whereas those at all frequencies applied are enhanced by an elevation of $[Ca^{++}]o$, noradrenaline and coupled pacing. Noradrenaline does not correct the contractile tension-driving rate relationship previously modified at low $[Na^+]o$ and low $[K^+]o$. It would be suggested that characteristic changes in the tension-rate curve seen in Na^+-deficient, K^+-free and ouabain-containing solutions are correlated with an inhibition of active processes in the cardiac cell membrane, which affect ionic movements across the membrane. It seems likely that mechanisms mediating adrenergic responses of the contractile tissue and the S-A node are associated with $[Ca^{++}]o$.

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