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RELATION OF SODIUM PUMP INHIBITION TO POSITIVE INOTROPY AT LOW CONCENTRATIONS OF OUABAIN IN RAT HEART MUSCLE

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SUMMARY

1. Low concentrations of ouabain which produce a positive inotropic effect on rat ventricular muscle do not inhibit the isolated Na^+–K^+-ATPase enzyme from this tissue, suggesting that these low-concentration inotropic effects are not related to sodium pump inhibition (Erdmann, Philipp & Scholz, 1980; Adams, Schwartz, Grupp, Grupp, Lee, Wallick, Powell, Twist & Gathiram, 1982).

2. We tested this hypothesis by continuously measuring intracellular Na^+ activity with Na^+-selective micro-electrodes and, separately, twitch tension of rat ventricular muscle during exposure to and wash-out of ouabain.

3. Intracellular Na^+ activity (a^i_{Na}) and transmembrane potential of quiescent muscle cells averaged $8{\cdot}5 \pm 2{\cdot}6$ mM (mean $\pm$ S.D., $n = 27$) and $-79{\cdot}2 \pm 2{\cdot}4$ mV ($n = 34$) respectively.

4. Low concentrations of ouabain (0·1, 0·5 and 1·0 μM) produced concentration-dependent increases in both a^i_{Na} and twitch tension. At lower concentrations of ouabain (0·01 and 0·05 μM), no detectable changes in a^i_{Na} and twitch tension were observed.

5. The data strongly indicate that in rat ventricular muscle sodium pump inhibition is present at low concentrations of ouabain which produce positive inotropy. This is consistent with previous results in canine and sheep cardiac Purkinje fibres.

INTRODUCTION

The mechanism of the inotropic action of cardiac steroids on heart muscle is still controversial. The controversy centres on the mechanism of action of cardiac steroids at concentrations which are at or slightly above the level at which inotropic effects are first seen, referred to in this paper as 'low concentrations'.

In cardiac Purkinje fibres an increase in intracellular Na^+ activity is closely related to positive inotropy during both onset and recovery periods of the effect of cardiac steroids at low and high concentrations (Lee, Kang, Sokol & Lee, 1980; Lee & Dagostino, 1982; Eisner, Lederer & Vaughan-Jones, 1983*a*; Wasserstrom, Schwartz

& Fozzard, 1983). Furthermore, the positive inotropic effect of strophanthidin requires that an increase in intracellular Na^+ activity be associated with suitable cellular Ca^{2+} availability (Vassalle & Lee, 1984). These data are consistent with the concept that cardiac steroids first specifically bind to the Na^+–K^+-ATPase (Matsui & Schwartz, 1968) causing an inhibition of the Na^+ pump which, in turn, increases the intracellular Na^+ level. The increase in intracellular Na^+ is expected to increase the intracellular Ca^{2+} by affecting Na^+–Ca^{2+} exchange (Reuter & Seitz, 1968; Glitsch, Reuter & Scholz, 1970; Sheu & Fozzard, 1982). The increase in intracellular Ca^{2+} results in the positive inotropic effect. According to this scheme, the inhibition of the Na^+ pump is a *necessary* step in the inotropic action of cardiac steroids, a concept first suggested by Repke (1963).

In contrast to the results cited above, other work has suggested a dissociation between pump inhibition and positive inotropy in the presence of low concentrations of cardiac glycosides (for review, see Noble, 1980). In rat ventricular muscle, ouabain produces a positive inotropic effect at low concentrations of 0·1–10 μM as well as at higher concentrations (> 10 μM). The low-concentration effect does not correlate with inhibition of the Na^+–K^+-ATPase activity in membrane preparations isolated from the same tissues, while at the higher concentration the degree of inotropy correlates well with inhibition (Erdmann, Philipp & Scholz, 1980; Adams, Schwartz, Grupp, Grupp, Lee, Wallick, Powell, Twist & Gathiram, 1982). This strongly suggests that low concentrations of ouabain do not increase intracellular Na^+ but still produce a positive inotropic effect (Erdmann *et al.* 1980). The suggestion, therefore, is that there are two inotropic mechanisms, one dependent on inhibition of the Na^+–K^+-ATPase (i.e. so-called 'low-affinity digitalis binding' site) and the other, at low concentrations of glycosides (i.e. so-called 'high-affinity digitalis binding' site) independent of the Na^+–K^+-ATPase and involving another receptor.

The main aim of the present study was to determine if low concentrations of ouabain do, in fact, affect the Na^+ pump in intact tissue and change the intracellular Na^+ level in rat ventricular muscle. The concept that we employed is that measurement of Na^+–K^+-ATPase activity of rat heart membranes *in vitro* may not be sensitive enough to detect the small but significant changes which may take place. Continuous measurement of intracellular Na^+ activity of the muscle with Na^+-selective microelectrodes during exposure to and the wash-out of ouabain is a highly sensitive technique. We therefore used this method in order to examine the effects of low concentrations of ouabain on Na^+–K^+-ATPase activity. Our study revealed that even low concentrations of ouabain that do not measurably inhibit Na^+–K^+-ATPase of rat ventricular membrane *in vitro*, do produce significant inhibition of the Na^+ pump in intact tissue (i.e. increase intracellular Na^+). Hence our data support only a single inotropic mechanism for digitalis.

Some of these data have been reported previously in abstract form (Lee, Im, Pecker, Grupp & Schwartz, 1983).

METHODS

Tissue and solutions

Male Sprague–Dawley rats weighing 175–200 g were killed by cervical dislocation and their hearts rapidly removed. The hearts were immersed in oxygenated Tyrode solution at room temperature and papillary muscles from the right ventricle were dissected. The muscles ranged in widest

diameter from 0·5 to 1·0 mm and were about 2·0–3·0 mm in length. The muscle was placed in a narrow channel of a muscle chamber and superfused with oxygenated Tyrode solution at 36–37 °C (Lee & Dagostino, 1982). One end of the muscle was fixed to the Sylgard floor of the muscle chamber by an insect pin and the other connected to a tension transducer by a 25 μm wire attached to one of the chordae. In order to stabilize the muscle for recording with electrodes, a cross-bar was placed lightly over the middle of the muscle. Considering this and the shape of the papillary muscle, the active part of the muscles was considerably smaller than the dimensions given above.

Oxygenated Tyrode solution was perfused through the channel at a constant rate adjusted so that solution exchanges around the muscle took place within a few seconds. Concentrations (mM) in the Tyrode solution were: NaCl, 137; KCl, 5·4; $MgCl_2$, 1·05; NaH_2PO_4, 0·45; $NaHCO_3$, 11·9; $CaCl_2$, 1·8; and dextrose, 5·0. In some experiments, 2·7 mM-$CaCl_2$ was used. Tyrode solution was saturated with 97% oxygen and 3% carbon dioxide; pH was 7·3–7·4.

Stock solutions of 1 mM-ouabain (Boehringen Mannheim) were prepared and diluted with oxygenated Tyrode solution for use in each experiment. Ouabain concentrations used were 0·01, 0·05, 0·1, 0·5, 1·0 and 5·0 μM.

Measurement of intracellular Na^+ and K^+ activities

Intracellular Na^+ activity (a^i_{Na}) was measured with Na^+-selective micro-electrodes made with the neutral carrier, ETH 227 (Steiner, Oehme, Ammann & Simon, 1979). Construction and calibration of the Na^+-selective micro-electrodes have been previously described in detail (Dagostino & Lee, 1982; Lee, 1981). Interference from K^+ and Ca^{2+} were accounted for as described previously (Dagostino & Lee, 1982; Lee & Dagostino, 1982; Vassalle & Lee, 1984). For this purpose intracellular K^+ activity (a^i_K) was measured separately (see Results section). Na^+-selective micro-electrodes were stable for longer than 10 h (Dagostino & Lee, 1982) and calibrated after each experiment. a^i_{Na} of quiescent muscles were measured continuously before, during and after exposure to ouabain.

Intracellular K^+ activity (a^i_K) of quiescent muscle of rat ventricle was measured with K^+-selective micro-electrodes. These micro-electrodes were made employing a K^+-selective liquid ion exchanger (W.P. Instruments, Inc., New Haven, CT, U.S.A.). Their construction and calibration were entirely analagous to that described for Na^+-selective micro-electrodes. The interference due to Na^+ or Ca^{2+} is negligible because of the high selectivity of the electrodes and large ratio of a^i_K to intracellular activity of competing cations.

We have been concerned about a possible shunt produced by poor sealing of the cell membrane around the ion-selective micro-electrode tip during impalement of the ventricular muscle cells. The cell membrane potential measured with an ion-selective micro-electrode must be the same as that measured with a conventional micro-electrode so that a significant error is not involved in the measurement of intracellular ion activities (Lee, 1981). To test for the possibility of such shunts we made micropipettes similar to those used for making the ion-selective micro-electrodes and filled them with 3 M-KCl. The micro-electrodes had resistances of 10–17 MΩ. Membrane potentials measured with these micro-electrodes were the same as those measured with the conventional micro-electrodes used in this study. This indicates that significant shunts were not involved in the measurements of intracellular ion activities of the ventricular muscle.

Measurement of contractile tension

To measure twitch tension, the ventricular muscles were stimulated with square pulses delivered to the electrodes from a stimulator (model 301-T, W.P. Instruments, Inc., New Haven, CT, U.S.A.) through a stimulus isolation unit. The stimulus voltage was 20–40% above threshold voltage. The muscle cells were driven continuously before, during and after exposure to ouabain at the rate of 0·5 or 1 Hz. Twitch tension of the muscle was measured with a force transducer (model 405, Cambridge Technology, Inc., Cambridge, MA, U.S.A.) at a sensitivity of 50 mV/mg. The muscle length for maximum twitch tension was determined by stretching the muscles in steps. The muscle length was then reduced until the twitch tension was about 80% of the maximum tension. Twitch tension was recorded on a chart recorder (model 280, Gould Inc., Cleveland, OH, U.S.A.) and also displayed on an oscilloscope together with the action potential.

A stable base line was required for over 5 min before exposure to ouabain. In the experiments where voltages were measured this meant that the variation had to be less than 0·5 mV. When twitch tension was measured, peak and resting tension were required not to vary by more than 0·25 mg. Changes had to be gradual and reversible after wash-out.

RESULTS

Intracellular ion activities

In each experiment a rat papillary muscle driven at the rate of 0·5 Hz had been equilibrated in normal Tyrode solution for at least 1 h before intracellular Na^+ activity (a^i_{Na}) was measured. In some experiments a muscle was driven at the rate

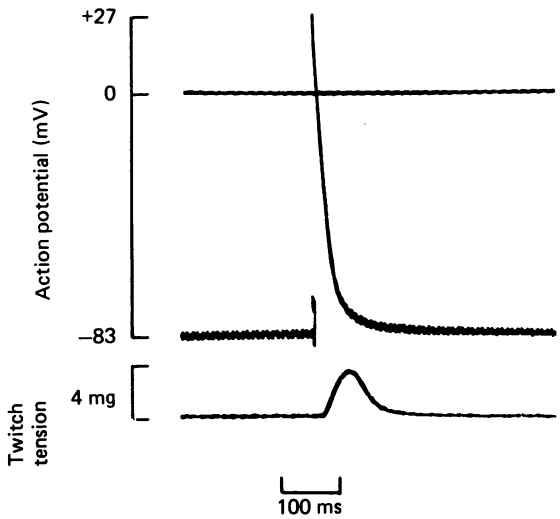

Fig. 1. Action potential and twitch tension of a rat ventricular muscle stimulated at a constant rate of 0·5 Hz. In this experiment, diastolic potential and overshoot of the action potential were −83 and +27 mV respectively.

of 1 Hz because twitch tension of some muscles driven at this higher frequency was more stable. Fig. 1 illustrates a representative action potential and twitch tension. The cardiac action potential duration was much shorter than that of other species. Maximum diastolic potential averaged $-81{\cdot}1 \pm 3{\cdot}3$ mV (mean ± S.D., twenty-five measurements in eleven tissues). The over-shoot of the action potential was $29{\cdot}5 \pm 4{\cdot}6$ mV (mean ± S.D., twenty measurements in eleven tissues).

To measure a^i_{Na} of quiescent muscle, the stimulation was stopped. When the intracellular potential recorded by the Na^+-selective micro-electrode (E^i_{Na}) became stable, transmembrane potential (V_m) of a resting cell as measured with a conventional micro-electrode was subtracted electronically from E^i_{Na}, and a^i_{Na} calculated. The a^i_{Na} in quiescent muscle averaged $8{\cdot}5 \pm 2{\cdot}6$ mM (mean ± S.D., twenty-seven measurements in eight tissues). The transmembrane potential of quiescent muscle cells (V_m) averaged $-79{\cdot}0 \pm 2{\cdot}4$ mV (mean ± S.D., thirty-four measurements in eighteen tissues).

Intracellular K^+ activity (a^i_K) of rat ventricular muscle was measured in a manner analagous to that described above for a^i_{Na}. The a^i_K value in quiescent muscle was found to be $115{\cdot}0 \pm 9{\cdot}0$ mM (mean ± S.D., thirty-one measurements in four tissues).

Effect of ouabain on a^i_{Na}

In order to examine the effect of ouabain on the Na^+ pump of rat ventricular muscle, a^i_{Na} was continuously measured before, during, and after exposure to ouabain. Ouabain concentrations used in this study were 0·01, 0·05, 0·1, 0·5, 1·0 and 5·0 μM. At each concentration, the exposure time was 5 min. This duration of

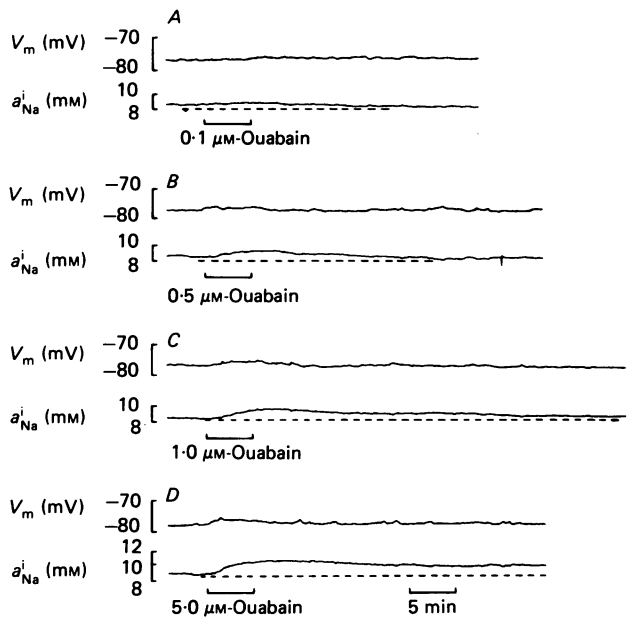

Fig. 2. Continuous recording of intracellular Na^+ activity (a^i_{Na}) and transmembrane potential (V_m) of a quiescent rat ventricular muscle before, during and after exposures to ouabain at concentrations of 0·1 (*A*), 0·5 (*B*), 1·0 (*C*) and 5·0 μM (*D*).

exposure was long enough to see an effect of ouabain on twitch tension at all concentrations which we used which have been previously reported to have an effect in this tissue. No detectable changes in a^i_{Na} were observed during exposure to ouabain at concentrations of 0·01 μM (four tests in two tissues) or 0·05 μM (seven tests in three tissues; see Fig. 3). Fig. 2 shows a representative experiment of the effects of 0·1, 0·5, 1·0 and 5·0 μM-ouabain on V_m (membrane potential) and a^i_{Na} of the same quiescent muscle. The threshold concentration of ouabain required to produce an effect was around 0·1 μM, as indicated by a small increase of a^i_{Na} (Fig. 2*A*). At this concentration of ouabain, no detectable changes in V_m were observed. Ouabain further increased a^i_{Na} as its concentration was increased (Fig. 2). At low concentrations of ouabain (0·1 and 0·5 μM), a^i_{Na} completely returned to the control levels after exposure to ouabain (Fig. 2*A* and *B*). Subsequent exposure of the tissue to previously used concentrations of ouabain gave reproducible results. After higher concentrations of ouabain (1·0 and 5·0 μM), however, recovery was often incomplete about 30 min after exposure, as shown in Fig. 2*C* and *D*.

Fig. 3 shows the relation between the change in a^i_{Na} (Δa^i_{Na}) and ouabain concentration. The a^i_{Na} change at each dose was measured as shown in Fig. 2 and represented the maximum change in a^i_{Na} during 5 min exposure to the drug. No detectable changes in a^i_{Na} were observed at ouabain concentrations of 0·01 and 0·05 μM. As

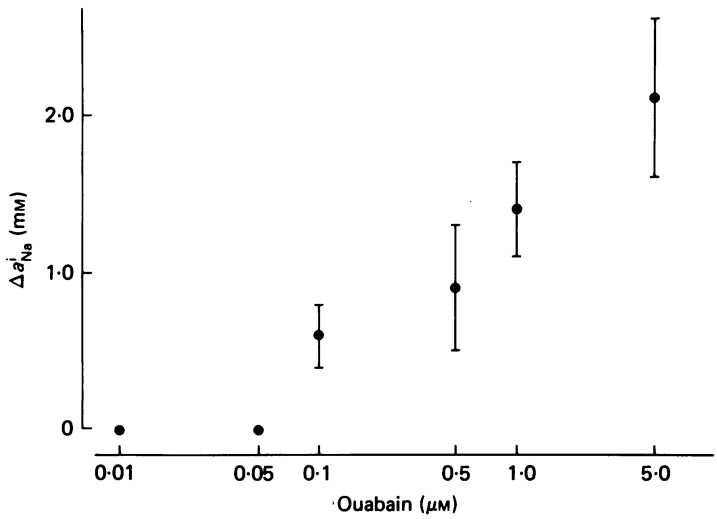

Fig. 3. Relation between change in a^i_{Na} (Δa^i_{Na}) and concentration of ouabain. The number of measurements at 0·01, 0·05, 0·1, 0·5, 1·0 and 5·0 μM were 4, 7, 15, 13, 10 and 6 respectively. Bars represent 1 S.D. on either side of the mean.

ouabain concentration was increased to 0·1, 0·5, 1·0 and 5·0 μM, a^i_{Na} increased in a concentration-dependent manner. This result clearly indicates that ouabain at these concentrations inhibits the Na^+ pump in rat ventricular muscle.

Effect of ouabain on twitch tension

Simultaneous measurements of twitch tension and a^i_{Na} from the same muscle are necessary to study the precise relation between changes in tension and intracellular ion activity. However, such simultaneous measurements were technically difficult in rat ventricular muscle (see Discussion). Therefore twitch tension was measured separately from a^i_{Na} in the same muscle or in different muscles. Twitch tension was continuously measured during exposure to and wash-out of ouabain at concentrations of 0·01, 0·05, 0·1, 0·5, 1·0 and 5·0 μM. At each concentration, the exposure time was 5 min. No detectable changes in twitch tension were observed during exposure to ouabain at concentrations of 0·01 μM (six tests in two tissues) and 0·05 μM (nine tests in three tissues; see Fig. 5). Fig. 4 shows a representative experiment of the effects of 0·1, 0·5, 1·0 and 5·0 μM-ouabain on twitch tension of a ventricular muscle driven at a constant rate of 0·5 Hz. The threshold concentration for ouabain's effect on twitch tension was around 0·1 μM as indicated by a small increase in twitch tension at this concentration (Fig. 4*A*). Ouabain produced concentration-dependent increases of twitch tension (Fig. 4*A–D*). At the lowest concentrations (0·1 and 0·5 μM), wash-out of ouabain resulted in complete recovery of twitch tension (Fig. 4). At higher

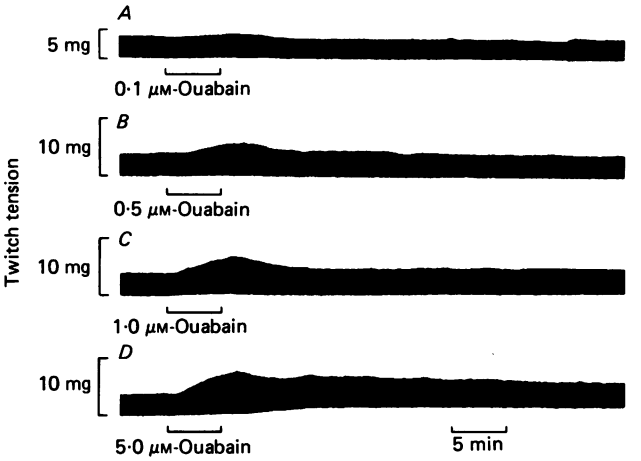

Fig. 4. Continuous recording of twitch tension of a rat ventricular muscle (stimulated at a rate of 0·5 Hz) before, during and after exposures to ouabain at concentrations of 0·1 (*A*), 0·5 (*B*), 1·0 (*C*) and 5·0 μM (*D*).

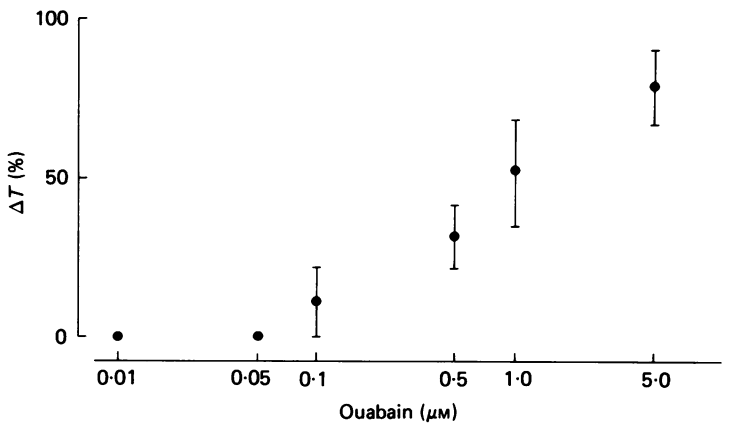

Fig. 5. Relation between change in twitch tension (ΔT) and concentration of ouabain. The number of measurements at 0·01, 0·05, 0·1, 0·5, 1·0 and 5·0 μM were 6, 9, 7, 7 and 4 respectively. Bars represent 1 S.D. on either side of the mean.

concentrations (1·0 and 5·0 μM), however, recovery was often incomplete 30 min after exposure (Fig. 4).

Fig. 5 shows the relation between the change in twitch tension (ΔT) and ouabain concentration. The twitch tension change at each concentration was measured as shown in Fig. 4 and represents the maximum change in twitch tension during 5 min exposure to ouabain. No detectable changes in twitch tension were observed at the concentrations of 0·01 and 0·05 μM. As ouabain concentration was increased to 0·1, 0·5, 1·0 and 5·0 μM, twitch tension increased in a concentration-dependent manner. A negative inotropic effect was not observed at any of the concentrations of ouabain employed.

Relation between a^i_{Na} and twitch tension

Although intracellular Na^+ activity (a^i_{Na}) and twitch tension were separately measured, their responses to ouabain were similar. At ouabain concentrations of 0·01 and 0·05 μM, no detectable changes in a^i_{Na} or twitch tension were observed. The 0·1 μM-ouabain produced small increases in a^i_{Na} and twitch tension (Figs. 2–5).

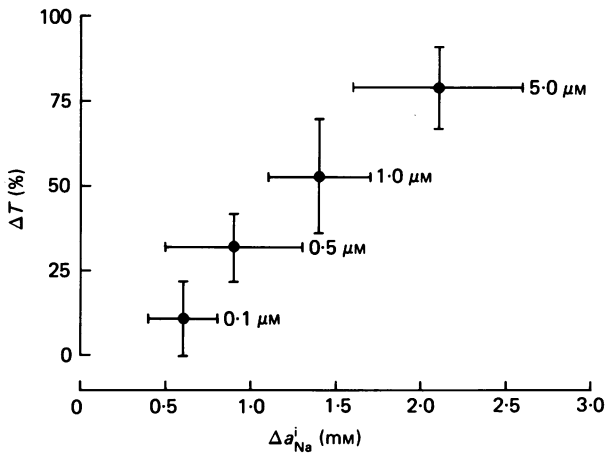

Fig. 6. Relation between the increase in twitch tension and that in a^i_{Na}. The change of the twitch tension (ΔT) at each ouabain concentration (0·1, 0·5, 1·0 or 5·0 μM) represents the percentage increase of the control twitch tension. The change of a^i_{Na} (Δa^i_{Na}) at each ouabain concentration represents the a^i_{Na} increase from the control a^i_{Na}. Bars represent 1 S.D. on either side of the mean.

Therefore, the threshold concentration of ouabain required to have an effect on both a^i_{Na} and twitch tension appeared to be around 0·1 μM. At ouabain concentrations greater than 0·1 μM, the drug produced concentration-dependent increases in a^i_{Na} and twitch tension. Fig. 6 is a plot of the increases in twitch tension (% of control tension) against the increases in a^i_{Na} at ouabain concentrations of 0·1, 0·5, 1·0 and 5·0 μM. It is apparent that, as a^i_{Na} rises, twitch tension increases. These results indicate that the increase in a^i_{Na} is related to the positive inotropic effect of ouabain.

DISCUSSION

Resting value of a^i_{Na} and a^i_K

The resting value of a^i_{Na} (8·5 ± 2·6 mM) found in the rat ventricular muscle is similar to that measured in rabbit and guinea-pig ventricular muscle (Lee & Fozzard, 1975; Cohen, Fozzard & Sheu, 1982; Kleber, 1983). However, the a^i_{Na} value is substantially lower than that (31·1 ± 1·62 mM, ± S.E. of mean) of rat ventricular muscle reported recently (Wasserstrom, 1983). We do not know the reason for this discrepancy.

The resting membrane potential (V_m) and action potential over-shoot that we

observed in this study are comparable with results of other workers (Watanabe, Delbridge, Bustamante & McDonald, 1983).

Our value of 115 mM for a_K^i in rat ventricular muscle is similar to previously reported values of 108 and 116 mM in guinea-pig ventricle (Baumgarten, Cohen & McDonald, 1981; Cohen *et al.* 1982) and somewhat higher than that (83 mM) in rabbit ventricular muscle (Lee & Fozzard, 1975). Therefore, the resting levels of a_{Na}^i and a_K^i in rat ventricular muscle are similar to those in ventricular muscle of other species.

Effect of low concentrations of ouabain

Results from several laboratories have suggested that low and high concentrations of cardiac glycosides produce inotropic effects by different mechanisms and in particular that low concentrations produce their positive inotropic effects in the absence of Na^+ pump inhibition (Erdmann *et al.* 1980; Godfraind & Ghysel-Burton, 1980; Adams *et al.* 1982; Kurobane, Nandi & Okita, 1983). These studies have utilized a variety of methods to estimate Na^+ pump inhibition including $^{86}Rb^+$ uptake and measurements of Na^+–K^+-ATPase activity in sarcolemmal preparations. Studies utilizing Na^+-selective electrodes have not shown a dissociation between positive inotropy and Na^+ pump inhibition due to cardiac glycosides (Lee *et al.* 1980; Lee & Dagostino, 1982; Cohen *et al.* 1982; Wasserstrom *et al.* 1983) but these studies have not been done in tissues where such a dissociation is proposed to occur.

Work in rat ventricular muscle has shown that the inotropic effects of low concentrations of ouabain in this tissue do not correlate with Na^+ pump inhibition as estimated from $^{86}Rb^+$ uptake (Erdmann *et al.* 1980) or Na^+–K^+-ATPase inhibition in rat myocyte sarcolemmal preparations (Erdmann *et al.* 1980; Adams *et al.* 1982). On the other hand, the inotropic effects of high concentrations of ouabain showed a good correlation with these estimates of pump inhibition. The low-concentration inotropic response did, however, correlate with an apparent high-affinity site detected by [^{3}H]ouabain binding studies (Erdmann *et al.* 1980; Adams *et al.* 1982). This suggests that in rat ventricular muscle the inotropic effects of low concentrations are independent of Na^+–K^+-ATPase inhibition. The purpose of the present study was to test this hypothesis utilizing Na^+-selective electrodes to measure changes in a_{Na}^i and, thereby, changes in Na^+ pump activity.

We therefore measured a_{Na}^i during exposure to and wash-out of ouabain as a direct measure of pump activity in living cells. At low concentrations (0·1–0·5 μM), ouabain produced small but distinct increases in a_{Na}^i which returned to control levels after exposure to the drug (Figs. 2 and 3). From this result, it seems reasonable to conclude that ouabain at these low concentrations indeed inhibits the Na^+ pump in rat ventricular muscle. A discrepancy exists between the results of the present study and those of previous studies with this tissue (Erdmann *et al.* 1980; Adams *et al.* 1982). It is possible that isolated Na^+–K^+-ATPase from rat heart behaves differently from the *in vivo* pump, although this is unlikely. It is perhaps more likely that the biochemical measurements of enzyme activity in membrane preparations were a less-sensitive method for detecting pump inhibition than the method used in this study. As regards the dissociation of positive inotropy and inhibition of $^{86}Rb^+$ uptake in the steady state, these would not be expected to correlate with each other (Eisner, Vaughan-Jones & Lederer, 1983*c*).

The effect of digitalis on a^i_{Na} in working ventricular muscle was reported in one study by Cohen *et al.* (1982), while most other studies were done with cardiac Purkinje fibres. They reported that an application of 0·3 μM-ouabain for 10 min to guinea-pig ventricular muscle stimulated at 0·2 Hz caused an increase of a^i_{Na} from 7·2 to 9·4 mM in one experiment, and from 6·6 to 9·0 mM in a second experiment. Our results show that 0·1, 0·5, 1 and 5 μM-ouabain exposed to quiescent rat ventricular muscle for 5 min increased a^i_{Na} by $0·6 \pm 0·2$, $0·9 \pm 0·4$, $1·4 \pm 0·3$ and $2·1 \pm 0·5$ mM, respectively. Similar estimates of the change in intracellular Na^+ concentration have also been obtained using radioisotope methods in rabbit myocardium (Langer & Serena, 1970).

It has been reported that ouabain at low doses (about 0·1 μM or less) decreases a^i_{Na} (Deitmer & Ellis, 1978), increases intracellular K^+ content (Ghysel-Burton & Godfraind, 1977), and stimulates $^{86}Rb^+$ transport (Lechat, Malloy & Smith, 1983). These results suggest that ouabain may stimulate the Na^+ pump in cardiac muscle cells (for a review, see Noble, 1980). In the present study, a^i_{Na} did not decrease during exposure to low concentrations of ouabain, which is consistent with other results recently reported (Lee & Dagostino, 1982; Wasserstrom *et al.* 1983).

A role for catecholamines in the low-dose effects of cardiac glycosides has been proposed by studies in guinea-pig left atria (Hougen, Spicer & Smith, 1981; Lechat *et al.* 1983). Our studies did not examine this possibility. In the present study, concentrations of ouabain producing positive inotropy also produced an increase in a^i_{Na}. Thus, at least in the rat ventricular muscle preparation we used, the threshold for any ouabain-induced catecholamine-mediated inotropy would be as high or higher than the threshold for positive inotropy associated with pump inhibition. Despite these considerations, a role for catecholamines is not ruled out by these experiments.

Relation between a^i_{Na} and twitch tension

The increases of a^i_{Na} at ouabain concentrations of 0·1, 0·5, 1·0 and 5·0 μM were plotted against those of twitch tension (Fig. 6). This result indicates that the increase in a^i_{Na} is related to the increase in twitch tension. The finding that a^i_{Na} and twitch tension are positively related even when small changes in a^i_{Na} occur is similar to results in Purkinje fibres (Lee *et al.* 1980; Eisner, Lederer & Vaughan-Jones, 1981; Lee & Dagostino, 1982; Eisner, Lederer & Vaughan-Jones, 1983*b*; Wasserstrom *et al.* 1983).

Simultaneous and continuous measurements of a^i_{Na} and twitch tension are necessary to study the temporal relation between a^i_{Na} and tension during ouabain exposure and wash-out. Such simultaneous and continuous measurements were, however, technically difficult in rat ventricular muscle, although they have been done in cardiac Purkinje fibres (Lee & Dagostino, 1982). This difficulty may be due to the small size and strong contraction of working ventricular muscle cells. Therefore, in the present study a^i_{Na} was measured continuously in quiescent muscle and twitch tension was separately measured in driven muscles.

The Na^+ pump activity and a^i_{Na} can be expected to be different in quiescent and active cells (cf. Deitmer & Ellis, 1978; Gadsby & Cranefield, 1979; Eisner & Lederer, 1980; Cohen *et al.* 1982; Lee & Dagostino, 1982). If these differences are present in rat ventricular muscle, the increase in a^i_{Na} produced by ouabain in quiescent cells would differ from the increase in active cells, and the tension–a^i_{Na} relation shown in Fig. 6 would be quantitatively different from that which would be obtained by simultaneous measurement of a^i_{Na} and twitch tension in active cells. Nevertheless,

such a quantitative change in this relation should not alter our conclusion that the increase in twitch tension is related to the increase in a^{i}_{Na}.

Our results indicate that a^{i}_{Na} increases over the full range of ouabain concentrations which increase twitch tension in rat ventricular muscle. This strongly suggests that in this tissue, as in others, the inotropic actions of even low concentrations of ouabain are accompanied and mediated by Na^+ pump inhibition. The most likely explanation for a 'unified' mechanism of action of cardiac glycosides involves first a binding to the Na^+–K^+-ATPase (Matsui & Schwartz, 1968), followed by inhibition of the enzyme, which leads to a significant enhancement of a^{i}_{Na} and increases in intracellular Ca^{2+} levels and positive inotropy (Schwartz, Lindenmeyer & Allen, 1975; Schwartz, 1976).

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