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# INTRACELLULAR SODIUM ACTIVITY AND ITS REGULATION IN GUINEA-PIG ATRIAL MYOCARDIUM

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SUMMARY

1. Intracellular $Na^+$ activity ($a^i_{Na}$) and membrane resting potential were studied in quiescent guinea-pig atrial and papillary muscles by means of $Na^+$-sensitive and conventional microelectrodes. The effects of the cardioactive steroid dihydroouabain (DHO) on $a^i_{Na}$, force of contraction and sarcolemmal $Na^+$, $K^+$-ATPase activity were also investigated.

2. In thirty atria and twenty-two papillary muscles, $a^i_{Na}$ amounted to $8{\cdot}0 \pm 0{\cdot}2$ and $4{\cdot}7 \pm 0{\cdot}3$ mM, respectively (means ± S.E.M.). When both tissues were from the same animal, with the same ion-sensitive microelectrode mean $a^i_{Na}$ values of $7{\cdot}9 \pm 0{\cdot}2$ and $5{\cdot}1 \pm 0{\cdot}5$ mM ($P < 0{\cdot}01$) were obtained from eight atrial and eight papillary muscles, respectively.

3. Membrane resting potentials ($E_m$) were significantly ($P < 0{\cdot}001$) more negative in the papillary muscles ($-83{\cdot}5 \pm 0{\cdot}7$ mV; $n = 8$) than in the atrium ($-78{\cdot}1 \pm 0{\cdot}5$ mV; $n = 8$). Deviation of $E_m$ from $E_K$ (determined by $K^+$-sensitive microelectrodes) was $3{\cdot}0 \pm 0{\cdot}2$ mV in ventricular ($P < 0{\cdot}05$) and $6{\cdot}1 \pm 0{\cdot}3$ mV in atrial preparations ($P < 0{\cdot}05$).

4. Inhibition of the $Na^+$ pump by DHO increased $a^i_{Na}$ of the atrium within 10 min by $0{\cdot}6 \pm 0{\cdot}1$ ($n = 7$), $1{\cdot}3 \pm 0{\cdot}1$ ($n = 5$) and $3{\cdot}2 \pm 0{\cdot}2$ mM ($n = 5$) at 5, 10 and 30 $\mu$M, respectively. In the papillary muscle, 10 $\mu$M DHO was without effect while $a^i_{Na}$ rose by $1{\cdot}0 \pm 0{\cdot}1$ ($n = 5$) and $2{\cdot}9 \pm 0{\cdot}2$ mM ($n = 6$) at 30 and 120 $\mu$M DHO.

5. Consistent with the $a^i_{Na}$ measurements, the potency of DHO to increase force of the isometric contraction was three times higher in atrium than in papillary muscle (stimulation frequency 0·2 Hz).

6. Hydrolytic activity of sarcolemmal $Na^+$,$K^+$-ATPase isolated from atria amounted to only one third of that detected in ventricles ($0{\cdot}07 \pm 0{\cdot}01$, $n = 6$, *versus* $0{\cdot}2 \pm 0{\cdot}01$ $\mu$mol phosphate released $min^{-1}$ (g tissue)$^{-1}$, $n = 5$). The inhibitory potencies of DHO on sarcolemmal $Na^+$,$K^+$-ATPase preparations were found to be identical in the enzymes from either tissue.

7. It is concluded that a lower $Na^+$ pump density is responsible for the higher $a^i_{Na}$ and for the lower resting membrane potential in atrial as compared to ventricular cells. The regulation of cellular $Na^+$ homeostasis in atrial muscle appears to be closer

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MS 1190

to the limits of its capacity than in ventricle, explaining the higher sensitivity of the atrium to interventions which impede $Na^+$ pump activity.

## INTRODUCTION

It is well known that the $Na^+$–$K^+$ exchange pump extrudes $Na^+$ from the cell interior and thus maintains a low intracellular $Na^+$ concentration with respect to the outside. In cardiac cells, the resulting $Na^+$ gradient across the cell membrane appears to be important for membrane transport of ions, such as $H^+$ and $Ca^{2+}$, and provides the driving force for inward $Na^+$ current during an action potential. In addition, intracellular $Na^+$ has been implicated in the regulation of cardiac contractility as exemplified by the force–frequency relationship (Cohen, Fozzard & Sheu, 1982; Wang, Chae, Gong & Lee, 1988) and the positive inotropic effect of several cardioactive drugs (Lee, Kang, Sokol & Lee, 1980; Brill & Wasserstrom, 1986; Schmied, Wang & Korth, 1991). Much of our present knowledge of cellular $Na^+$ regulation has emerged from studies utilizing $Na^+$-sensitive microelectrodes which allow intracellular $Na^+$ activity ($a^i_{Na}$) to be monitored continuously over time and sensitively enough to detect even small changes in ion activity. $Na^+$-sensitive microelectrodes have proved to be a powerful tool in elucidating the mechanism of action of cardiac steroids (Lee, 1985) and particularly in resolving the controversial issue on the positive inotropic effect of low ouabain concentrations in rat heart (Grupp, Im, Lee, Lee, Pecker & Schwartz, 1985).

While all the relevant data on the regulation of $a^i_{Na}$ in cardiac cells have been obtained from ventricular preparations or Purkinje strands, there is no information available on the regulation of $a^i_{Na}$ in atrial cells. This seems surprising, the more so since atrial preparations have been widely used to study the action of cardioactive steroids on contractility, binding characteristics and $Na^+$,$K^+$-ATPase activity. In addition, several reports suggest that the regulation of $a^i_{Na}$ and its perturbation by cardioactive steroids may be different in atrial and in ventricular preparations (Poole-Wilson & Cameron, 1975; Young & Lingrel, 1987; Schmidt, Svendsen, Haunsø & Kjeldsen, 1990).

In the present study ion-sensitive microelectrodes were used to monitor $a^i_{Na}$ in atrial and ventricular preparations under control conditions and under the influence of the cardioactive steroid dihydroouabain. These experiments were complemented by the measurement of contractile force and of $Na^+$,$K^+$-ATPase activity. The results demonstrate that resting $a^i_{Na}$ and the effectiveness of $Na^+$ pump inhibition is substantially higher in atrial than in ventricular myocardium. This finding is probably due to different numbers of $Na^+$,$K^+$-ATPase molecules in the cell membranes of both tissues.

## METHODS

*Measurement of force of contraction*

Guinea-pigs of either sex weighing 250–350 g were killed by cervical dislocation. Right ventricular papillary muscles (diameter 0·5–0·8 mm) and fine trabeculae from the left atrium (2–3 mm in length and 0·20–0·25 mm in diameter) were dissected from the isolated heart and mounted in a two-chambered organ bath with internal circulation of the bath solution (volume, 50 ml) as described previously (Reiter, 1967). The bath solution was constantly gassed and kept in circulation by 95% $O_2$–5% $CO_2$; the temperature was maintained at 35 °C, pH 7·5. The composition of the bath solution was (mM): NaCl, 115; KCl, 4·7; $MgSO_4$, 1·2; $CaCl_2$, 3·2; $NaHCO_3$,

25; $KH_2PO_4$, 1·2; and glucose, 10. The muscles were stimulated through two punctate platinum electrodes with square wave pulses of 1 ms and an intensity slightly above threshold. Force of contraction was recorded isometrically by means of an inductive force transducer (Q-11, 10p, Hottinger Baldwin Meßtechnik, Darmstadt, Germany) connected to an oscilloscope and a pen recorder. The resting force was kept constant at 4 mN throughout the experiment. An equilibration period of at least 1 h at a stimulation frequency of 1 Hz preceded each experiment. Subsequently, the frequency of stimulation was lowered to 0·2 Hz, and the drug intervention was started as soon as force of contraction had reached a steady state.

*Measurement of ion activities*

To measure $a^i_{Na}$ and $a^i_K$, papillary muscles and atrial trabeculae were mounted horizontally in a perfusion chamber (volume, 0·4 ml) perfused at a constant rate of 5 ml $min^{-1}$. Exchange of bath solution was complete within 1 min. The voltage-recording electrodes had tip resistances of 20 MΩ, and small tip potentials, when filled with 3 M KCl, acidified to a pH of 2 with HCl. The construction and calibration of the ion-sensitive microelectrodes with either the $Na^+$ liquid ion exchange resin ETH 227 (Steiner, Oehme, Ammann & Simon, 1979) or the $K^+$ liquid ion exchanger (Corning Glass Works, lot 477317) have been described in detail elsewhere (Sheu & Fozzard, 1982; Baumgarten, Singer & Fozzard, 1984). The muscles were impaled with a conventional electrode and an ion-sensitive microelectrode so that the impalements were as close as possible. From the potentials measured with the two microelectrodes, ion activities of the cell were calculated by means of the following equation:

$$E^i - E_m = E_0 + S\log(a^i_X + k_{X,Y}a^i_Y), \quad (1)$$

where $E^i$ is the transmembrane potential measured with the ion-sensitive microelectrode with respect to the reference electrode in the bath, $E_m$ is the transmembrane potential measured with the conventional microelectrode, $E_0$ is a constant potential of the ion-sensitive microelectrode, and $S$ is the slope of the ion-sensitive microelectrode (ranging from 50 to 61 mV per decade) as determined in KCl and NaCl solutions containing 0·1 mM EGTA. $a^i_X$ and $a^i_Y$ are the ion activities and the subscripts X and Y refer to the principal and interfering ion (either $K^+$ or $Na^+$). $k_{X,Y}$ is the selectivity coefficient of the ion-sensitive microelectrode which ranged from 0·01 to 0·02. The selectivity coefficient of the $Na^+$-sensitive microelectrode with respect to $Ca^{2+}$, $k_{Na,Ca}$ was in the range 1·7–2·5 (Dagostino & Lee, 1982), indicating that the electrode was more selective to $Ca^{2+}$ than $Na^+$. However, the interference of $Ca^{2+}$ on $a^i_{Na}$ measurements is very small and probably uniform in guinea-pig atrial and ventricular cells because intracellular resting $Ca^{2+}$ activity is low (about 1/100000 of that of $a^i_{Na}$) and nearly identical in both tissues (Beuckelmann & Wier, 1988; Bals, Bechem, Paffhausen & Pott, 1990). Before and after each experiment, the electrodes were calibrated at 35 °C with pure solutions of KCl or NaCl and with mixtures of NaCl and KCl, with the sum ($Na^+ + K^+$) kept constant at 150 mM. Conventional and ion-sensitive microelectrodes were connected to a dual-channel high-impedance electrometer (model 773, World Precision Instruments, New Haven, CT, USA). The signals were displayed separately and electronically subtracted on a pen recorder and on digital panel meters. The panel meter readings were used for calculating $a^i_{Na}$ and $a^i_K$.

*Measurement of $Na^+,K^+$-ATPase activity*

$Na^+,K^+$-ATPase of guinea-pig (150–200 g) hearts were assayed in preparations each of which originated from twenty atria and five ventricles respectively. The isolation procedure was described previously (Ebner, 1990). Protein concentration was determined as described elsewhere (Lowry, Rosebrough, Farr & Randall, 1951). The enzyme activity was tested with the coupled enzyme assay (pyruvate kinase–lactate dehydrogenase, PK–LDH) at 35 °C. In a final volume of 1·3 ml, the reaction medium contained (mM): KCl, 5·9; NaCl, 140; $MgCl_2$, 5; $NaN_3$, 5; ethyleneglycol-bis-(β-aminoethylether)*N,N,N',N'*-tetraacetic acid (EGTA), 0·5; Tris(hydroxymethyl)aminomethane (Tris), 50 (pH 7·4); Tris-ATP, 3 (pH 7·4); phosphoenolpyruvate, 0·5; NADH, 0·24; $(NH_4)_2SO_4$, 16·8; pyruvate kinase, 7 U; lactate dehydrogenase, 10 U. After preincubation for 30 min, $Na^+,K^+$-ATPase was added (5–15 μg protein in 100 μl histidine (10 mmol $l^{-1}$) pH 7·4). After a further 10 min, water (control) or dihydroouabain solution was injected in a volume of 20 μl and the time course of enzyme activity was followed for 15–30 min. NADH absorbance was monitored at 334 nm. The data were fed into a computer via a RS-232 interface at 1 s intervals. The tests were run in duplicate. Preincubation with 5 μg sodium dodecylsulphate per millilitre did not affect either enzyme (see Ebner, 1990). The experiments were evaluated with a special integrated rate equation

(Ebner, 1990) which describes the onset of inhibition independently of enzyme decay during the assay with a pseudo-first-order rate constant $k_1$ and fractional inhibition $EI/E$ at steady state in a monoexponential function,

$$S = S_0 + (1 - EI/E)(bt + ct^2 + dt^3) - \exp(-k_1 t)\, EI/E\, (b/k_1 + 2c/k_1^2 (k_1 t + 1) + 3d\,(t^2/k_1^1 + 2t/k_1^2 + 2/k_1^3)) + EI/E\,(b/k_1 + 2c/k_1^2 + 6d/k_1^3), \quad (2)$$

where $S$ is substrate concentration at time $t$ and $S_0$ at time 0; b, c, d are coefficients of a cubic polynomial fitted to the control curve of $S$. Fit of eqn (2) to data (Gauss–Newton method) provides estimates of a pseudo-first-order rate constant of the onset of inhibition and its steady-state value as fractional inhibition. Enzyme activity was calculated under control conditions at $t = 0$ as estimated by the coefficient b of the polynomial.

*Chemicals*

The following drugs were used (abbreviations and sources in parentheses): dihydroouabain (Hommel AG, Adliswil, Switzerland); Tris(hydroxymethyl)aminomethane; adenosine-5′-triphosphate (disodium salt of ATP) (Merck, Darmstadt, Germany); ethyleneglycol-bis-(β-aminoethylether)*N,N,N′,N′*-tetraacetic acid (EGTA); lactate dehydrogenase–pyruvate kinase mixture in 2·2 mol $l^{-1}$ ammonium sulphate (PK–LDH); albumin (Sigma, München, Germany); phosphoenolpyruvate (tricyclohexylammonium salt); nicotinamide-adenine dinucleotide phosphate ($Na_2$NADH) (Boehringer, Mannheim, Germany); Tris-ATP was prepared from $Na_2$-ATP with Dowex 50 W × 8.

*Evaluation of results*

Results are either presented as arithmetic means ± S.E.M. or as original records. The positive inotropic effect ($\Delta F_c$), i.e. the increase in force of contraction over its basal value, was normalized as a percentage of the maximal effect. Significance tests were performed by the two-tailed $t$ test for paired or unpaired observations. Differences between means were regarded statistically significant at $P < 0.05$.

## RESULTS

### *Intracellular activities of $Na^+$ and $K^+$*

In each experiment a guinea-pig papillary muscle and atrial trabecula driven at a rate of 1 Hz had been equilibrated in normal Krebs–Henseleit solution for at least 1 h. To measure intracellular $Na^+$ ($a^i_{Na}$) and $K^+$ ($a^i_K$) activities of quiescent muscles, the stimulation was stopped. When the intracellular potentials recorded with the ion-sensitive and the conventional microelectrode became stable, ion activities were calculated in both tissues. In thirty atrial trabeculae and twenty-two papillary muscles, the mean values of $a^i_{Na}$ amounted to 8·0 ± 0·2 and 4·7 ± 0·3 mM, respectively. When both tissues were obtained from the same heart and impaled sequentially with the same ion-sensitive electrode, mean $a^i_{Na}$ values of 7·9 ± 0·2 and 5·1 ± 0·5 mM were obtained from eight atrial and eight papillary muscles, respectively (see Table 1). Thus, the present data demonstrate a significant ($P < 0.01$) and nearly 60% higher value of $a^i_{Na}$ in atrial as compared to ventricular cells. As can be further seen from Table 1, the $a^i_K$ values showed a reciprocal distribution with respect to $a^i_{Na}$. Also presented in Table 1 are values of membrane resting potentials, $E_m$, and calculated potassium electrochemical equilibrium potentials, $E_K$. The data show that $E_m$ was significantly ($P < 0.001$) less negative in atrial than in ventricular cells. Although atria had a lower $a^i_K$ than papillary muscles ($P < 0.05$), the lower resting potential of atria was mainly due to a distinct deviation of $E_m$ from $E_K$. The difference between $E_m$ and $E_K$ was 3·0 ± 0·2 mV in ventricular ($P < 0.05$) and 6·1 ± 0·3 mV in atrial preparations ($P < 0.05$).

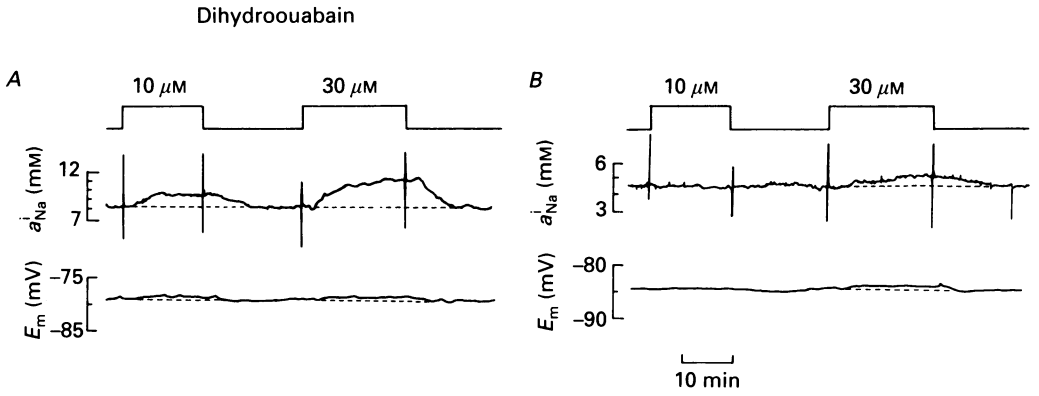


Fig. 1. Effects of dihydroouabain on intracellular $Na^+$ activity ($a^i_{Na}$) and membrane resting potential ($E_m$) in guinea-pig atrial (*A*) and ventricular myocardium (*B*). Note that all dihydroouabain effects could be reversed by washing the muscles with drug-free solution. Papillary muscle and atrium were obtained from the same animal.

TABLE 1. Comparison of electrophysiological parameters as determined in atrial and ventricular preparations obtained from the same guinea-pig heart

| | Ventricle (8) | Atrium (8) |
|---|---|---|
| $E_m$ (mV) | $-83{\cdot}5 \pm 0{\cdot}7$ | $-78{\cdot}1 \pm 0{\cdot}5$*** |
| $E_K$ (mV) | $-86{\cdot}5 \pm 0{\cdot}8$ | $-84{\cdot}5 \pm 0{\cdot}7$* |
| $a^i_{Na}$ (mM) | $5{\cdot}1 \pm 0{\cdot}5$ | $7{\cdot}9 \pm 0{\cdot}2$** |
| $a^i_K$ (mM) | $114{\cdot}0 \pm 4{\cdot}5$ | $103{\cdot}0 \pm 4{\cdot}3$* |

Values are given as means ± S.E.M. with number of preparations in parentheses. $E_K$ was calculated using the Nernst equation.

Significant *vs.* ventricle: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$; Student's paired *t* test.

### *Effect of dihydroouabain on $Na^+$ activity*

In order to examine the concentration-dependent effect of dihydroouabain on the $Na^+$ pump of guinea-pig ventricular and atrial myocardium, $a^i_{Na}$ and $E_m$ were continuously measured before, during and after exposure to various concentrations of the cardioactive steroid. Figure 1 shows a representative experiment in which the threshold concentration of dihydroouabain required to increase $a^i_{Na}$ was determined in a quiescent atrial trabecula (Fig. 1*A*) and a papillary muscle (Fig. 1*B*). Both tissues were obtained from the same heart. Application of 10 μM dihydroouabain to the superfusing solution caused $a^i_{Na}$ of the atrium to increase from 8·1 to 9·6 mM and $E_m$ to decrease from 78 to 77 mV. After the effect of dihydroouabain on $a^i_{Na}$ and $E_m$ was stable, the muscle was again superfused with drug-free solution, and $a^i_{Na}$ and $E_m$ returned to their predrug control level. Subsequent superfusion of the atrium with 30 μM dihydroouabain resulted in an increase in $a^i_{Na}$ from 8·1 to 11·2 mM and in a decrease of $E_m$ from 78 to 77 mV. Reversal of $a^i_{Na}$ and $E_m$ during wash-out was complete within 10 min. In contrast to atrium, no detectable changes in $a^i_{Na}$ and $E_m$ were observed during exposure of the papillary muscle to 10 μM dihydroouabain. The threshold concentration of dihydroouabain required to produce an effect was 30 μM, as indicated by the small increase in $a^i_{Na}$ from 4·5 to 5·2 mM (Fig. 1*B*). The decrease in $E_m$, however, from −84 to −83 mV, did not differ from effects observed with 10

and 30 $\mu$M dihydroouabain in resting atrial preparations. Figure 2 shows the relationship between the change in $a^i_{Na}$ ($\Delta a^i_{Na}$) and the dihydroouabain concentration in atrial (○) and ventricular (●) myocardium. The $a^i_{Na}$ change at each concentration was measured as shown in Fig. 1 and represented the steady-state effect of the drug.

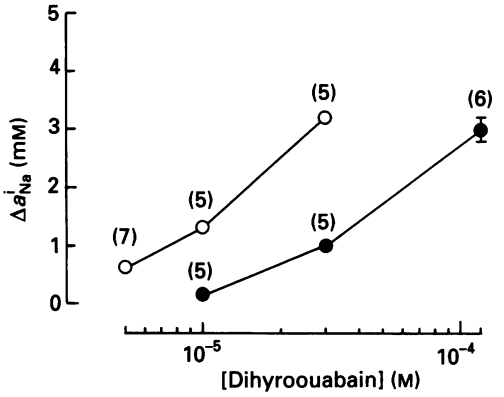


Fig. 2. Concentration-dependent effects of dihydroouabain on intracellular $Na^+$ activity ($a^i_{Na}$) of guinea-pig atrial (○) and ventricular myocardium (●). The number of preparations is given in parentheses. Note that atrial and ventricular preparations exposed to 10 and 30 $\mu$M dihydroouabain were obtained from the same heart, respectively. Symbols represent arithmetic means ± S.E.M. shown as vertical bars if exceeding the size of the symbols. Ordinate scale: increase in $a^i_{Na}$ over its basal value.

In a total of seventeen atrial preparations, dihydroouabain increased $a^i_{Na}$ from 7·8±0·3 to 8·4±0·3 mM at 5 $\mu$M ($n = 7$), from 8·2±0·2 to 9·5±0·2 mM at 10 $\mu$M ($n = 5$) and from 8·5±0·4 to 11·7±0·5 mM at 30 $\mu$M ($n = 5$). In five papillary muscles exposed to 10 $\mu$M dihydroouabain, no significant increase in $a^i_{Na}$ was observed. Increasing the concentration of dihydroouabain to 30 and 120 $\mu$M produced a rise in $a^i_{Na}$ from 5·0±0·4 to 6·0±0·4 mM ($n = 5$) and from 4·7±0·6 to 7·6±0·6 mM ($n = 6$), respectively. The results clearly indicate a higher potency of dihydroouabain to raise $a^i_{Na}$ in atrial than in ventricular myocardium.

*Effect of dihydroouabain on force of contraction*

Simultaneous measurements of force of contraction and $a^i_{Na}$ from the same muscle would be desirable to study the precise relation between changes in force and intracellular ion activity. However, such simultaneous measurements are technically difficult, especially in the presence of cardioactive steroids. Therefore, force of contraction was measured separately from $a^i_{Na}$ in different muscles. Figure 3 shows concentration–effect curves for the positive inotropic effect of dihydroouabain in six atrial preparations (○) and in eleven papillary muscles (●). Contraction frequency was consistently 0·2 Hz. As shown in Fig. 3, dihydroouabain had a higher inotropic potency in atrial than in ventricular myocardium. The lowest effective concentration of dihydroouabain was 3 $\mu$M in atrial preparations while 30 $\mu$M dihydroouabain was necessary to produce a similar increase in force of contraction in papillary muscles. Half-maximally effective concentrations, log $EC_{50}$, were −4·9±0·1 and −4·2±0·2 in the atrial and ventricular myocardium, respectively.

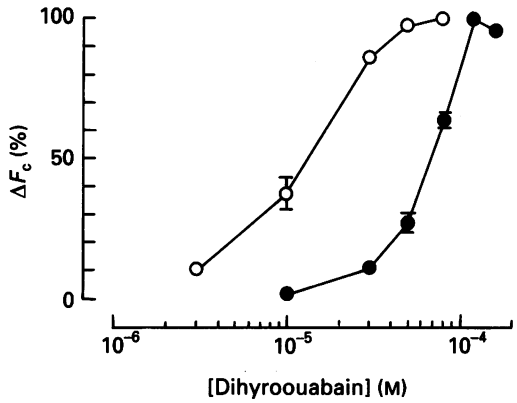


Fig. 3. Concentration-dependent positive inotropic effects of dihydroouabain in guinea-pig atrial trabeculae (○) and papillary muscles (●). Arithmetic means ± S.E.M. of 6 (○) and 11 (●) muscles are shown. Ordinate scale: positive inotropic effect, $\Delta F_c$, expressed as a percentage of the respective maximal effect of dihydroouabain ($\Delta F_c$ 100% = 11·3 ± 3·9 mN (○) and 23·1 ± 2·7 mN (●). Pre-drug control force was 5·2 ± 1·9 mN (○) and 3·6 ± 0·9 mN (●)). Contraction frequency 0·2 Hz.

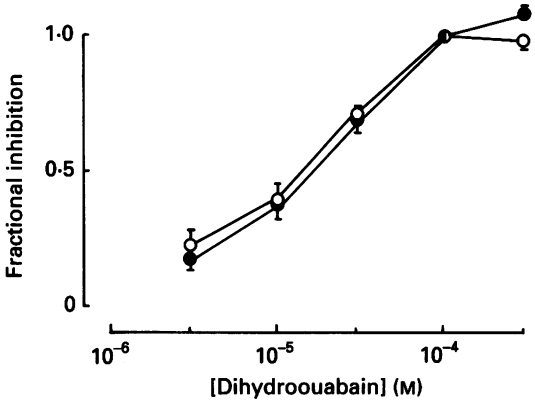


Fig. 4. The inhibitory potency of dihydroouabain on $Na^+,K^+$-ATPase in enzyme preparations from guinea-pig atria (○) and ventricles (●). Steady-state inhibition (ordinate scale: fractional inhibition) of different concentrations of dihydroouabain is shown. Symbols represent arithmetic means ± S.E.M. of 5 to 8 preparations. The inhibition of 100 $\mu$M dihydroouabain was set to 1. In the preparations from ventricles 68 and from atria 54% of total ATPase activity was maximally inhibited by dihydroouabain. The solution contained (mM): $Na^+$, 145; $K^+$, 5·9; ATP, 3; EGTA, 0·5; $NaN_3$, 5; 35 °C.

### *$Na^+,K^+$-ATPase activity and its inhibition by dihydroouabain*

Different activity of the $Na^+$ pump and an altered susceptibility to pump inhibition by dihydroouabain could have contributed to the observed difference in $Na^+$ homeostasis of atria and ventricles. We therefore studied $Na^+,K^+$-ATPase activity in both tissues. While the inhibitory potency of dihydroouabain on $Na^+,K^+$-ATPase was identical in preparations from either tissue (Fig. 4), the dihydroouabain (300 $\mu$mol $l^{-1}$)-sensitive fraction of total ATPase activity of ventricles was sig-

nificantly ($P < 0.001$) greater than observed for atria irrespective of a reference to protein content or tissue wet weight. Mean values of five ventricular and of six atrial enzyme assays amounted to $0.21 \pm 0.03$ ($0.20 \pm 0.01$) and $0.07 \pm 0.01$ ($0.07 \pm 0.01$) $\mu$mol phosphate released $min^{-1}$ and (g tissue)$^{-1}$, respectively (values in parentheses refer to $\mu$mol phosphate released $min^{-1}$ (mg protein)$^{-1}$. The result was independent whether the atrial or ventricular enzyme was isolated from tissues of the same or of different animals.

## DISCUSSION

The mean resting value of 8·0 mM ($n = 30$ preparations) for $a^{i}_{Na}$ in guinea-pig atria obtained in the present study was substantially higher than in ventricular cells of twenty-two papillary muscles (4·7 mM). The significance of the difference between both tissues ($P < 0.01$) was also clearly established when sequential measurements were carried out with identical ion-sensitive microelectrodes in tissues obtained from the same heart (Table 1). The resting values of $a^{i}_{Na}$ found in guinea-pig papillary muscle are close to those previously reported in the same type of preparation and under similar experimental conditions: 5·8 mM (Cohen *et al.* 1982) and 4·0 mM (Wang *et al.* 1988). In contrast to ventricular tissue, measurements of $a^{i}_{Na}$ in mammalian atria have not yet been described.

Two explanations can be given for the higher values of $a^{i}_{Na}$ in guinea-pig atrial as compared to ventricular cells: (1) the $Na^+$ elimination from atrial cells via the $Na^+$ pump is less effective and/or (2) the resting $Na^+$ influx into atrial cells is much higher. The $Na^+$ pump has been identified as a membrane-bound ATP-hydrolysing enzyme system, which pumps $Na^+$ out of and $K^+$ into the cell, thereby generating an electrochemical gradient that is essential in maintaining membrane potential and electrical activity of excitable cells (Schwartz, Lindenmayer & Allen, 1975). Three isozymes of the catalytic $\alpha$-subunit of $Na^+,K^+$-ATPase have been cloned and sequenced (Shull, Greeb & Lingrel, 1986), and marked differences in their affinities for cardiac glycosides have been observed (Sweadner, 1989). Expression of isozymes varies among species and tissues and may even be subject to changes during development (Sweadner & Farshi, 1987; Ng & Akera, 1987*b*) or hormonal stimulation (Ng, Yao & Akera, 1989; Orlowski & Lingrel, 1990). In heart muscle, two isozymes exhibiting different affinities for cardioactive steroids have been identified. Some species appear to have nearly equivalent amounts of the two isozymes while others have predominantly one form (Ng & Akera, 1987*a*; Sweadner & Farshi, 1988). Recently, the two isozymes of $Na^+,K^+$-ATPase have been identified with cDNA probes in the rat heart and were found to be differently distributed in atrium and ventricle (Young & Lingrel, 1987). In guinea-pig heart, however, the distribution of isozymes seems to be less well defined (Sweadner, 1989). Since dihydroouabain exhibited a higher potency in increasing $a^{i}_{Na}$ and force of contraction in atrial than in ventricular myocardium, a high affinity isozyme of $Na^+,K^+$-ATPase could have prevailed in this type of tissue. On the other hand, the higher level of $a^{i}_{Na}$ in atrial cells could have enhanced the turnover rate of the $Na^+$ pump and thus increased the apparent affinity of $Na^+,K^+$-ATPase for the cardioactive steroid (Herzig & Mohr, 1985; Stimers, Lobaugh, Liu, Shigeto & Lieberman, 1990). Our measurement of $Na^+,K^+$-ATPase activity in preparations from guinea-pig ventricles and atria showed

that the inhibitory potency of dihydroouabain on $Na^+,K^+$-ATPase was identical in both tissues (Fig. 4). This finding clearly excludes the possibility that two functionally different types of $Na^+,K^+$-ATPase are responsible for the tissue-specific sensitivities for dihydroouabain. A plausible explanation for the higher $a^i_{Na}$ and the

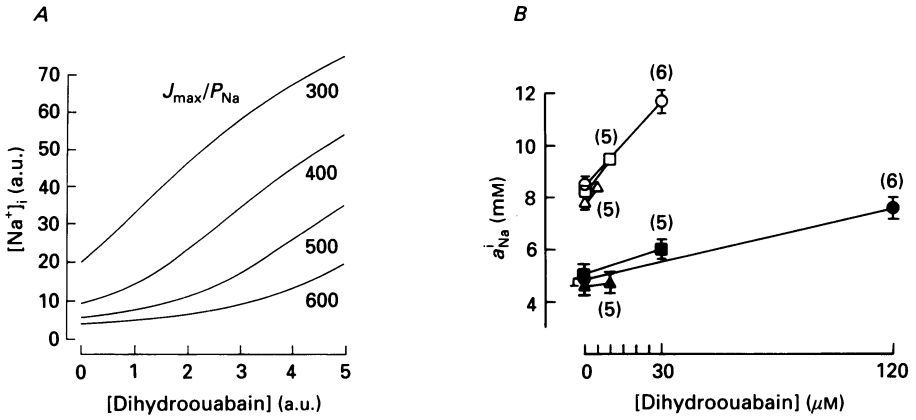


Fig. 5. *A*, intracellular $Na^+$ $[Na^+]_i$ in relation to $Na^+$ pump inhibition. Model calculations. The $Na^+$ influx into the cell, driven for simplicity sake by the concentration difference instead of the electrochemical gradient ($J_{in} = P_{Na}S(Na^+_0 - Na^+_i)$) is balanced by the $Na^+$ pump ($J_p = J_{max}S/(1 + K_{Na}/Na^+_i + K_p + K_pK_A A)$), where $K_{Na}$ reflects the affinity of $Na^+_i$ to the $Na^+$ pump, $K_p$ the equilibrium constant governing the transition between the conformational states of the enzyme $E_1$ and $E_2$ facing the intra- and extracellular space, respectively, $K_A$ the affinity of the pump inhibitor A to $E_2$, and $S$ the surface area of the cell, the other symbols are self-explanatory. $J_p$ results from a model where $Na^+_i$ combines with the inward facing conformation of the enzyme $E_1$, which after transition to $E_2$, reacts with the inhibitor A. $J_p$ is assumed to be proportional to the $E_1Na^+_i$ complex. For different degrees of pump inhibition as affected by dihydroouabain (abscissa scale), $[Na^+]_i$ (ordinate scale) was calculated at different values of $J_{max}/P_{Na}$; $K_{Na}$ was 10, $K_p$ 1, $K_A$ 1; all units are arbitrary (a.u.). *B*, intracellular $Na^+$ activity ($a^i_{Na}$) in relation to $Na^+$ pump inhibition by different concentrations of dihydroouabain in guinea-pig atrial (open symbols) and ventricular preparations (filled symbols). The number of preparations is given in parentheses. Symbols represent arithmetic means ± S.E.M. Ordinate scale: $a^i_{Na}$ in mM; abscissa scale: dihydroouabain in μM (linear scale).

enhanced potency of dihydroouabain in atrial cells therefore refers to the $Na^+$ pump density per unit plasma membrane which we found in atrial cells to be one third of that in ventricular cells. In addition, quantification of the total $Na^+,K^+$-ATPase concentration in atria and ventricles from porcine and canine hearts by measuring [$^3$H]ouabain binding to intact myocardial preparations, revealed that the concentration of binding sites was about 2–3 times lower in atria than in ventricles (Schmidt *et al.* 1990). The consequence of such a different $Na^+$ pump distribution on intracellular $Na^+$ can be deduced from model calculations shown in Fig. 5*A*. In ventricular cells, and probably in many other cells, the $Na^+$ pump may be regarded to have a high reserve capacity under normal physiological conditions (Akera & Ng, 1991). This condition is expressed in Fig. 5*A* as a high numerical value for $J_{max}/P_{Na}$ (maximal $Na^+$ efflux/$Na^+$ permeability). Moderate inhibition of the pump by a

cardioactive steroid produces only a marginal increase in $a^i_{Na}$ because the activity of the remaining pump units matches the rate of $Na^+$ influx. Only when the degree of the pump inhibition is large enough, a rise in $a^i_{Na}$ occurs as a result of a decreased reserve capacity of the pump. However, when the number of $Na^+$,$K^+$-ATPase molecules in the sarcolemmal membrane is reduced as might be the case in atrial cells (shown as decreasing $J_{max}/P_{Na}$ values in Fig. 5*A*), resting $a^i_{Na}$ becomes elevated and moderate inhibition of the pump results in a significant increase in $a^i_{Na}$. Furthermore, the model predicts that the relationship between $Na^+$ pump inhibition and $a^i_{Na}$ becomes progressively steeper when $J_{max}/P_{Na}$ decreases. As shown in Fig. 5*B*, the experimental data are in accordance with the theoretical predictions and support the idea that the ratio of maximum $Na^+$ extrusion capacity to $Na^+$ influx is smaller in atrial than in ventricular muscle, consistent with a lower pump-site density in atrium. Since the model shown in Fig. 5 cannot discriminate between reduced $Na^+$ efflux capacity and/or increased $Na^+$ influx, a contribution of the latter to the higher $a^i_{Na}$ level in atrial cells cannot completely be excluded. An elevated $a^i_{Na}$ due to an enhanced $Na^+$ influx, however, should markedly stimulate the $Na^+$ pump and hence increase intracellular $K^+$ activity. The opposite was found: $a^i_K$ was significantly lower in atrial than in ventricular cells (see Table 1), a finding which is compatible with a reduced rather than with an enhanced $Na^+$ pump activity. It should be emphasized, however, that we do not have direct experimental evidence to exclude the possibility that resting $Na^+$ influx may be larger in atrium than in ventricle. The role of a geometric factor, namely different surface:volume ratios in atrial and ventricular cells, on steady-state $a^i_{Na}$ can be ruled out on the basis of the equations presented in the legend to Fig. 5. Since both, $Na^+$ influx and $Na^+$ efflux are proportional to the surface area of the cell, this factor cancels out under equilibrium conditions and does not need to be considered any further, although the approach to pre-steady state may have been influenced by cell geometry. Although we did not measure $a^i_{Na}$ in contracting preparations, the higher inotropic potency of dihydroouabain in atria indicates that the difference between both tissues is still preserved during repetitive activity.

In accordance with previous studies, $E_m$ was significantly ($P < 0{\cdot}05$) more positive to $E_K$ in atrial (by 6·1 mV) than in ventricular myocardium (by 3·0 mV; see also Lee & Fozzard, 1975; Baumgarten *et al.* 1984). This large deviation of $E_m$ from $E_K$ in atrial cells seems to favour the modulatory influence of acetylcholine on $E_m$ by its influence on membrane $K^+$ permeability, $P_K$ (Trautwein & Dudel, 1958). A rather simple explanation for the depolarized $E_m$ in atrial cells might be a higher $P_{Na}/P_K$ ratio in this type of tissue, but in light of the present results, a reduced $Na^+$ pump density which produces less outward current could also contribute to the lower $E_m$ (Gadsby, 1984).

The physiological significance of the relatively high $a^i_{Na}$ in atrial cells remains unclear. By reducing the driving force for $Ca^{2+}$ extrusion via $Na^+$–$Ca^{2+}$ exchange and by competing less effectively with the sarcoplasmic reticulum $Ca^{2+}$ pump, a high $a^i_{Na}$ level ( and a low diastolic $E_m$) could contribute to the higher $Ca^{2+}$ loading of the atrial as compared to the ventricular myocardium (Fukuda, 1975). Such an arrangement may be essential for atrial cells which have to generate force very rapidly because atrial contraction time is strongly limited by the following ventricular contraction.

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## REFERENCES

AKERA, T. & NG, Y.-C. (1991). Digitalis sensitivity of $Na^+,K^+$-ATPase, myocytes and the heart. *Life Sciences* **48**, 97–106.

BALS, S., BECHEM, M., PAFFHAUSEN, W. & POTT, L. (1990). Spontaneous and experimentally evoked $[Ca^{2+}]_i$-transients in cardiac myocytes measured by means of a fast Fura-2 technique. *Cell Calcium* **11**, 385–396.

BAUMGARTEN, C. M., SINGER, D. H. & FOZZARD, H. A. (1984). Intra- and extracellular potassium activities, acetylcholine and resting potential in guinea pig atria. *Circulation Research* **54**, 65–73.

BEUCKELMANN, D. J. & WIER, W. G. (1988). Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. *Journal of Physiology* **405**, 233–255.

BRILL, D. M. & WASSERSTROM, J. A. (1986). Intracellular sodium and the positive inotropic effect of veratridine and cardiac glycoside in sheep Purkinje fibers. *Circulation Research* **58**, 109–119.

COHEN, C. J., FOZZARD, H. A. & SHEU, S.-S. (1982). Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circulation Research* **50**, 651–662.

DAGOSTINO, M. & LEE, C. O. (1982). Neutral carrier $Na^+$–$Ca^{2+}$-selective microelectrodes for intracellular application. *Biophysical Journal* **40**, 199–207.

EBNER, F. (1990). Factors influencing the onset of ouabain inhibition of Na,K-ATPase from guinea-pig myocardium. *British Journal of Pharmacology* **101**, 337–343.

FUKUDA, Y. (1975). Difference in calcium content of atrial and ventricular muscle. *Japanese Journal of Physiology* **25**, 467–479.

GADSBY, D. C. (1984). The Na/K pump of cardiac cells. *Annual Review of Biophysics and Bioengineering* **13**, 373–398.

GRUPP, I., IM, W.-B., LEE, C. O., LEE, S.-W., PECKER, M. S. & SCHWARTZ, A. (1985). Relation of sodium pump inhibition to positive inotropy at low concentrations of ouabain in rat heart muscle. *Journal of Physiology* **360**, 149–160.

HERZIG, S. & MOHR, K. (1985). Sodium load and high affinity ouabain binding in rat and guinea-pig cardiac tissue. *British Journal of Pharmacology* **84**, 685–688.

LEE, C. O. (1985). 200 years of digitalis: the emerging central role of the sodium ion in the control of cardiac force. *American Journal of Physiology* **248**, C367–378.

LEE, C. O. & FOZZARD, H. A. (1975). Activities of potassium and sodium ions in rabbit heart muscle. *Journal of General Physiology* **65**, 695–708.

LEE, C. O., KANG, D. D., SOKOL, J. H. & LEE, K. S. (1980). Relation between intracellular Na ion activity and tension of sheep cardiac Purkinje fibers exposed to dihydro-ouabain. *Biophysical Journal* **29**, 315–330.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

NG, Y.-C. & AKERA, T. (1987*a*). Two classes of ouabain binding sites in ferret heart and two forms of $Na^+$-$K^+$-ATPase. *American Journal of Physiology* **252**, H1016–1022.

NG, Y.-C. & AKERA, T. (1987*b*). Relative abundance of two molecular forms of $Na^+,K^+$-ATPase in the ferret heart: developmental changes and associated alterations of digitalis sensitivity. *Molecular Pharmacology* **32**, 201–205.

NG, Y.-C., YAO, A.-Z. & AKERA, T. (1989). Tissue-specific isoform regulation of $Na^+$-$K^+$-ATPase by thyroid hormone in ferrets. *American Journal of Physiology* **257**, H534–539.

ORLOWSKI, J. & LINGREL, J. B. (1990). Thyroid and glucocorticoid hormones regulate the expression of multiple Na,K-ATPase genes in cultured neonatal rat cardiac myocytes. *Journal of Biological Chemistry* **265**, 3462–3470.

POOLE-WILSON, P. A. & CAMERON, I. R. (1975). ECS, intracellular pH, and electrolytes, of cardiac and skeletal muscle. *American Journal of Physiology* **229**, 1299–1304.

REITER, M. (1967). Die Wertbestimmung inotrop wirkender Arzneimittel am isolierten Papillarmuskel. *Arzneimittelforschung* **17**, 1249–1253.

SCHMIDT, T. A., SVENDSEN, J. H., HAUNSØ, S. & KJELDSEN, K. (1990). Quantification of the total Na,K-ATPase concentration in atria and ventricles from mammalian species by measuring $^3$H-ouabain binding to intact myocardial samples. Stability to short term ischemia reperfusion. *Basic Research in Cardiology* **85**, 411–427.

SCHMIED, R., WANG, G.-X. & KORTH, M. (1991). Intracellular $Na^+$ activity and positive inotropic effect of sulmazole in guinea pig ventricular myocardium. *Circulation Research* **68**, 597–609.

SCHWARTZ, A., LINDENMAYER, G. E. & ALLEN, J. C. (1975). The sodium–potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacological Reviews* **27**, 3–134.

SHEU, S.-S. & FOZZARD, H. A. (1982). Transmembrane $Na^+$ and $Ca^{2+}$ electrochemical gradients in cardiac muscle and their relationship to force development. *Journal of General Physiology* **80**, 325–351.

SHULL, G. E., GREEB, J. & LINGREL, J. B. (1986). Molecular cloning of three distinct forms of the $Na^+$,$K^+$-ATPase $\alpha$-subunit from rat brain. *Biochemistry* **25**, 8125–8132.

STEINER, R. A., OEHME, D., AMMANN, D. & SIMON, W. (1979). Neutral carrier sodium ion-sensitive microelectrode for intracellular studies. *Analytical Chemistry* **51**, 351–353.

STIMERS, J. R., LOBAUGH, L. A., LIU, S., SHIGETO, N. & LIEBERMAN, M. (1990). Intracellular sodium affects ouabain interaction with the Na/K pump in cultured chick cardiac myocytes. *Journal of General Physiology* **95**, 77–95.

SWEADNER, K. J. (1989). Isozymes of the $Na^+/K^+$-ATPase. *Biochimica et Biophysica Acta* **988**, 185–220.

SWEADNER, K. J. & FARSHI, S. K. (1987). Rat cardiac ventricle has two $Na^+$,$K^+$-ATPases with different affinities for ouabain: developmental changes in immunologically different catalytic subunits. *Proceedings of the National Academy of Sciences of the USA* **84**, 8404–8407.

SWEADNER, K. J. & FARSHI, S. K. (1988). Two forms of the Na,K-ATPase catalytic subunit detected by their immunological and electrophoretic differences. Alpha and alpha+ in mammalian heart. *Progress in Clinical and Biological Research* **268B**, 149–156.

TRAUTWEIN, W. & DUDEL, J. (1958). Zum Mechanismus der Membranwirkung des Acetylcholin an der Herzmuskelfaser. *Pflügers Archiv* **266**, 324–334.

WANG, D. Y., CHAE, S. W., GONG, Q. Y. & LEE, C. O. (1988). Role of $a^i_{Na}$ in positive force–frequency staircase in guinea pig papillary muscle. *American Journal of Physiology* **255**, C798–807.

YOUNG, R. M. & LINGREL, J. B. (1987). Tissue distribution of mRNAs encoding the $\alpha$ isoforms and $\beta$ subunit of rat $Na^+$,$K^+$-ATPase. *Biochemical and Biophysical Research Communications* **145**, 52–58.