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INTERDEPENDENCE OF ION TRANSPORT AND THE ACTION OF OUABAIN IN HEART MUSCLE

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1 The influence of ouabain (0.4 μM) on contractile force and cellular Na and K concentrations was investigated in isolated left atria of the guinea-pig at rest and at different beat frequencies. Simultaneously the binding of ouabain to the tissue was determined.

2 Strict dependence of rates of onset of positive iontropic action and of binding of ouabain on beat frequency are limited to conditions where no alterations of cellular Na and K concentrations occur. A correlation was observed between sodium flux per unit time and the development of positive inotropism and binding to the receptors of ouabain.

3 Ouabain exerts its positive inotropic effect without affecting the intracellular Na and K concentrations in spite of the fact that under these conditions even the majority of binding sites, i.e. Na-K-adenosine triphosphatases (Na-K-ATPases), are occupied by the drug. The positive inotropic effect may be explained by a ouabain-induced conformational alteration of the Na-K-ATPase which leads to structural alterations of the plasmalemma connected with an increased availability of coupling calcium.

4 Increasing the frequency of stimulation over a critical value, which appears to be determined by an overloading of the Na pump, induces a decrease in contractile force, cellular accumulation of Na and loss of K, and eventually contracture.

5 The rate of binding of ouabain appears to depend on the actual concentration of particular conformations of the Na-K-ATPase with high affinity for ouabain. These conformations transiently occur during a pumping cycle and their concentration may therefore be dependent on the frequency of cycling which in turn is determined by the frequency of contraction.

6 Ouabain can easily be washed out from the tissue irrespective of the condition of the muscle. If, however, the intracellular Na and K homeostasis is impaired, the inhibition of the pump persists even if ouabain is released from the binding sites upon wash-out. It is suggested that the inhibition of the pump is maintained by an increased intracellular Ca ion concentration and a depletion of ATP.

7 A kinetic model is proposed for the interaction between cardiac glycosides and the Na-K-ATPase in intact heart muscle cells.

Introduction

The dependence of the actions of cardiac glycosides on frequency of contraction of heart muscle is still a matter of discussion. The dependence of rate of onset of cardiac glycoside-induced intoxication upon beat frequency is well known and repeatedly documented (Weizsäcker, 1913; Holland & Sekul, 1961). Weizsäcker (1913) stated that the number of contractions rather than the time of incubation determine the onset of toxic effects after addition of ouabain in frog ventricular muscle. However, cardiac glycosides are not completely inactive in the resting heart and will produce contracture (Weizsäcker, 1913). Yet, contractile activity considerably enhances the onset of toxic signs. While the majority of investigators agree on a beat-dependent onset of toxicity (Weizsäcker, 1913; Holland & Sekul, 1961; Gersmeyer & Holland, 1963) conflicting results are obtained with respect to a frequency-dependent rate of development of the cardiac glycoside-induced positive inotropism.

In general, at bath temperatures below 35°C, heart muscles of different species develop increased contractile force upon administration of cardiac glycosides in a strictly beat-dependent way (Wilbrandt, Brawand & Witt, 1953; Sanyal & Saunders, 1958; Gersmeyer & Holland, 1963; Holland, 1964; Moran, 1963; 1967; Byrne & Dresel, 1969). At a bath temperature of 35°C there still exists some beat-dependence at the higher frequencies of contraction and an obvious dependence upon time of

incubation at lower frequencies (Vinzenci, 1967). If heart muscle is incubated at temperatures above 35°C the development of the positive inotropic effect becomes mainly time-dependent, though a certain influence of frequency remains involved (Garb & Penna, 1957; Koch-Weser & Blinks, 1962; Koch-Weser, 1962; 1971).

One of the basic actions of cardiac glycosides is to shorten the duration of the action potential concomitantly with the induction of enhanced contractile force. Recently it was demonstrated by Brinkmann & Ravens (1976) that shortening of the action potential below a critical duration will counteract the development of the positive inotropic effect. If both actions of cardiac glycosides, shortening of the action potential duration and positive inotropism, are initiated via one common mechanism, then the time course of development of the positive inotropic effect may be altered by the concomitantly occurring electrophysiological changes. Calculations of the respective half lives may be false and a correlation between frequency of contraction and time course of development of positive inotropism may be obscured. Both high temperature and high beat frequency *per se* tend to shorten the duration of the action potential (Shanes, 1958), and upon addition of cardiac glycosides the critical shortening will be attained earlier. In consequence one may speculate that it is only at low temperatures and within a limited range of beat frequency that the development of positive inotropism can be correlated to the frequency of contraction. From the foregoing it is understandable that several investigators sought to gain more insight into the relation between beat frequency and molecular mode of action of cardiac glycosides by means of binding studies which can be considered to be a more direct approach (Weizsäcker, 1913; Roth-Schechter, Anderson & Richardson, 1970; Deutscher, Harrison & Goldman, 1972). Unfortunately the experiments were carried out only with hydrophobic cardiac glycosides which are predominantly taken up by cardiac tissue in a nonspecific way, i.e. time course of uptake gives no information on the time course of binding of the drugs to a specific saturable compartment which may be linked to the inotropic effect (Kuschinsky, Lahrtz, Lüllmann & van Zwieten, 1967; Lüllmann, Peters & Ravens, 1975a). Accordingly, no correlation between uptake and frequency of contraction could be observed.

For the reasons outlined above, the present investigation was carried out at a comparatively low temperature (32°C) and with ouabain which, at the concentration applied, is taken up nonspecifically by heart muscle to a minor extent, the major amount being bound specifically to a saturable binding compartment (Godfraind & Lesne, 1972; Lüllmann *et al.*, 1975a). The purpose of the study was to gain further information concerning (1) the influence of frequency of contraction on the binding rate of ouabain, (2) the possible correlation between binding and the positive inotropic action of ouabain, and (3) the interdependence between transmembrane excitation sodium fluxes, Na- and K-transport, adenosine triphosphatase (ATPase)-activity and the processes of binding and positive inotropic action of ouabain.

In order to investigate the problems outlined, mechanical activity, Na- and K-content, and the binding of ouabain were determined at different frequencies in isolated left auricles of the guinea-pig. A ouabain concentration was chosen to provide a pure inotropic response at low frequencies. Based upon the results obtained, a hypothetical model will be discussed which involves the activity of the cardiac glycoside receptor (the Na-K-activated ATPase) in frequency-dependent actions of cardiac glycoside on heart muscle.

Some of these results were presented to the German Pharmacological Society (Bentfeld, Peters & Schreiner, 1976).

Methods

Mechanograms of left auricles isolated from guinea-pigs weighing about 300 g were recorded isometrically by means of a strain gauge; the auricles were preloaded with 0.5 g, and electrically stimulated by rectangular pulses of 4 ms duration, 3–6 V, and with frequencies as indicated below. The Tyrode solution (mM: NaCl 135, KCl 2.7, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.21 and glucose 5.5) was aerated by a mixture of 95% O_2 and 5% CO_2; the temperature was kept at 32°C.

Sodium and potassium were determined by flame photometry. The tissue samples were divided in two parts, one of which was used for [^{3}H]-ouabain measurement (see below) the other for determination of the ions. After different incubation periods the auricles were removed, blotted by a standard procedure (Lüllmann & van Zwieten, 1967) ashed to dryness in 1 ml of a mixture of equal amounts of HNO_3 (65%) and $HClO_4$ (60%), and re-dissolved in 0.1N HCl (Zepf, 1966). For each set of experiments a calibration curve was established and the Na and K backgrounds were separately determined. Each muscle sample was subjected to a double determination. The size of the extracellular space (ECS) was taken from earlier experiments performed under identical conditions and amounted to 0.3 ml/g wet wt. in non-intoxicated and 0.26 ml/g wet wt. in contracted muscles (Lüllmann & van Zwieten, 1967). The cellular Na and K contents were calculated by subtracting the respective extracellular amounts from the total tissue contents, and are expressed as mmol/kg cell.

Ouabain binding was measured in the second part

of each auricle by means of tritiated ouabain (sp. act. 10 Ci/mmol, NEN, Chicago). After blotting, the samples were dissolved in Soluene (Packard Instr.), and subjected to liquid scintillation counting. The concentration of [^{3}H]-ouabain determined in the tissue (corrected for by the amount present in the ECS) relative to medium concentration was expressed as tissue medium ratio (T/M ratio = concentration per g cell divided by concentration per ml of Tyrode solution).

Results

Effects on contractile force and binding of ouabain at different beat frequencies

The action of ouabain was investigated at stimulation frequencies, of 0, 1, 2, 4, and 5 Hz. The ouabain concentration of 0.4 μM proved to act purely inotropically at 1 Hz, but induced toxicity at the higher frequencies; the onset of both actions was accelerated with increasing frequencies. For any investigated beat frequency the mechanograms were recorded and the cellular Na and K content as well as ouabain binding were determined for time periods up to 270 minutes.

In the resting state the Na and K content of the auricles remained unchanged for the entire period of observation, as shown in Figure 1. The figure also depicts rate and amount of ouabain binding. After 270 min, the T/M ratio attained a value of about 1.4, but had not yet reached the final equilibrium level. Under resting conditions, the binding of ouabain proceeded comparatively slowly but nevertheless the glycoside was accumulated.

At 1 Hz, ouabain almost doubled the contractile force. The maximum inotropic response was reached within approximately 90 min (Figure 1). The ion content of the muscles remained unaltered by ouabain throughout the entire incubation period as compared

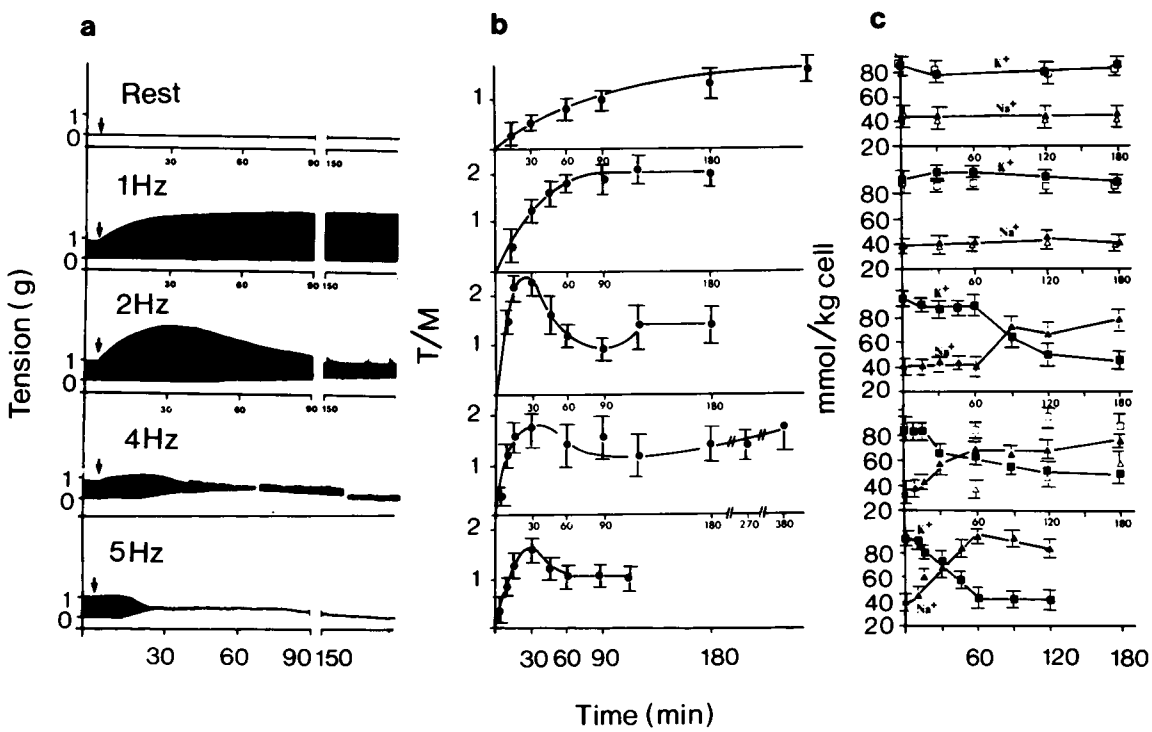

Figure 1 Influence of frequency of contraction on the effect of ouabain on contractile force, cellular Na and K concentration and binding of the drug to isolated left auricles of guinea-pig. (a) Ordinate scale: contractile tension (g); (b) ordinate scale: T/M-ratio (= radioactive ouabain per g tissue divided by radioactive ouabain per ml Tyrode solution); (c) ordinate scale: Na and K (mmol per kg cell). In (a), (b) and (c) abscissa scale: time (min). Open symbols = controls; solid symbols = in the presence of ouabain. Squares: cellular K; triangles: cellular Na. Reading from above, downwards the frequencies are: 1, 2, 4 and 5 Hz. Note that the abscissa scale is different for the recordings of mechanical activities. Points on the curves are mean values of at least 8 individual experiments. Vertical lines show s.e. means.

to the untreated control muscles. A small but significant increase in the K content could be observed between 30 and 120 min when the heart muscle developed its maximum contractile force. As also demonstrated in Figure 1, the ouabain binding reached a plateau after about 120 min at a T/M ratio of 2.2. In comparison to the resting muscle the rate of binding was considerably faster and the amount of ouabain bound was higher.

At 2 Hz, ouabain at the same concentration produced a transient positive inotropic effect with a maximum increase of about 100% after 30 min of incubation. Thereafter the muscles displayed toxic signs, i.e. decay of maximum systolic tension, arrhythmia, and contracture (Figure 1). The cellular Na and K content remained constant for 60 min in spite of the continuous presence of ouabain. Then a progressive loss of K and a gain of Na could be observed which coincided with the occurrence of toxic signs (Figure 1). The ouabain binding proceeded at an even higher rate as compared to that at 1 Hz. The maximum binding (T/M of 2.3) was already attained within about 30 min of exposure to ouabain. But in contrast to the preceding experimental condition, the binding of ouabain at the higher frequency was not maintained but fell within 60 min to a T/M value of about 0.8, in spite of the continued presence of ouabain. Thereafter, the binding of ouabain slowly increased again (Figure 1). The reduction of the T/M values which indicates a release of previously bound ouabain, corresponded to the onset of toxicity.

At a beat frequency of 4 Hz, ouabain at a concentration of 0.4 μM, evoked only a small (about 15%) transient positive inotropic effect within 15 min (Figure 1). This response was followed by intoxication. During the short phase of increased contractile force, the Na and K content remained unaffected. Immediately upon the onset of intoxication, the auricles started to lose K and to gain Na. Initially, loss of K and gain of Na proceeded rapidly, but progressively slowed and final values for the cellular ion concentrations were approached between 180 and 270 min (Figure 1). Ouabain was bound at a rate similar to that observed at 2 Hz, the maximum T/M value was, however, reduced to 1.8, and subsequently declined to about 1.0 in the continued presence of ouabain. Thereafter, the T/M ratios slowly increased with time, reaching finally a value of 1.4 which is identical with the value obtained under resting conditions after the same time of exposure to ouabain.

At a beat frequency of 5 Hz, 0.4 μM ouabain no longer had a positive inotropic action, but immediately upon addition induced intoxication (Figure 1). Simultaneously the cellular Na content increased and K was lost. Both processes attained final equilibria for the Na and K distribution within 60 min of exposure of the auricles to the drug (Figure 1). The ouabain binding curve was quite similar to that obtained at 4 Hz but the peak value was found to be considerably lower. The subsequent decline of the T/M ratios reached its minimum also after about 60 min of incubation with ouabain (Figure 1).

Reversibility of binding and effects of ouabain

To test the reversibility of binding and the effects of ouabain, the auricles were incubated for 120 min with [^{3}H]-ouabain at a concentration of 4×10^{-7} M at three different frequencies. Thereafter the organs were transferred to a drug-free Tyrode solution. Contractile parameters, cellular Na and K content and T/M ratios (M is now concentration of [^{3}H]-ouabain in the Tyrode solution before the wash-out phase) were determined for time periods up to 180 minutes. The frequencies 0, 1 and 4 Hz were chosen since they provided three different situations as far as actions and binding of ouabain are concerned. As demonstrated in Figure 2, similarly shaped wash-out curves for ouabain were obtained in resting, strongly contracting and contractured auricles, although the amounts of ouabain bound before the beginning of the wash-out experiments were different under the three conditions studied (see uptake experiments and legend to Figure 2). Upon wash-out the tissue levels of ouabain declined to half their values in about 12 min, independent of the condition, and reached background values after about 100 minutes. The mechanograms in Figure 2 demonstrate that the inotropic response declined at a similar rate whereas the contractured muscle remained intoxicated although ouabain had been completely removed. In the latter case cellular loss of K and gain of Na proceeded irrespective of the release of ouabain from the tissue: at the beginning of the wash-out period the cellular contents of Na and K were 70 and 55 mmol/kg respectively. At the end of the 2 h wash-out period the respective values amounted to 100 mmol/kg for Na and 40 mmol/kg for K. In resting muscles and in muscles beating at 1 Hz no changes for the cellular ion concentrations could be observed during the uptake and wash-out periods.

Discussion

In order to find a link between the actions of cardiac glycosides on contractile parameters and on the transmembrane ionic transport system, the aim of the present investigation was to study the influence of frequency of contraction on glycoside binding to atrial tissue, and simultaneously the effects of cardiac glycosides on contractile force and cellular Na^+ and K^+ concentrations. Conditions were chosen which allowed one particular ouabain concentration, merely by an alteration of the stimulation frequency, to elicit (1) a sustained positive inotropic response, or (2)

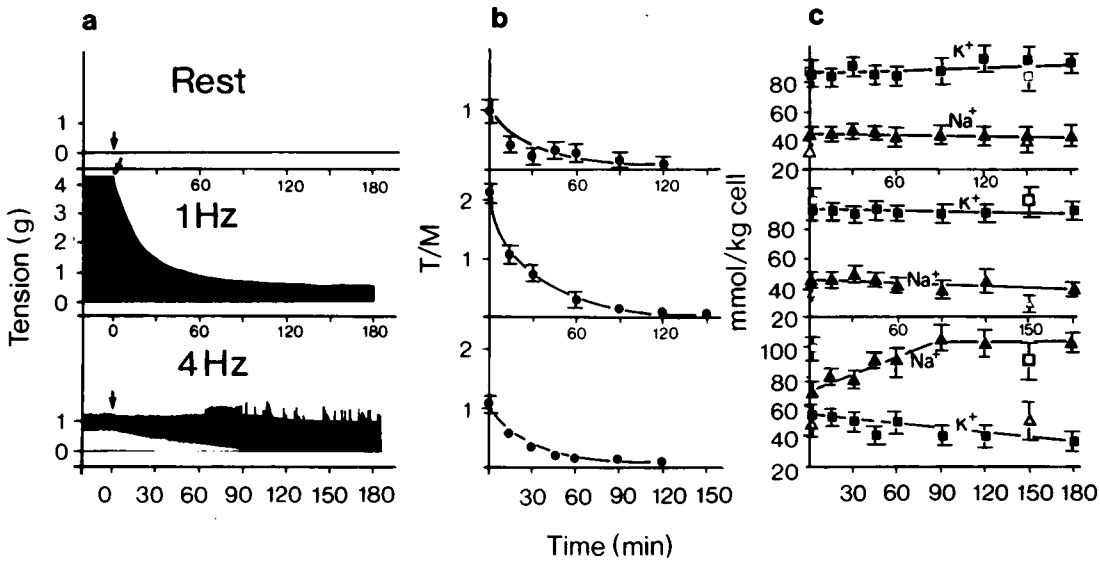

Figure 2 Reversibility of the effects of ouabain on contractile force and cellular Na and K concentrations and release of the drug from the tissue upon wash-out. (a) Ordinate scale: contractile tension (g); (b) ordinate scale: T/M-ratio; (c) ordinate scale: cellular Na and K concentrations. In (a), (b) and (c) abscissa scale: time (min). Arrows indicate beginning of the wash-out of ouabain. Points on the curves represent means of at least 6 individual experiments. Vertical lines show s.e. means.

transitory positive inotropic effects followed by intoxication effects, or (3) exclusively toxic effects.

As can be seen in Figure 1, the mechanical responses obtained upon addition of ouabain were frequency-dependent. The time to maximum positive inotropic effect was about 60 min at a frequency of 1 Hz, 30 min at 2 Hz, and 15 min at 4 Hz, corresponding to about 3600 beats for these three conditions. This strong correlation between number of beats and time to positive inotropic maximum agrees well with the data of Wilbrandt *et al.* (1953), Sanyal & Saunders (1958), Gersmeyer & Holland (1963), Holland (1964) and Moran (1967). At 5 Hz the positive inotropic action is almost immediately overcome by toxic effects, and such an effect may also already be involved at 4 Hz. As far as the onset of intoxication is concerned, at 2, 4 and 5 Hz again, a beat-dependence becomes obvious for the negative inotropic phase as well as for the increase in diastolic tension: the former being visible after about 3600, the latter beginning after roughly 5000 beats. A similar beat-dependence for the onset of toxicity has been demonstrated by Weizsäcker (1913), Holland & Sekul (1961) and Gersmeyer & Holland (1963) (for review see Lee & Klaus, 1971).

For the rate of binding of ouabain a frequency-dependence can only be seen at 1 Hz in comparison to 2 Hz: under both conditions the binding maxima are attained after about 3600 beats. Possibly, this may still hold true for 4 Hz, since the T/M ratios at 15 and 30 min are not significantly different from each other (Figure 1). If the binding rates are compared to the influx of sodium per unit of time, the rate of binding at rest should also be included in the comparison. According to Shanes (1958) and Langer (1968) about 0.5 mmol Na/kg enter the cell per min under resting conditions. Each depolarization adds another 0.04 mmol/kg and increases the actual Na concentration by about 0.1%. Thus the following amounts of Na (which will have to be extruded from the cell by the Na-pump) enter the cells: rest 0.008; 1 Hz 0.04; 2 Hz 0.08; 4 Hz 0.16 and 5 Hz 0.20 mmol Na kg^{-1} s^{-1}. In resting muscles the equilibrium of binding of ouabain was attained after about 300 min which corresponds to a Na-flux of 150 mmol/kg cell. At 1, 2, 4 and 5 Hz, this value is attained within about 60, 30, 15 and 12 min, respectively. Except for the highest frequency this correlation agrees with the experimental results suggesting that the binding process might depend upon the Na-flux.

During the development of the positive inotropic effect no alterations of cellular Na- and K-concentrations were measurable. It seems much more obvious that as soon as cellular accumulation of sodium and loss of potassium occur the muscles develop an increase in diastolic tension.

Only at a beat frequency of 1 Hz does the development of the positive inotropic effect evoked by ouabain parallel the binding of the drug, and is associated with a slight and transient increase in the cellular K-concentration (which would rather reflect a stimulation than an inhibition of the ATPase). The cellular Na-concentration remains completely unaffected. The maximum positive inotropic effect is attained when the majority of binding sites is occupied by ouabain (T/M = 2). Data of Kuschinsky, Lüllmann & van Zwieten (1968), Godfraind & Lesne (1972), Erdmann & Hasse (1975) and Lüllmann *et al.* (1975a) suggest about 90% of the adsorbed ouabain to be specifically bound to a saturable compartment identical with the Na-K-ATPase.

An increase of the stimulation frequency to 2, 4 and 5 Hz progressively shortened the positive inotropic state, induced transitory maxima of binding of ouabain, and even reduced the maximum binding and positive inotropic effect (Figure 1). According to Brinkmann & Ravens (1976) the decline of contractile tension, which at higher beat frequencies follows the positive inotropism induced by cardiac glycosides, and the reduced positive inotropic effect, are correlated to a critical shortening of the action potential duration. The early repolarization phase seems to prevent a complete activation of contractile elements by calcium ions which become rebound to their binding sites (Lüllmann & Peters, 1976). Neither the binding maxima nor the following decay of ouabain binding correlates to changes in cellular Na- or K-concentrations (Figure 1). It is suggested that the release of ouabain, in spite of its continuous presence in the the bath may be caused by a lowered concentration of ATPase conformations which readily bind ouabain (e.g. depletion of ATP, accumulation of calcium).

The cellular loss of K and gain of Na at higher beat frequencies coincides with the increased diastolic tension. Arrhythmias may occur simultaneously. Decreased gradients for K and Na are accompanied by a lowered transmembrane potential, which in combination with a shortened action potential favours the occurrence of extrasystoles and arrhythmias. Similarly, during increase in diastolic tension an increase of cellular calcium concentration due to a decreased calcium outward transport was reported by Kasparek (1976). The cellular accumulation of calcium will in turn hinder relaxation and, according to Godfraind & De Pover (1976) will itself inhibit the Na-K-ATPase. Thus in spite of wash-out of ouabain, the Na-K-ATPase remains inhibited as can be seen in Figure 2. During the phase of contracture a slow binding of ouabain reappears, which may reflect a continuous accumulation of inorganic phosphate in the cytosol (Lee, Ju, Lee & Burstein, 1960). As is known from isolated ATPase preparations, in the presence of inorganic phosphate, Mg^{2+}, and Na, all ATPase molecules will occupy a binding conformation for cardiac glycosides and lose their transport activity (Schwartz, Lindenmayer & Allen, 1975).

Possible determinants for the rate of binding of ouabain

The specific binding site for cardiac glycosides is the Na-K-ATPase which upon binding cardiac glycosides, loses its ability to transport Na^+ and K^+ (Repke, 1964, for review see Schwartz *et al.*, 1975). During the pumping process the ATPase undergoes several conformational steps with different binding affinities for cardiac glycosides (Post, Kume, Tobin, Orcutt & Sen, 1969). The intracellular Na concentration seems to play a major role for the cycling frequency of the ATPase. The rate of binding of cardiac glycosides to the isolated enzyme increases with increasing Na concentrations (Schwartz *et al.*, 1975). For this reason it is tempting to correlate the amount of Na which enters atrial cells per unit of time to the activity of the ATPase and thus to the rate of binding of cardiac glycosides. Figure 1 shows that the rate of binding is enhanced at higher beat frequencies. The essential requirement, i.e. an increased intracellular Na concentration is, however, not fulfilled, indicating that according to the law of mass action the individual ATPase centre will not be stimulated by the increased frequency. Yet, the depolarization-dependent influx of Na will occur more frequently if the beat frequency is increased. Consequently, the ATPase is faced with the same concentration but a higher amount of Na^+ to be extruded. This necessitates additional cycling of the transport system, i.e. extra pumping activity. The question arises, whether in the intact cell increased frequencies provide conditions under which per unit time the concentration of ATPase-conformations with high affinity for cardiac glycosides is augmented.

An increase of the 'high affinity receptor' concentration would also explain the higher rate of formation of the ouabain-ATPase complex in contracting as compared to resting muscles. As outlined above, there is a close correlation between size of Na influx/min at rest and Na influx/min at different frequencies and the rates of binding of ouabain under these conditions and it may be suggested that also under *in vivo* condition the loading of the ATPase by Na^+ is responsible for the rate of onset of action and binding ouabain.

As a working hypothesis it is possible to offer a model accounting for the experimental observation that the actions of ouabain on atrial muscle under specific conditions are frequency-dependent. Since under control conditions the cellular Na concentration did not increase even at high beat frequencies (under the present conditions, up to 5 Hz), the ATPase seems to extrude the extra amount of Na in very short

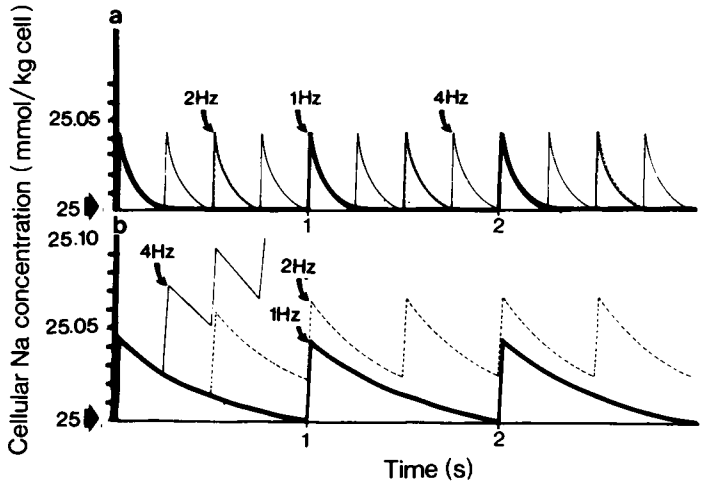

Figure 3 Hypothetical presentation of intracellular undulations of Na by means of cumulative Bateman functions (Dost, 1968). (a) Depicts control conditions; ordinate scale: cellular Na concentration in mmol/kg cell; abscissa scale: time (s). The upstrokes represent depolarization-dependent Na-influxes. The respective frequencies are indicated by arrows. (b) Presents the situation after partial inhibition of ATPase by ouabain. Depending on frequency, i.e. load of the inhibited ATPase, cellular Na concentration remains constant or increases. For details see text.

periods of time. The time necessary for the extra pumping activity of the ATPase is limited by the interval between two beats and thus should be less than 200 milliseconds. Above 5 Hz cellular Na starts to rise. This indicates that a critical beat frequency exists with respect to an overloading of the membrane ATPase. Hypothetically the undulations of the cellular Na concentrations at different frequencies are demonstrated in Figure 3.

The ATPase activity can be stimulated by increasing Na concentrations in the range between 1 and 100 mM (for review see Schwartz *et al.*, 1975), such that small alterations of the Na concentrations like those occurring during an action potential, do not significantly activate the pump. In conclusion, in intact cells, the ATPase activity appears to be governed rather by the frequency of electrical events than by altered cellular Na concentrations. A reduction of the number of available ATPase molecules should lead to a lower critical frequency at which the cellular Na concentration will rise, because the extrusion process demands more time to deal with the excitation Na influx in order to warrant an adequate end-diastolic Na concentration (Figure 3). Ouabain in fact reduces the critical beat frequency as shown above. Similar observations have been made by Issekutz (1915) and Gersmeyer & Holland (1963). The interpretation may be that, whenever the critical frequency is exceeded, due to reduction of the number of active ATPases, the cellular Na concentration progressively increases and the K concentration falls accordingly to an equilibrium value corresponding to the respective frequency (Figure 3).

Interaction of cardiac glycoside with Na-K-ATPase seems to induce conformational changes beyond the protein of the membrane bound enzyme (Lüllmann, Peters, Preuner & Rüther, 1975b). This change is thought to alter the characteristics of calcium binding to the plasmalemma resulting in an enhanced availability of coupling calcium which will improve the effectiveness of the excitation-contraction-coupling process and thus induce a positive inotropic effect (Lüllmann & Peters, 1976; 1977). In fact, experimental evidence exists that cardiac glycosides may alter the calcium binding characteristics of the Na-K-ATPase (Schwartz, 1976; Proppe, 1976). From the foregoing it may be suggested that the positive inotropic effect of ouabain does not result from an inhibited ion transport (cf. Figure 1). A possible mode of interaction between ouabain and the functional states of the ATPase is drawn schematically in Figure 4. The dual effects of ouabain on pump activity and on Ca binding sites are indicated.

While the rate of association of ouabain is determined primarily by the cycling frequency of the ATPase, the dissociation rates were found to be independent of the activity of the ATPase and of the situation of the muscle. As can be seen from the wash-out experiments in resting muscles and in muscles in a pure inotropic state, ouabain can dissociate from the ATPase, which continues the transport cycle and returns to its normal binding characteristics

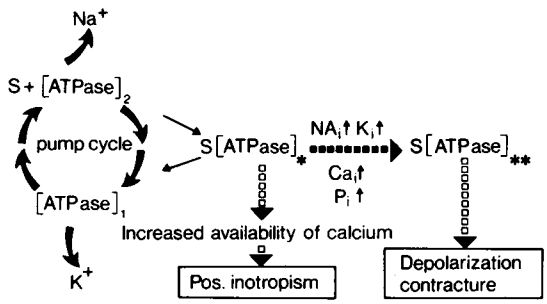

Figure 4 Hypothetical model depicting binding of ouabain to the Na-K-ATPase in intact cells and the interdependence between conformational alterations of the eynzyme and impairment of ion transport. S = ouabain; $ATPase_1$ = enzyme in a conformational state with low affinity for cardiac glycosides; $ATPase_2$ = enzyme with high affinity for cardiac glycosides; bent solid arrows indicate transition of additional conformational steps during one activity cycle; S [ATPase]* = ouabain-ATPase complex; the conformation of the enzyme has changed, enzymatic and transport activities are reversibly blocked, availability of calcium is enhanced; Na_i; K_i, Ca_i = intracellular sodium, potassium, and calcium concentrations; Pi = inorganic phosphate concentrations; ↑ = increase, ↓ = decrease; S [ATPase]** = further conformational change of the enzyme which eventually becomes irreversibly inhibited by lack of ATP or increased diastolic cellular Ca^{2+}-concentrations even if ouabain becomes removed from the ATPase. As long as the proportion of uninhibited ATPase molecules is sufficient to counteract Na influx at a given frequency, the increased availability of calcium underlying the positive inotropic action will be the only manifestation of the ouabain-ATPase interaction.

(Figure 4). The latter is supported by the finding that upon wash-out of ouabain the positive inotropic effect and the contractility concomitantly return to the control level (Figure 2), and the muscle again becomes susceptible to ouabain.

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