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Electrophysiological studies of some semisynthetic cardiac glycoside derivatives in isolated papillary muscle of the guinea-pig

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1 The effects of digitoxin, 3α-methyl-digitoxigenin-3β-monoglucoside (3α-MDM), 3α-methyl-digitoxigenin (3α-MD), proscillaridin, 4, 5-methylene-procillaridin (4, 5-MP), and 3β-hydroxy-4, 5-methylene-A, B-*trans*-scillarenin (3β-HMTS) on force of contraction and on the transmembrane action potentials were examined in isolated papillary muscles of guinea-pigs.

2 All derivatives exhibited the typical cardiac glycoside effects: i.e. they increased the force of contraction and shortened the action potential duration at 20% (plateau phase) and 90% of repolarization. With digitoxin, 3β-HMTS and 4, 5-MP a transient prolongation in action potential duration was observed at the lower concentrations. The action potential amplitude and the resting membrane potential were reduced consistently only with the higher concentrations used.

3 The onset of the positive inotropic effects of 3α-MDM, 3α-MD and 3β-HMTS was more rapid than that of digitoxin and proscillaridin. The increment in contractile force reached a maximum well before the full shortening effect on the action potential duration had developed. The shortening of the action potential is thought to be responsible for the biphasic nature of the positive inotropic effect.

4 With 3α-MD and 3α-MDM even toxic effects, e.g. increase in baseline tension, were completely reversible after washing in drug-free solution.

5 The dose-response curves for the positive inotropism can only be compared reliably once the equilibrium of drug action has been established. This steady state is probably reflected by the development of the full shortening in action potential duration.

Introduction

The Mg^{2+}-dependent, Na^+ and K^+-activated adenosine triphosphatase (Na, K-ATPase) is generally considered to represent the receptor for cardiac glycosides (Repke & Portius, 1963; Schwartz, Lindenmayer & Allen, 1975; Lüllmann & Peters, 1979). Recently, several cardiac glycoside derivatives were obtained by chemical modification, e.g. 3α-methyl-digitoxigenin-monoglucoside (3α-MDM), 3α-methyl-digitoxigenin (3α-MD), 4, 5-methylene-proscillaridin (4.5-MP), and 3β-hydroxy-4.5-methylene-A, B-*trans*-scillarenin (3β-HMTS) (Albrecht, 1977), which can be distinguished from conventional cardiac glycosides by their effects on the Na, K-ATPase (Fath, Kraft, Mancke, Peters & Pulss, 1979; Lüllmann, Peters & Ziegler, 1979; Kraft & Peters, 1980). In general, they exhibit a lower affinity for the enzyme as well as enhanced dissociation-rates.

In addition to the enhancement of force of contraction, cardiac glycosides affect the transmembrane action potentials in a characteristic manner (for references see Lüllmann & Ravens, 1973). It is not clear whether this effect is related to the interaction of cardiac glycosides with their receptor, the Na, K-ATPase, or whether it represents an independent unspecific influence on membrane conductances. Nevertheless, the effects on the shape of the cardiac action potentials provide additional information to characterize the new derivatives. We therefore investigated their effects on transmembrane action potentials and force of contraction in guinea-pig isolated papillary muscles. A preliminary account of the results has been published (Niehus, Pulss & Ravens, 1980).

Methods

Guinea-pigs of either sex weighing 300 to 400 g were killed by cervical dislocation. Their hearts were rapidly removed and right ventricular papillary muscles of less than 1 mm in diameter were selected. The non-tendinous end was fixed with a stainless steel clamp in a 3 ml organ bath which was perfused with oxygenated Tyrode solution at approximately 15 ml min^{-1}. A small glass hook was attached to the tendinous end of the muscle and connected rigidly to an isometric force transducer (Statham UC-2 cell). The resting length of the muscle was adjusted by a stretching force of 3–5 mN to yield an optimal force of contraction. The frequency of electrical stimulation via two platinium electrodes was 1 Hz, square impulses of twice the threshold voltage were used. The temperature in the muscle bath was maintained at 32.0 ± 0.3°C. The pH of the Tyrode solution was maintained at 7.4 ± 0.1 by equilibrating the solution with a mixture of 3% CO_2 and 97% O_2.

Transmembrane action potentials were recorded with conventional glass microelectrodes filled with KCl, 3 mol l^{-1}. The tip resistance was 10–20 MΩ. A high impedance (10^{11}Ω) capacitance compensated preamplifier was used to display the transmembrane potentials on a dual beam oscilloscope (Tektronix 502 A). A Hellige amplifier which powered the force transducer was connected to the second channel of the oscilloscope. For documentation purposes the oscilloscope screen was photographed at regular time intervals during the whole course of the experiment. The individual pictures were projected to measure the following parameters: force of contraction, action potential amplitude, resting membrane potential, and duration of the action potential from the upstroke to 20% and 90% of repolarization. Since it was only occasionally possible to maintain a stable microelectrode impalement during the whole period of drug exposure, several measurements were averaged. The differences between the means were evaluated statistically by Student's *t* test.

Each experiment began with an equilibration period of 90 to 120 min during which the parameters measured stabilized. The perfusion medium was then changed to a solution containing the cardiac glycoside in the final concentration. After further 120 min the preparations were washed in drug-free Tyrode solution for a period of 90–120 min to test the reversibility of the effects.

Digitoxin, 3α-MDM, 3α-MD, proscillaridin, 4, 5-MP and 3β-HMTS were dissolved as concentrated stock solutions in dimethylsulphoxide and propyleneglycol (1:9) and stored in the refrigerator until final use but not longer than three weeks. Adequate volumes of stock solution were added to the Tyrode solution to obtain the final concentrations. The concentration of solvent in the Tyrode solution never exceeded 0.6% by volume; in control experiments this solvent concentration had no effect on the parameters measured. Dose-response curves for the positive inotropic effect in atria were used to select approximately equieffective concentrations of the various compounds (Fath, 1982).

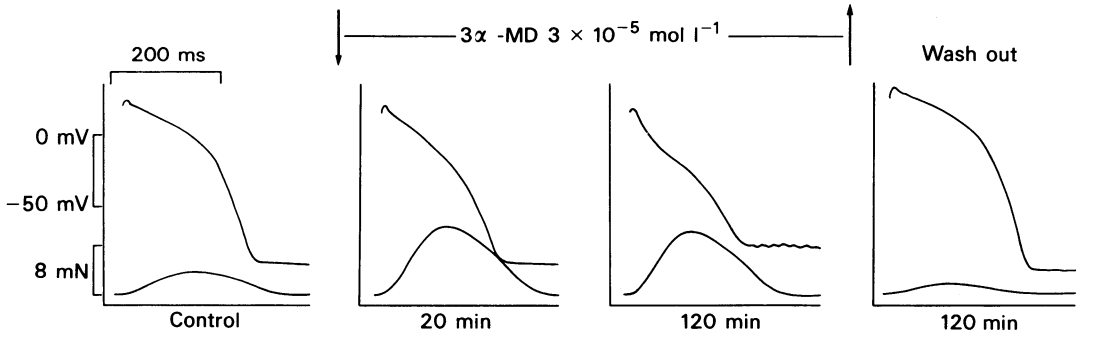

Figure 1 Original tracings of transmembrane action potentials (upper beam) and force of contraction (lower beam) of isolated papillary muscle of the guinea-pig during a representative experiment with 3α-methyl-digitoxigenin (3α-MD). The control recording was obtained at the end of an equilibration period of 120 min, the next two frames show tracings after 20 and 120 min of exposure to 3α-MD, 3×10^{-5} mol l^{-1}, the right frame was obtained after 120 min of washout in drug-free Tyrode solution. The downward and upward arrows symbolize the addition and removal of the compound, respectively. Stimulation frequency 1 Hz. Calibrations as indicated by the bars.

The composition of the Tyrode solution (mmol l^{-1}) was as follows: NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 12.0, NaH_2PO_4 0.21 and glucose 5.5. Digitoxin, proscillaridin, 3α-MDM, 3α-MD, 4, 5-MP, and 3β-HMTS were kindly supplied by Knoll AG, Ludwigshafen.

Results

The 3α-methylated derivatives of digitoxigenin and its monoglucoside (3α-MD and 3α-MDM, respectively) increased the force of contraction and shortened the action potential duration in isolated papillary muscles. The concentrations required were higher than for digitoxin which served as a control substance. The effects are illustrated in Figures 1 and 2.

After 20 min of exposure with 3α-MD (3×10^{-5} mol l^{-1}) the positive inotropic effect reached its maximum. At this time the shape of the action potential was altered mainly at the plateau level. The full effect on the action potential required 120 min to develop. It included shortening in action potential duration at 20% and 90% of repolarization, decrease in action potential amplitude and membrane depolarization. During this prolonged exposure period, the force of contraction did not remain at the maximum level of augmentation but decreased slightly. The effects of 3α-MD were rapidly reversible on washing in drug-free solution. The decrease in contractile force during 3α-MD exposure and after washout was larger than the spontaneous decline in control experiments of comparable duration. This seems to be a peculiarity of guinea-pig

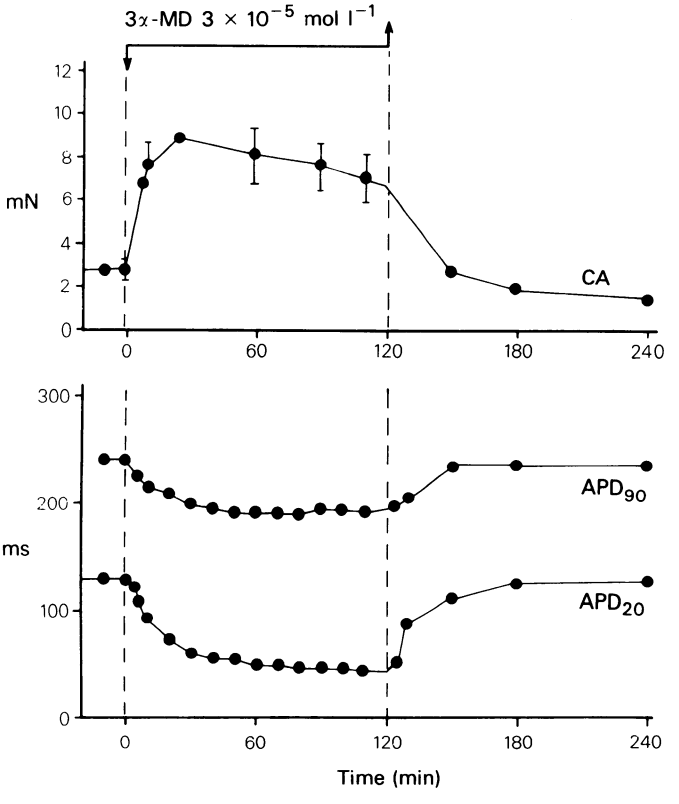

Figure 2 Time courses of the effects of 3α-methyldigitoxigenin (3α-MD), 3×10^{-5} mol l^{-1} on force of contraction (CA) and action potential duration at 90% and 20% of repolarization (APD_{90} and APD_{20}, respectively) of guinea-pig isolated papillary muscle. The symbols represent mean values from 5 experiments; s.e.means shown by vertical lines except for the s.e.means APD_{90} and APD_{20} which are smaller than the size of the symbols. The period of exposure to 3α-MD is indicated by the bar. The time course of the positive inotropic effect and the shortening in APD are different; both effects are reversible upon washout in drug-free Tyrode solution.

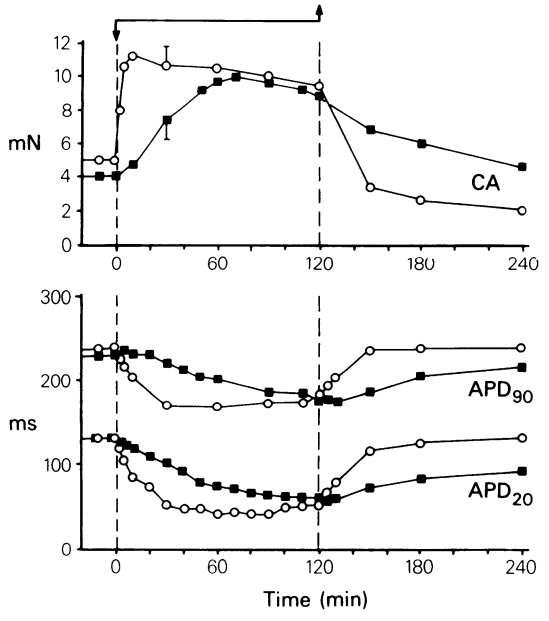

Figure 3 Time courses of the effects of 3α-methyl-digitoxigenin-3β-monoglucoside (3α-MDM, 1×10^{-5} mol l^{-1}, ○) and digitoxin (3×10^{-7} mol l^{-1}, ■) on force of contraction (CA) and action potential duration at 90% and 20% of repolarization (APD_{90} and APD_{20}, respectively) of guinea-pig isolated papillary muscle. Lay-out as in Figure 2. Note that the effects of 3α-MDM are more rapid in onset than with digitoxin and are completely reversible after washout.

papillary muscle since it is absent in cat papillary muscle (Peters, Ravens & Ziegler, 1980).

Principally similar results as with 3α-MD were obtained with 3α-MDM (10^{-5} mol l^{-1}) and with digitoxin (3×10^{-7} mol l^{-1}, Figure 3), i.e. the positive inotropic effect developed more rapidly than the shortening in action potential duration. The effects of digitoxin, however, were slower in onset (70 min were required for the maximum positive inotropic effect), they occurred at lower concentrations and they were not completely reversible after 120 min of washout in drug-free solution.

Of the compounds investigated, proscillaridin (3×10^{-8} mol l^{-1}) and its 4,5-methylene derivative (4, 5-MP, 3×10^{-8} mol l^{-1}) showed the slowest time courses of their effects (Figure 4). After 120 min of exposure the force of contraction had just reached its maximum. The shortening in action potential duration began after a delay of some 50 min and certainly did not reach an equilibrium value during the exposure period. The effect persisted during the 120 min washout period. In contrast to these two drugs, the onset of the effects of the A, B-*trans*-derivative 3β-HMTS (7.5×10^{-7} mol l^{-1}) was enhanced, the positive inotropic effect passed through a maximum after 40 min and then declined appreciably. The action potential duration was shortened at both repolarization levels with an equilibrium value reached after 120 min of exposure. All effects of 3β-HMTS were fully reversible after 120 min of washout.

Previously we were unable to demonstrate an initial prolongation in action potential duration as documented for example during the first 2–5 min of ouabain exposure in cat papillary muscle (Dudel & Trautwein, 1958). In Figure 4 the shortening in action potential duration induced by 4, 5-MP starts only after a delay of about 60 min. This could have been due to an initial prolongation at least in some individual experiments counterbalanced by an immediate shortening in others. To test this we examined the initial 10 min of drug exposure more closely (Figure 5). With digitoxin, 4, 5-MP and 3β-HMTS an initial prolongation was observed in 5 out of 6, 7 out of 7, and 8 out of 9 experiments, respectively, whereas with 3α-MDM, 3α-MD and proscil-

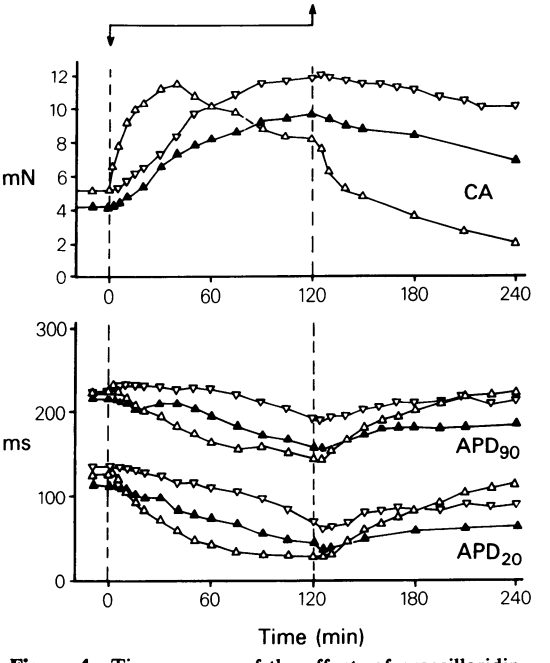

Figure 4 Time courses of the effects of proscillaridin (3×10^{-8} mol l^{-1}, ▲), 4,5-methylene-proscillaridin (4,5-MP, 3×10^{-8} mol l^{-1}, ▽), and 3β-hydroxy-4,5-methylene-A,B-*trans*-scillarenin (3β-HMTS, 7.5×10^{-7} mol l^{-1}, △) on force of contraction (CA) and action potential duration at 90% and 20% of repolarization (APD_{90} and APD_{20}, respectively) of guinea-pig isolated papillary muscle. The values are means from 7 to 9 experiments, s.e.mean is not given for reasons of clarity, but is of the same order of magnitude as in Figure 3. Similar lay-out to Figure 3.

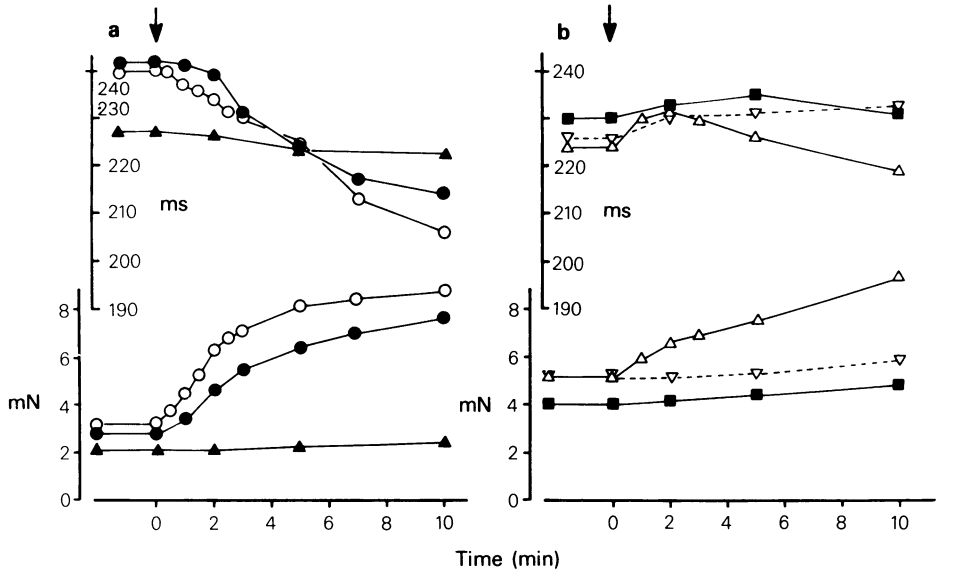

Figure 5 Time courses of the effects on force of contraction (lower part) and on action potential duration at 90% of repolarization (upper part) during the initial 10 min of exposure to different cardiac glycosides (arrow indicates start). (a) Effects of 3α-methyl-digitoxigenin-3β-monoglucoside (3α-MDM, 1×10^{-5} mol l^{-1}), 3α-methyldigitoxigenin (3α-MD, 3×10^{-5} mol l^{-1}, ●), and proscillaridin (3×10^{-8} mol l^{-1}, ▲). All three cardiac glycosides cause an immediate shortening in action potential duration. (b) Effects of digitoxin (3×10^{-7} mol l^{-1}, ■), 3β-hydroxy-4,5-methylene-A,B-*trans*-scillarenin (3β-HMTS, 7.5×10^{-7} mol l^{-1}, △), and 4,5-methylene-proscillaridin (4,5-MP, 3×10^{-8} mol l^{-1}, ▽). All three substances cause an initial prolongation in action potential duration. Note that the prolongation in action potential does not relate to the rate of positive inotropic effect. Data points are mean values of the same experiments depicted in Figures 3 and 4.

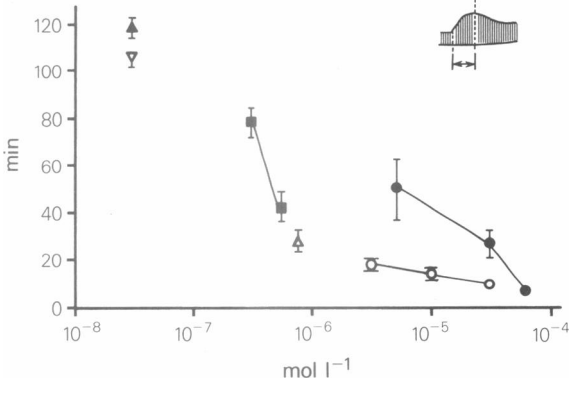

Figure 6 Time required for maximum contractile augmentation in relation to the concentration of the various cardiac glycosides. Ordinate scale: time in min between the beginning of exposure and the maximum force of contraction (see arrow in the mechanogram inset); abscissa scale: concentration of cardiac glycoside: (▲) proscillaridin; (▽) 4,5-methylene-proscillaridin (4,5-MP); (△) 3β-hydroxy-4,5-methylene-A,B-*trans*-scillarenin (3β-HMTS); (■) digitoxin; (●) 3α-methyl-digitoxigenin (3α-MD), and (○) 3α-methyl-digitoxigenin-3β-monoglycoside (3α-MDM).

laridin the action potential duration shortened immediately after drug exposure. It should be noted that prolongation in action potential duration does not parallel the rate of inotropic effect (compare proscillaridin to digitoxin, or 3α-MD to 3β-HMTS).

In Figure 6 the time of exposure at which the maximum of the positive inotropic effect occurred is plotted against the concentration of the various compounds. With increasing concentrations of each drug the time required for maximal positive inotropy was shortened. It should be noted that although 3α-MD and 3α-MDM were effective in the same concentration range, maximum positive inotropy was reached significantly earlier with 3α-MDM.

The biphasic nature of the positive inotropic effect made it difficult to establish dose-response curves for the compounds (Figure 7). When plotting the values at the time of maximal positive inotropic response (Figure 7a) the shortening in action potential duration was underestimated. On the other hand, plotting the values obtained after 120 min of exposure (Figure 7b), when the shortening in action potential duration had fully developed, resulted in much smaller differences in apparent positive inotropic potency.

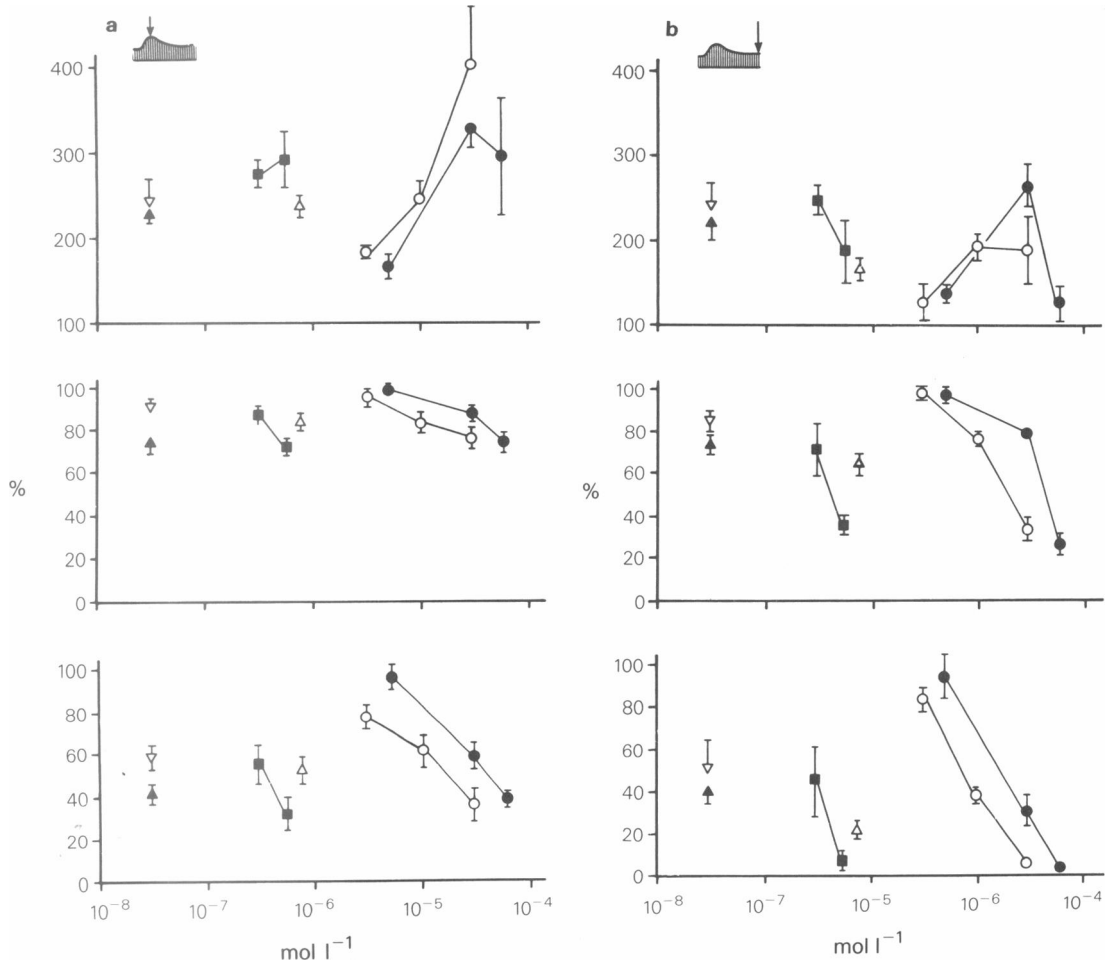

Figure 7 Concentration-response relationship of several cardiac glycosides and their semi-synthetic derivatives. Upper ordinate scales: increase in force of contraction as percentage of control (= 100%); middle and lower ordinate scale: action potential duration at 90% and 20% of repolarization, respectively, as percentage of the control (= 100%). (a) Relationship at the time of the maximum of contractile augmentation; (b), relationship after the development of the full action potential duration shortening (see arrows in the respective mechanogram insets). Symbols as in Figure 5.

In the experiments with moderate concentrations (Figures 2–4) the effects induced by 3α-MDM, 3α-MD and 3β-HMTS were rapidly and completely reversible during washout. Figure 8 illustrates the effects of washout after contracture had occurred as a sign of intoxication. For the conventional cardiac glycoside digitoxin (5.5×10^{-7} mol l^{-1}) the contracture even continued to develop during the first few minutes of washout and was poorly reversible during the remainder of the washing period. The electrical parameters, i.e. shortening in action potential duration, decrease in resting membrane potential and action potential amplitude, showed little recovery towards their pre-drug value. However, with 3α-MDM (3×10^{-5} mol l^{-1}), all changes observed were completely reversible after only 30 min of washout even though contracture had lasted for a longer period than with digitoxin (30 versus 15 min). Similar results with one exception (see below) were obtained in 4 experiments with 3α-MD (6×10^{-5} mol l^{-1}).

With the low concentrations of digitoxin, 3α-MD and 3α-MDM there were no significant changes in action potential amplitude or resting membrane potential, but with higher concentrations both parameters decreased (Table 1, Figure 8). The reversibility upon washout was sometimes different for either parameter, e.g. with 3α-MD, 6×10^{-5} mol l^{-1}, the action potential amplitude did not recover to its

Table 1 The effects of several cardioactive steroids on the amplitude of the action potentials and the resting membrane potential of isolated papillary muscles of the guinea-pig

Compound	Concentration ($mol\,l^{-1}$)	*Amplitude of the action potential* (mV) Control[1]	Drug[2]	Washout[3]	*Resting membrane potential* (mV) Control	Drug	Washout
Digitoxin	5.5×10^{-7} (6)	113.5±1.3	103.7±2.5†	97.1±4.5†	−92.6±1.1	−86.3±1.3*	−85.5±3.9**
3α-MDM	3×10^{-5} (5)	118.6±2.3	92.7±1.9†	115.3±5.7NS	−92.7±2.1	−80.9±1.4†	−93.9±4.4NS
3α-MD	3×10^{-5} (5)	123.5±1.7	105.5±1.8†	121 ±2.6NS	−97.1±1.8	−83.9±1.6†	−96.8±1.9NS
	6×10^{-5} (4)	115.8±1.4	83.1±2.1†	106.7±2.4**	−90.3±1.4	−77.3±1.4†	−88.0±1.4NS
Proscillaridin	3×10^{-8} (7)	117.3±1.7	105.1±1.8†	109.8±2.5**	−92.2±1.7	−85.2±1.5**	−90.6±2.2NS
4,5-MP	3×10^{-8} (9)	120.8±1.5	114.1±1.9**	117.4±2.1NS	−100.4±1.3	−91.1±1.6†	−93.7±1.5**
3β-HMTS	7.5×10^{-7} (7)	117.3±1.3	98.0±2.0†	115.6±1.8NS	−91.6±1.8	−83.5±1.2†	−91.3±2.0NS

Results (mean ± s.e.mean) were obtained from at least 3 measurements in each preparation. In parentheses: number of experiments.
[1]Control values measured from 90–120 min of the equilibration period before adding the drug. [2]Mean value obtained at the end of exposure time. [3]Mean value obtained after 90–120 min of washout in drug-free Tyrode solution.
*$P<0.05$; **$P<0.01$; †$P<0.001$; NS not significant.

pre-drug value, whereas the membrane depolarization was fully reversible (similarly with proscillaridin, $3 \times 10^{-8}\,mol\,l^{-1}$, but *vice versa* with 4,5-MP, $3 \times 1^{-8}\,mol\,l^{-1}$).

Discussion

It is generally agreed that during the positive inotropic effect of cardiac glycosides more calcium ions per beat are available in the cytosol for activation of the contractile proteins (for recent reviews of the subject see Lüllmann & Peters, 1979; Noble, 1980), but there is some controversy as to how this is linked to the binding of cardiac glycosides to and the subsequent inhibition of the Na, K-ATPase (e.g. compare Lüllmann & Peters, 1976, with Akera & Brody, 1978).

All the cardiac glycosides and their derivatives investigated in the present paper induce the typical positive inotropic effect as well as the typical changes in the shapes of the action potential. Proscillaridin and its 4, 5-methylene derivative are similar in action, particularly with respect to the slow onset of the effects. However, the semisynthetic compounds 3α-MDM, 3α-MD and 3β-HMTS share three interesting differences when compared to their parent compounds: the onset of action is rapid, all effects are completely reversible but higher concentrations are required. These effects are closely paralleled by the characteristics of the interaction of these compounds with the Na, K-ATPase: they have a higher rate of dissociation (and therefore equilibrium of drug action occurs more rapidly) and a lower affinity for the enzyme than conventional cardiac glycosides (Fath *et al.*, 1979; Kraft & Peters, 1980) so that higher concentrations are required. Direct evidence that the semisynthetic derivatives interact with the same cardiac glycoside receptor as the parent compounds was obtained from binding studies in heart muscle homogenate (Lüllmann & Mohr, 1982).

The cardiac glycoside-induced changes in the shape of the action potentials can be related to changes in membrane currents as measured in voltage clamp experiments (McDonald, Nawrath & Trautwein, 1975). The shortening in action potential duration at the plateau level is in accordance with a decrease in slow inward current, and the shortening at 90% of repolarization can be traced back to the increase in time-dependent potassium conductance as well as the diminished slow inward current (McDonald *et al.*, 1975). The time-independent potassium conductance also increases and serves as a plausible explanation for the decrease in action potential amplitude (Greenspan & Morad, 1975). Membrane depolarization is observed only with higher concentrations which cause effective pump inhibition and therefore result in substantial intracellular potassium loss (Müller, 1965; Bentfeld, Lüllmann, Peters & Proppe, 1977; Browning, Guarnieri & Strauss, 1981). Also, during effective pump inhibition the electrogenic contribution to the membrane potential is diminished (Daut & Rüdel, 1982) which causes further depolarization. It is not known whether the effects of cardiac glycosides on the transmembrane potential are due to direct interaction with the channel proteins (in which case there should be binding sites in addition to the Na, K-ATPase) or whether they are indirectly mediated by the consequences of drug interaction with the Na, K-ATPase.

In the present experiments we observed a marked difference between the time course of the positive inotropic effect and the shortening in action potential duration which confirms the results of an earlier

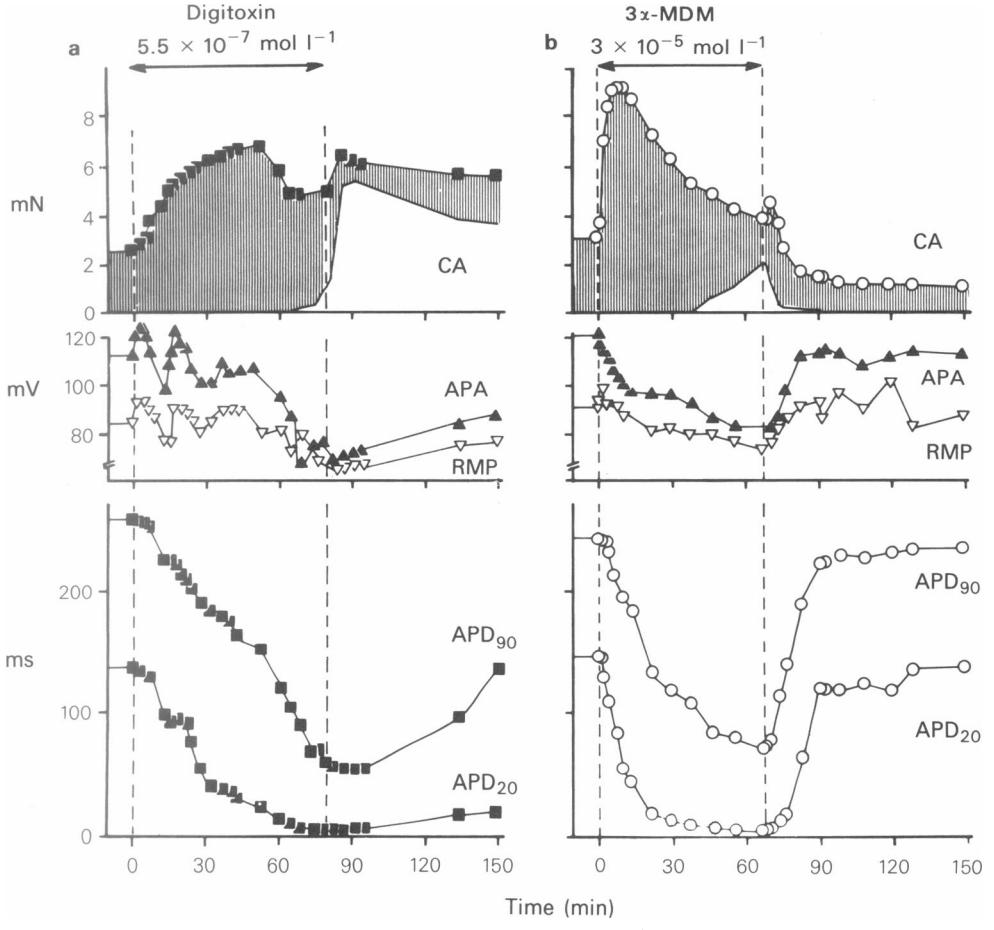

Figure 8 Time courses of the effects of digitoxin (5.5×10^{-7} mol l^{-1}, a) and 3α-methyl-digitoxigenin-3β-monoglucoside (3α-MDM, 3×10^{-5} mol l^{-1}, b) on the force of contraction (CA), the action potential amplitude (APA) the resting membrane potential (RMP) and the action potential duration at 90% and 20% of repolarization (APD_{90} and APD_{20}, respectively) of guinea-pig papillary muscle in two representative experiments. The non-shaded area above the abscissae for contractile force represents the increase in baseline tension (contracture). Note the rapid onset of the positive inotropic effect with 3α-MDM and the complete reversibility of all effects upon washout.

investigation with different cardiac glycosides (Lüllmann & Ravens, 1973). At that time the difference was interpreted as meaning that more than one site of action was involved in producing the different effects of cardiac glycosides. However, studies with radioactively labelled glycosides have provided evidence for only one high affinity binding site, the additional low affinity binding occurring at a much higher concentration range (Godfraind & Lesne, 1972). Since both positive inotropic effect and changes in action potential shape are observed at similar concentrations, it seems reasonable to assume that both biological effects result from drug interaction with one high affinity binding site. In binding studies, two non-identical sites of similar affinity could only be distinguished if they exhibit different association and dissociation kinetics, in which case biexponential time courses of drug binding and release should be measured. However, this was not the case either with ouabain (Lüllmann, Peters & Ravens, 1975) or with 3α-MDM (Mohr, 1983), but monoexponential time courses were found instead. Therefore, from experimental evidence only one binding site is apparent.

The question remains how drug interaction with the Na, K-ATPase can lead to such different effects as positive inotropism and membrane permeability changes and how they can occur with different time courses. In this context the concept of digitalis-

induced positive inotropism proposed by Lüllmann & Peters (1981) may provide an explanation. Briefly, these authors suggest that cardiac glycosides increase the releasability of calcium ions from a plasmalemmal calcium pool which is located in the lipid portion of the Na, K-ATPase molecules and contains the major portion of calcium ions released after depolarization to induce contraction (Lüllmann & Peters, 1977). During a pummping cycle the Na, K-ATPase molecules pass through different conformations which are thought to induce a disturbance of the lipid portion of the enzyme. It is one of the particular conformations which is preserved when cardiac glycosides bind to the Na, K-ATPase and therefore causes the enhanced releasability of calcium ions. Disturbance of the phospholipid component of the plasmalemma could also affect the passive membrane properties or influence membrane channels, thus leading to changes in the shape of the action potentials.

The temporal dissociation between the positive inotropic effect and the action potential changes remains unclear. It could be speculated that the positive inotropic effect develops so rapidly because of the existence of some amplification or positive feedback mechanism as suggested by Marban & Tsien (1982). In any case, it cannot be expected that the time course of the positive inotropic response is a simple reflection of the increase of receptor occupation with exposure time. Under control conditions a certain amount of calcium is released into the cytosol resulting in a certain concentration of free calcium ions. The systolic calcium ion concentration determines the activation of the contractile proteins by a dose-response curve which has been measured for cardiac actomyosin (Solaro, Wise, Shiner & Briggs, 1974) and obeys the mass action law. Under our experimental conditions, enough calcium ions are released to activate the contractile proteins up to a level within the steep part of this dose-response curve. At the beginning of the glycoside binding an enhancement of the calcium ion release per beat will be more effective, since the efficiency will be more than proportional in the steep part of the dose-response curve, in contrast to a similar enhancement close to the saturation level of the calcium ion-actomyosin dose-response curve.

As for the changes in shape of the action potentials, they must be the result of changes in membrane permeability and in membrane currents but no generally accepted concept about the underlying molecular mechanism has yet emerged. Although a saturable binding site for [^{3}H]-ouabain was found with a slow time course which was similar to the one observed for the shortening in action potential duration (Lüllmann & Ravens, 1973; Lüllmann *et al.*, 1975) the electrical changes cannot reflect the time course of receptor occupation directly, because biphasic responses were observed with three cardiac glycosides, i.e. digitoxin, 4, 5-MP and 3β-HMTS. On the other hand, shortening in action potential duration may be enhanced due to the high systolic calcium ion concentration which is known to increase potassium conductance (Bassingthwaighte, Fry & McGuigan, 1976).

From the pronounced biphasic nature of the positive inotropic effect, particularly with high concentrations of 3α-MDM and 3α-MD, it is proposed that in addition to the force-enhancing process there may be a second one counteracting the contractile augmentation. Our results suggest that the shortening in action potential duration is responsible for the decline of the positive inotropic effect, because the action potential duration limits the time available during an action potential for complete diffusion of released calcium ions to the contractile proteins (Morad & Trautwein, 1968; Morad & Orkand, 1971).

Finally, we would like to propose the following sequence of events. After addition of a cardiac glycoside the drug binding proceeds with a time course which is determined by the properties of the individual glycoside. The experimental evidence from binding studies available so far, points to the existence of only one high affinity binding site which is identical with the Na, K-ATPase. The occupation of the high affinity binding site is responsible for the two biological effects investigated in this paper, i.e. the positive inotropic effect and the changes in action potential duration proceeding with different time courses. The transformation of binding into either effect is largely unknown; however, in the absence of direct effects of glycosides on the contractile proteins the positive inotropism indicates that the systolic calcium ion concentration in the cytosol increases with time of exposure. Because of the non-linear relation between the cytosolic calcium ion concentration and force of contraction, it is impossible to draw direct conclusions from the time course of the positive inotropic effect about receptor occupation. Since no details are known about the transformation of glycoside binding into the changes in action potential duration, the time courses of these changes yield even less information about the occupation of the receptors. In other words, the observation of two different time courses for two biological events does not exclude the binding of a drug to only one high affinity binding site.

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References

AKERA, T. & BRODY, T.M. (1978). The role of Na^+, K^+-ATPase in the inotropic action of digitalis. *Pharmac. Rev.*, **29,** 187–220.

ALBRECHT, H.P. (1977). Synthese von Glykosiden C-3-verzweigter Cardenolide. *Liebigs Ann. Chem.*, **1977,** 1429–1434.

BASSINGTHWAIGHTE, J. B., FRY, C. H. & McGUIGAN, J. A. S. (1976). Relationship between internal calcium and outward current in mammalian ventricular muscle: a mechanism for the control of the action potential duration? *J. Physiol.*, **262,** 15–37.

BENTFELD, M., LÜLLMANN, H., PETERS, T. & PROPPE, D. (1977). Interdependence of ion transport and the action of ouabain in heart muscle. *Br. J. Pharmac.*, **61,** 19–27.

BROWNING, D.J., GUARNIERI, T. & STRAUSS, H.C. (1981). Ouabain effects on intracellular potassium activity and contractile force in cat papillary muscle. *J. clin. Inv.*, **68,** 942–956.

DAUT, J. & RÜDEL, R. (1982). The electrogenic sodium pump in guinea-pig ventricular muscle: Inhibition of pump current by cardiac glycosides. *J. Physiol., Lond.*, **330,** 243–264.

DUDEL, J. & TRAUTWEIN, W. (1958). Elektrophysiologische Messungen zur Strophanthinwirkung am Herzmuskel. *Naunyn Schmiedebergs Archs exp. Path. u. Pharmak.*, **232,** 393–407.

FATH, R. (1982). *Über die Wirkung C_3- und C_{16}-substituierter Cardenolide an isolierten linken Vorhöfen von Meerschweinchenherzen.* Doctoral Thesis, Medical Faculty of the University of Kiel.

FATH, R., KRAFT, D., MANCKE, A., PETERS, T. & PULSS, W. (1979). Pharmacological characterization of some new semi-synthetic cardiac glycosides. *Naunyn-Schmiedebergs Arch. Pharmac.*, **307,** R37.

GODFRAIND, T. & LESNE, M. (1972). The uptake of cardiac glycosides in relation to their actions in isolated cardiac muscle. *Br. J. Pharmac.*, **46,** 488–497.

GREENSPAN, A.M. & MORAD, M. (1975). Electromechanical studies on the inotropic effects of acetylstrophanthidin in ventricular muscle. *J. Physiol.*, **253,** 357–384.

KRAFT, D. & PETERS, T. (1980). Studies on the structure-activity relationship of C-3-branched cardiac glycosides on the Na-K-ATPase preparation of guinea-pig heart. *Naunyn-Schmiedebergs Arch. Pharmac.*, **311,** R40.

LÜLLMANN, H. & MOHR, K. (1982). On the binding of a 3-α-methylated digitoxigenin-glucoside to ouabain receptors in heart muscle homogenate. *Biochem. Pharmac.*, **31,** 2489–2494.

LÜLLMANN, H. & PETERS, T. (1976). On the sarcolemmal site of action of cardiac glycosides. In *Recent Advances in Studies on Cardiac Structure and Metabolism*, Vol. 9, The Sarcolemma, pp. 311–328. Baltimore: University Park Press.

LÜLLMANN, H. & PETERS, T. (1977). Plasmalemmal calcium in cardiac excitation-contraction coupling. *Clin. exp. Pharmac. Physiol.*, **4,** 49–57.

LÜLLMANN, H. & PETERS, T. (1979). Action of cardiac glycosides on the excitation-contraction coupling in heart muscle. *Progr. Pharmac.*, **2,** (2), 1–57.

LÜLLMANN, H. & PETERS, T. (1981). Influence of cardiac glycosides on cell membrane. In *Handbook of experimental Pharmacology*, Vol. 56/1, pp. 395–406. ed. Greeff, K. Berlin, Heidelberg, New York: Springer-Verlag.

LÜLLMANN, H., PETERS, T. & RAVENS, U. (1975). Studies on the kinetics of [^{3}H]-ouabain uptake and exchange in the isolated papillary muscle of the guinea-pig. *Br. J. Pharmac.*, **53,** 99–107.

LÜLLMANN, H., PETERS, T. & ZIEGLER, A. (1979). Kinetic events determining the effects of cardiac glycosides. *Trends Pharmac. Sci.*, Dec. 1979, 102–106.

LÜLLMANN, H. & RAVENS, U. (1973). The time courses of the changes in contractile force and in transmembrane potentials induced by cardiac glycosides in guinea-pig papillary muscle. *Br. J. Pharmac.*, **49,** 377–390.

MARBAN, E. & TSIEN, R.W. (1982). Enhancement of calcium current during digitalis inotropy in mammalian heart: positive feed-back regulation by intracellular calcium? *J. Physiol., Lond.*, **329,** 589–614.

McDONALD, T.F., NAWRATH, H. & TRAUTWEIN, W. (1975). Membrane currents and tension in cat ventricular muscle treated with cardiac glycosides. *Circulation Res.*, **37,** 674–682.

MOHR, K. (1983). Kinetics of binding of the semi-synthetic α-methyl-digitoxigenin-glucoside to cardiac Na^+/K^+-ATPase. *Biochem. Pharmac.*, (in press).

MORAD, M. & ORKAND, R.K. (1971). Excitation-contraction coupling in frog ventricles: Evidence from voltage clamp studies. *J. Physiol. Lond.*, **219,** 167–189.

MORAD, M. & TRAUTWEIN, W. (1968). The effect of the duration of the action potential on contraction in the mammalian heart muscle. *Pflügers Arch.*, **299,** 66–82.

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

NIEHUS, U., PULSS, W. & RAVENS, U. (1980). The effects of digitoxigenin-, proscillaridin- and scillarenin-derivatives on the electrical and mechanical activities of guinea-pig papillary muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **311,** R41.

NOBLE, D. (1980). Mechanism of action of therapeutic levels of cardiac glycosides. *Cardiovasc. Res.*, **14,** 495–514.

PETERS, T., RAVENS, U. & ZIEGLER, A. (1980). The effects of positive inotropic interventions on the maintenance of contractile force in heart muscle preparations after isolation. *Naunyn-Schmiedebergs Arch. Pharmac.*, **313,** R40.

REPKE, K. & PORTIUS, H.J. (1963). Über die Identität der Ionenpumpen-ATPase in der Zellmembran des Herzmuskels mit einem Digitalis-Rezeptorenzym. *Experientia*, **19,** 452–458.

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

SOLARO, R.J., WISE, R.M., SHINER, J.S. & BRIGGS, F.N. (1974). Calcium requirements for cardiac myofibrillar activation. *Circulation Res.*, **34,** 525–530.

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