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The time courses of the changes in contractile force and in transmembrane potentials induced by cardiac glycosides in guinea-pig papillary muscle

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Summary

1. The effects of ouabain, digoxin, digoxigenin monodigitoxoside, digoxigenin bisdigitoxoside and digitoxigenin bisdigitoxoside on the force of contraction and on the transmembrane action potential were compared in isolated papillary muscles of guinea-pigs.

2. All cardiac glycosides studied had a dose-dependent positive inotropic effect and simultaneously shortened the duration of the action potential at all levels of repolarization from the start of drug action.

3. In every instance, the reduction of the action potential duration developed more slowly than the increment in contractile force. However, the ratios between the two rates were independent of the concentrations used and seemed to be characteristic for the individual cardiac glycosides.

4. All cardiac glycosides had a biphasic effect on the time-to-peak tension. An initial increase was followed by a dose-dependent decrease.

5. The results are discussed with respect to the possible sites of action. Taking into account the different rates as well as the different ratios, it is proposed that more than one site of action is involved in producing the different effects of cardiac glycosides on heart muscle.

Introduction

During recent years, the effects of cardiac glycosides on the contractile force and the transmembrane potential of isolated mammalian heart muscle has been studied extensively (Dudel & Trautwein, 1958; Vassalle, Karis & Hoffman, 1962; Kassebaum, 1963; Müller, 1963 & 1965; Edmands, Greenspan & Fisch, 1967; Prasad & Callaghan, 1970; Prasad, Singh & Callaghan, 1971); in order to gain evidence for a possible mechanism of positive inotropism. There is good agreement that at a late stage of cardiac glycoside action, the increased force of contraction is accompanied by a marked shortening of the action potential duration at the plateau level as well as in the total duration. However, the early changes in the shape of the action potential are a subject of controversy; they seem to depend mainly on experimental conditions or to be species-specific.

In cat isolated papillary muscle, Dudel & Trautwein (1958) observed first a prolongation, later a shortening of the action potential duration; the force of contraction was enhanced throughout the experiment. This initial increase in action potential duration could not be demonstrated in canine papillary and trabecular muscle (Edmands *et al.*, 1967; Vassalle *et al.*, 1962) and in similar

preparations of sheep (Müller, 1965). In these experiments, a certain period of latency, during which no changes in the shape of the action potential took place, was followed by a progressive shortening in the action potential duration, whereas the positive inotropic effect developed immediately after the exposure to the drug. Prasad (1972) reported that the augmentation of contractile force of human papillary muscles by ouabain was associated with a shortening of the action potential duration from the very beginning. Kassebaum (1963) investigated the dependency on stimulation frequency in sheep trabecular muscle of the cardiac glycoside-induced changes in the shape of the action potential. He observed an increase in duration only at a low rate of stimulation of 0·5 Hz, whereas at the higher stimulation rate of 1 Hz, only a progressive decrease in action potential duration took place. At both stimulation frequencies the force of contraction was increased.

These findings therefore suggest, that the positive inotropic effect of cardiac glycosides cannot be related directly to the shortening of the action potential duration. Nevertheless, there is probably a relation between these two important parameters of muscle function. We therefore attempted to analyse the time course of the changes in the force of contraction and of the action potential. In addition, we compared the time courses of several cardiac glycosides at different concentrations with respect to their inotropism and action potential changes, because possible differences should indicate different affinities or kinetics regarding their sites of action.

Methods

Guinea-pigs of either sex weighing 300–400 g were killed by a blow on the head. Their hearts were rapidly removed and placed in Tyrode solution equilibrated with 95% O_2 and 5% CO_2. Right ventricular papillary muscles of less than 1 mm in diameter were excised and mounted with a silk thread in an 8 ml organ bath. A small glass hook was attached to the tendinous end of the muscle and connected rigidly to a strain gauge. The resting length of the muscle was adjusted by a stretching force of 300–500 mg to yield an optimal force of contraction. The preparations were stimulated with 2 ms square impulses of twice the threshold voltage at a rate of 1 Hz. The muscle chamber was perfused at a rate of 24 ml/min with Tyrode solution equilibrated with 95% O_2 and 5% CO_2 in the cold. The temperature in the muscle bath was maintained at $32{\cdot}0 \pm 0{\cdot}2°$ C.

Transmembrane action potentials were recorded with flexibly mounted glass microelectrodes filled with 3 M KCl and having a resistance of 10–20 Ω. By means of a capacitance compensated preamplifier of high impedance (10^{11} Ω) they were displayed on a dual beam oscilloscope (Tektronix 502 A). A Hellige carrier amplifier which powered the strain gauge for isometric tension recordings was connected to the second channel of the oscilloscope. For documentation purposes, the oscilloscope screen was photographed at well defined time intervals during the whole course of the experiment. The individual pictures were projected to measure the following parameters: force of contraction, time-to-peak tension, amplitude of action potential, membrane potential, and duration of action potential from the upstroke (phase O) to 20%, 50% and 90% of repolarization.

Each experiment began with a control period of 120 min during which the muscle chamber was perfused with Tyrode solution. The perfusion medium was then changed to a solution containing a glycoside at the final concentration, in which the muscle normally remained for a further 120 minutes. Sixty to 90 min washing in glycoside-free Tyrode solution completed the experiment. To determine the time course of the glycoside-induced changes in one experimental group, the statistical mean of the individual values measured at comparable times during the course of the experiments were calculated and plotted against time. The differences between the means were evaluated by Student's *t*-test.

Ouabain was dissolved in distilled water. Digoxin, digoxigenin monodigitoxoside, digoxigenin bisdigitoxoside and digitoxigenin bisdigitoxoside were dissolved in 70% methanol in a concentration of 1×10^{-3}M. To obtain the final concentrations used, an adequate volume of stock solution was added to the Tyrode solution in the storage vessel. The alcohol concentration in the perfusion medium never exceeded 0·1% and was without measurable effect on the parameters studied.

The composition of the Tyrode solution (mM) 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; Glucose 5·5. The pH of the solution was adjusted to 7·2 by gassing with a mixture of 95% O_2 and 5% CO_2.

Results

Effects of ouabain

At the concentration of $3{\cdot}5\times10^{-7}$M, ouabain acted on the transmembrane potential and the force of contraction as illustrated by a typical experiment in Figure 1. The first frame in this series was taken at the end of an equilibration period of 120 min and served as a control. In comparison with the next recordings, a marked enhancement of the contractile force at 10 min and 30 min of ouabain action was noted, and the shape of the action potential had changed slightly: there was an immediate, though not very pronounced shortening in the action

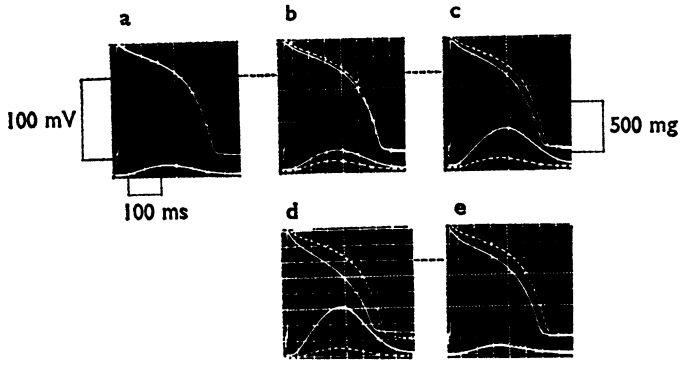

FIG. 1. Original tracings of transmembrane action potential (upper beam) and the force of contraction (lower beam) of guinea-pig isolated papillary muscle during a representative experiment. The control recording at the end of an equilibrium period of 120 min (a) is superimposed in dotted lines on the other recordings: 10 min (b), 30 min (c) and 80 min (d) after exposure to $3{\cdot}5\times10^{-10}$M ouabain; after 90 min of wash-out (e). Horizontal calibration: 100 ms. The interrupted horizontal line is the level of zero potential. Note that the change in shape of the action potential is not fully reversible but the force of contraction returns to control values.

potential duration. After 80 min of exposure to ouabain, the action potential was shortened considerably and also, the membrane potential was decreased. The force of contraction was not increased much further. The positive inotropic effect was fully reversible after 90 min wash-out in glycoside-free Tyrode solution and the action potential was prolonged again but did not recover to control values.

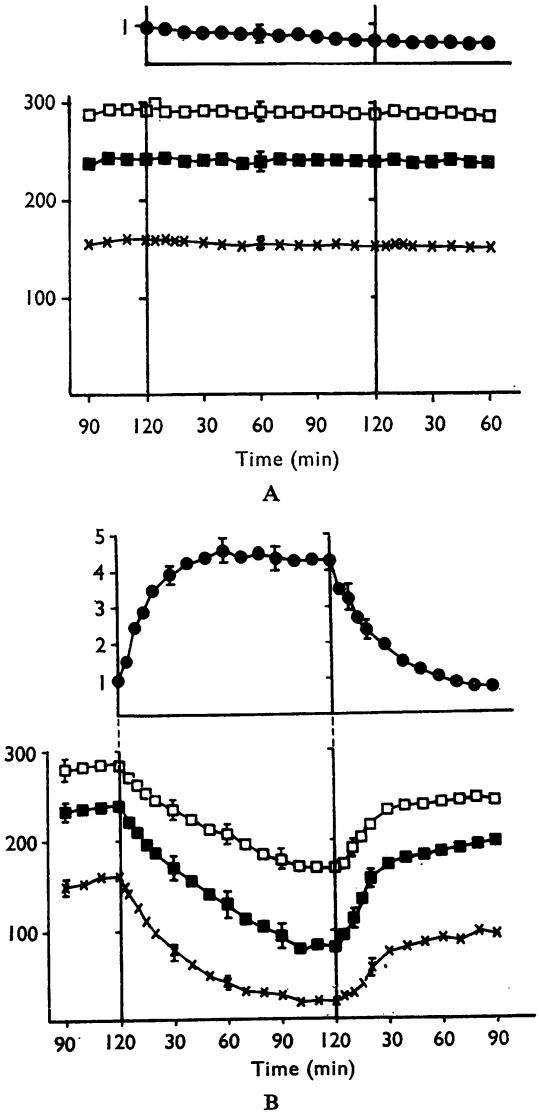

FIG. 2. Time course of the force of contraction (●——●) and the action potential duration at 90% (□——□), 50% (■---■), and 20% (×---×) of repolarization of guinea-pig isolated papillary muscle. The contractile force is expressed as a multiple of the value measured at the end of an equilibration period of 120 minutes. Standard errors of the mean were determined for each value, but to clarify the figures only representative S.E.M.'s are illustrated. (A) Control group. The symbols reresent the mean values of 10 exeriments at any particular instant. Note that the force of contraction is diminished throughout the experiment; the action potential duration remains constant. (B) Ouabain 7×10^{-7}M. The symbols represent the mean of 7 experiments. The maximum positive inotropic effect is reached after 50 min, the action potential duration continues to decrease up to 120 min, and does not return to control values after wash-out.

Figure 2B summarizes the changes observed at the concentration of 7×10^{-7}M ouabain. The action potential duration at 20%, 50% and 90% repolarization in milliseconds is plotted against time in minutes. The contraction amplitude is expressed as a multiple of the control amplitude measured at 90, 100, 110 and 120 min of the control period and each experiment was its own control. Since the contractile force did not remain constant throughout the experiment, every value was corrected for the spontaneous decline in contractile force at its particular time during the experiment. The correction factor was estimated from 10 control experiments during which a decline of about 50% of the contraction amplitude was observed in the course of 3 h following an equilibration period of 2 hours. In contrast, the action potential remained constant in shape (Fig. 2A) throughout the control period, and did not have to be corrected.

As clearly indicated in Fig. 2B, the positive inotropic effect and the shortening in action potential duration due to ouabain followed a different time course; the largest possible enhancement of the force of contraction at this concentration had developed 50 min after the addition of the drug, while the maximum in reduction of the action potential duration was reached much later at about 120 minutes.

For mathematical description, the curves of Fig. 2B were considered to be simple exponential functions, since plotted in a semilogarithmic system they yielded straight lines. The individual values were expressed as percent of the maximal effect so that the changes in both parameters could be compared (Figure 3). Their half-life times differed considerably: the increase in the force of contraction had a $T_{\frac{1}{2}}$ of 12·5 min, whereas that of the decline in action potential duration at 20% repolarization was 21 minutes. Thus it seems that ouabain at a concentration of 7×10^{-7}M acts more slowly on the action potential duration than on the force of contraction.

Similar findings could be demonstrated at all 'non-toxic' concentrations of ouabain studied ($0{\cdot}7\times10^{-7}$M, $2{\cdot}1\times10^{-7}$M, $3{\cdot}5\times10^{-7}$M, 7×10^{-7}M). The positive inotropic effect was always accompanied by a shortening in the action potential

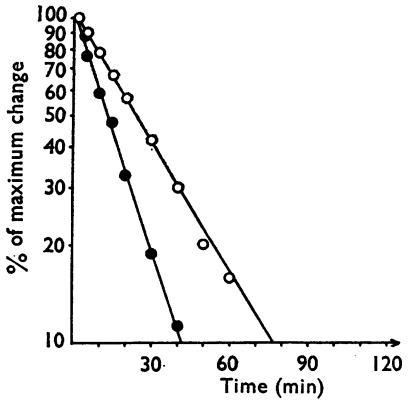

FIG. 3. Semilogarthmic plot of the time course of the increase in force of contraction (●——●) and the decrease in action potential duration at 20% repolarization (○——○) of guinea-pig isolated papillary muscle after exposure to 7×10^{-7}M ouabain. Ordinates: change at any particular instant expressed in percent of the maximal change. Abscissae: time in minutes. The two processes are simple exponential functions and distinctly differ in their half-lives ($T_{\frac{1}{2}}$).

TABLE 1. *Effect of ouabain on the force of contraction, the action potential duration and their time courses ($T_{\frac{1}{2}}$ in isolated papillary muscles of guinea-pig) at 32° C*

Concentration of ouabain in the perfusion medium (M)	Force of contraction: Increase in % of control	Force of contraction: $T_{\frac{1}{2}}$ (min)	Action potential duration: 20% repolarization: Control[1] (ms)	20% repolarization: Lowest[2] value measured (ms)	20% repolarization: a–b (ms)	20% repolarization: $T_{\frac{1}{2}}$ (min)	90% repolarization: Control[1] (ms)	90% repolarization: Lowest[2] value measured (ms)	90% repolarization: a–b (ms)	90% repolarization: $T_{\frac{1}{2}}$ (min)	Ratio of $T_{\frac{1}{2}}$ $T_{\frac{1}{2}}$ of APD_{20} / $T_{\frac{1}{2}}$ of f.o.c.
$0{\cdot}7\times10^{-7}$ (6)	28±6	30	173±8	146±6	27	50	300±8	289±6	11	50	1·67
$2{\cdot}1\times10^{-7}$ (8)	207±37	19	151±7	92±5	59	30	280±7	245±4	35	34	1·58
$3{\cdot}5\times10^{-7}$ (8)	329±38	23	141±7	56±8	85	37	279±5	235±4	44	33	1·61
$7{\cdot}0\times10^{-7}$ (7)	357±23	12·5	160±11	20±4	140	21	285±10	168±11	117	34	1·68
$2{\cdot}1\times10^{-7}$ (6)[3]	263±28	22	109±4	61±8	48	33	211±7	185±2	26	46	1·50

Number of individual experiments in parentheses. [1], Control value measured at the end of the equilibration period before adding ouabain; [2], value obtained at the end of ouabain exposure (120 min); [3], this group of experiments was performed at the temperature of 37° C. APD_{20} action potential duration at 20% repolarization; f.o.c. force of contraction.

TABLE 2. *Effect of digoxin and some metabolites of digitoxin on the force of contraction and the action potential duration at 20% repolarization in isolated papillary muscles of guinea-pig*

Name of compound	Concentration of drug in the perfusion medium (M)	Force of contraction: Increase in % of control	Force of contraction: $T_{\frac{1}{2}}$ (min)	Action potential duration at 20% repolarization: Control (ms)	Lowest value measured (ms)	Difference (ms)	$T_{\frac{1}{2}}$ (min)	Ratio of $T_{\frac{1}{2}}$ $T_{\frac{1}{2}}$ of APD_{20} / $T_{\frac{1}{2}}$ of f.o.c.
Digoxin	3×10^{-7} (7)	100±17	21·5	180±4	112±11	68	34	1·58
	1×10^{-6} (8)	321±41	15	161±4	24±4	137	27	1·80
Digoxigenin monodigitoxoside	3×10^{-7} (8)	96±14	11	167±6	101±7	66	37	3·36
	5×10^{-7} (7)	135±22	10	183±7	100±4	82	30	3·0
	1×10^{-6} (8)	339±25	8	153±7	42±8	111	29	3·63
Digoxigenin bisdigitoxoside	1×10^{-6} (7)	361±27	15	137±4	14±2	123	30	2·0
Digitoxigenin bisdigitoxoside	1×10^{-7} (6)	211±19	33	138±6	75±6	63	37	1·12

Number of experiments in parentheses. APD_{20}=Action potential duration at 20% repolarization; f.o.c.=force of contraction.

duration, which developed more slowly. Both effects were dose-dependent (Figure 4). At increasing concentrations of the drug, the final effect was reached faster, i.e. the rate of drug action was also dose-dependent. The data for the different half-lives ($T_{\frac{1}{2}}$) are listed in Table 1.

At the concentration of $1{\cdot}4\times10^{-6}$M, ouabain had an initial positive inotropic effect with a half-time of 6 minutes. The $T_{\frac{1}{2}}$ for the shortening of the action potential duration at 20% repolarization was 11 minutes. After 30 min of

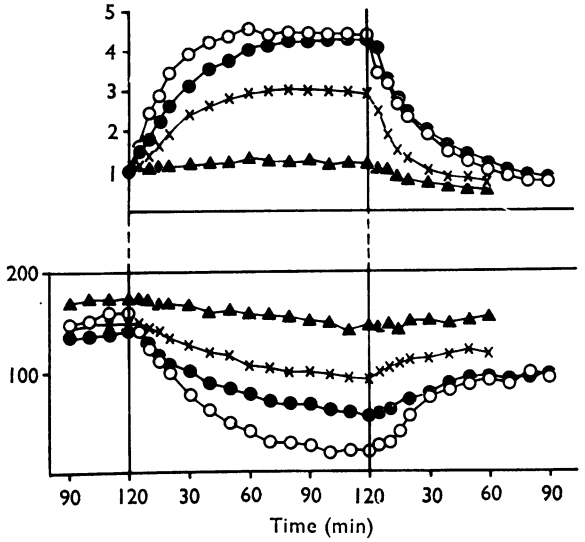

FIG. 4. Time courses of the changes in force of contraction and action potential duration at 20% repolarization after exposure to different concentrations of ouabain: $0{\cdot}7\times10^{-7}$M (▲——▲), $2{\cdot}1\times10^{-7}$M (×—×), $3{\cdot}5\times10^{-7}$M (●——●), 7×10^{-7}M (○- - -○). See also legend to Fig. 2.

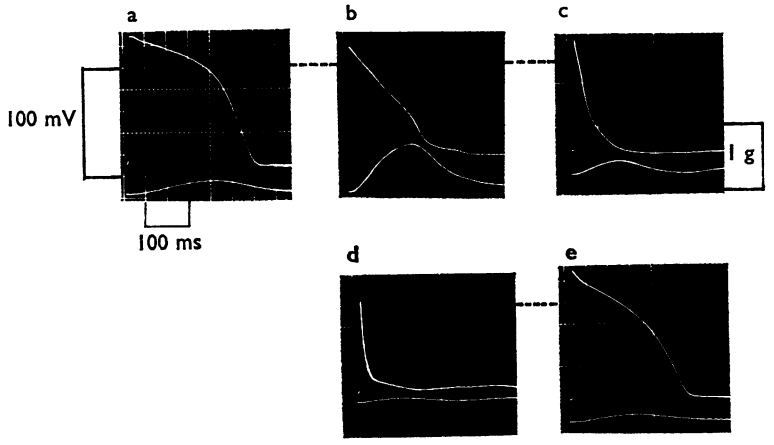

FIG. 5. Original tracing of the transmembrane action potential (upper beam) and the force of contraction (lower beam) of guinea-pig isolated papillary muscle during exposure to a toxic ($1{\cdot}4\times10^{-6}$M) concentration of ouabain. The control recording (a) was taken at the end of an equilibration period of 120 min, the following recordings were taken after 40 min (b), 60 min (c), and 90 min (d) of ouabain action, and after 80 min of wash-out in glycoside-free Tyrode (e). Calibrations are indicated by the bars, the interrupted horizontal line represents the level of zero potential. Note the elevated base-line tension in recordings c and d.

exposure to this high concentration, toxic symptoms occurred, i.e. resting tension increased, the initial enhancement of the contractile force completely vanished, and the action potential approached the shape of a spike (see Figure 5). No signs of arrhythmia could be recorded, and after 90 min of wash-out the actcion potential recovered remarkably. The contracture, however, was not reversed completely.

Effects of digoxin, digoxigenin bisdigitoxoside, and digoxigenin monodigitoxoside

In the same manner as described for ouabain the changes in the contractile force and the action potential duration induced by digoxin (3×10^{-7}M, 5×10^{-7}M), digoxigenin bisdigitoxoside (1×10^{-6}M), and digoxigenin monodigitoxoside (3×10^{-7}M, 5×10^{-7}M, 1×10^{-6}M, 2×10^{-6}M) were analysed. The results are presented in Table 2. They were essentially the same as those observed with ouabain, i.e. an immediate, though more slowly developing shortening in the action potential duration together with the positive inotropic effect. Some quantitative differences in the time course of the changes require a more detailed comparison. For this purpose, the effects of digoxin and digoxigenin monodigitoxoside, both at a concentration of 1×10^{-6}M, are shown together in Figure 6. Comparison of the time courses of action of the two glycosides shows that digoxigenin monodigitoxoside enhanced the force of contraction faster than digoxin and depressed the duration of the action potential not only more slowly but also to a lesser degree. The half times of the equivalent processes Fig. 7A and B) were 8 and 15 min for the increase in the force of contraction and 29 and 27 minutes for the shortening in the action potential duration in the case of digoxigenin monodigitoxoside and digoxin, respectively. This suggests that the rate of augmentation of contractile force is not necessarily linked to a certain rate of shortening of the action potential, but both parameters can be dissociated and

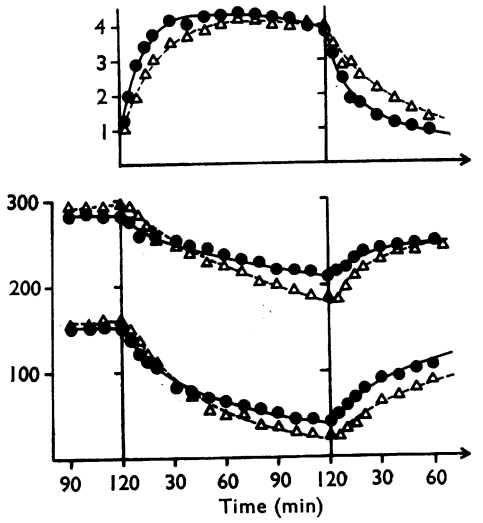

FIG. 6. Comparison of the changes in the force of contraction and the action potential duration at 20% and 90% repolarization as induced by 1×10^{-6}M digoxin (△---△) and 1×10^{-6}M digoxigenin monodigitoxoside (●——●) in guinea-pig isolated papillary muscle. Layout as in Fig. 2. Digoxigenin monodigitoxoside reached the maximal positive inotropic effect earlier than digoxin, but it shortened the action potential duration at 20% and 90% repolarization more slowly and to a lesser degree than digoxin, as can be seen by the crossing over of the curves for the action potential duration.

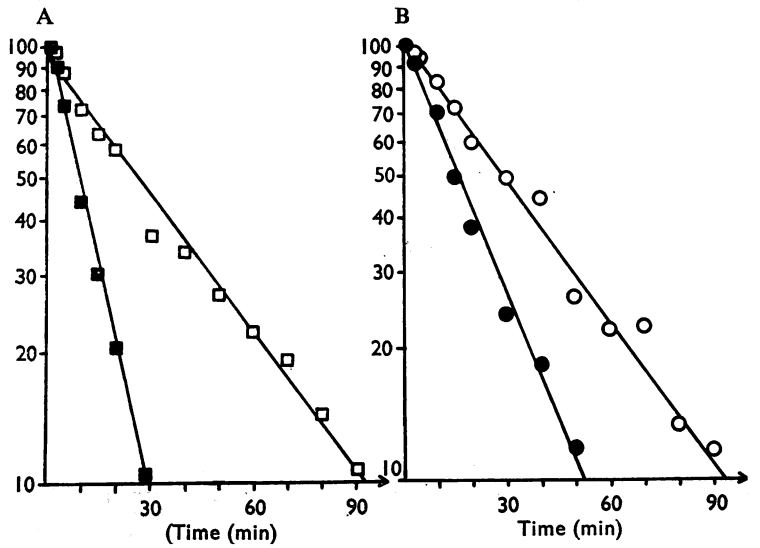

FIG. 7. Comparison of the semilogarithmic plots of the time courses of the glycoside-induced changes in guinea-pig isolated papillary muscle. (A) Digoxigenin monodigitoxoside 1×10^{-6}M: Force of contraction (■——■), T =8 min; action potential duration at 20% repolarization (□——□), $T_{\frac{1}{2}}$ =29 min. (B) Digoxin, 1×10^{-6}M: force of contraction (●——●), $T_{\frac{1}{2}}$=15 min; action potential duration at 20% repolarization (○——○) $T_{\frac{1}{2}}$=27 min.

the degree of dissociation seems to be glycoside-specific. More evidence can be obtained from the ratio of the half times of the depression of the action potential duration to that of the increase in force of contraction. The factors calculated were 1·65 and 3·5 (Table 1, last column and Table 2, last column) for all concentrations of ouabain and digoxigenin monodigitoxoside respectively. The ratios estimated for digoxin and digoxigenin bisdigitoxoside (Table 2, last column) were 1·7 and 2·0 respectively. In other words, digoxigenin monodigitoxoside shortened the action potential duration approximately 3·5 times more slowly than it enhanced contractile force.

Not only the time course but also the absolute depression of the action potential duration at a given increment of contractile force deserves attention, because it also implies a shortening of the refractory period and thus renders the myocardium more liable to answer to ectopic stimuli. In Fig. 8, the enhancement of the force of contraction at three times the $T_{\frac{1}{2}}$ is plotted versus the reduction in action potential duration at the same time, expressed as a percent of the control value at the end of the equilibration period. The graphs of ouabain and digoxigenin monodigitoxoside cross over at higher concentrations, which indicates, that digoxigenin monodigitoxoside shortened the action potential duration at a certain positive inotropic effect to a lesser degree than ouabain. At lower concentrations, however, this relation is reversed. Digoxin had in any case the most 'unfavourable' ratio, i.e. at any given increment of contraction amplitude, the shortening of action potential was more pronounced.

Membrane potential and action potential amplitude

At high concentrations, all cardiac glycosides studied decreased the action potential amplitude as well as the membrane potential. The data are presented in Table 3. The decline in the amplitude of the action potential takes place

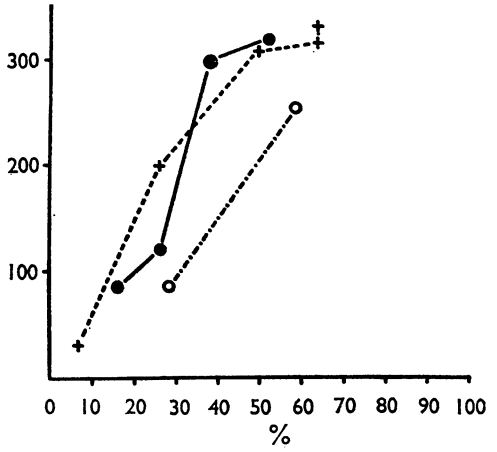

FIG. 8. Increase in force of contraction (ordinates) measured at three times the value of $T_{\frac{1}{2}}$ plotted against the decrease in action potential duration at 20% repolarization, expressed as % of control values measured at the same instant (abscissae). (●——●) digoxigenin mondigitoxoside (+ - - - +) ouabain, and (○.-.○) digoxin. Points to the right of the figure are for increasing concentration of drug.

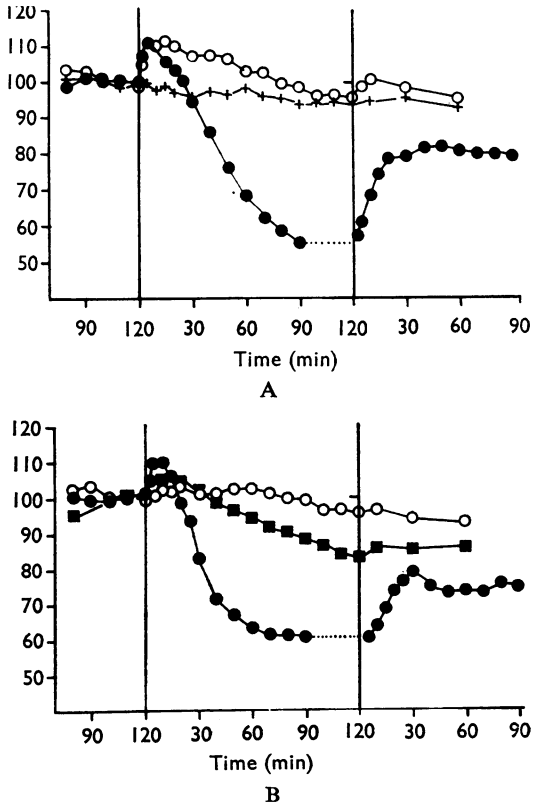

FIG. 9. The time course of the change in time-to-peak tension after exposure of guinea-pig isolated papillary muscles to different concentrations of digoxigenin monodigitoxoside (A) and ouabain (B). Ordinates: time-to-peak tension in % of the control (100% = the mean of the values measured at 90, 100, 110 and 120 min of the equilibration period). Abscissae: time in minutes, vertical lines denote exposure to the drug. (A) Digoxigenin monodigitoxoside 3×10^{-7}M (○ - - - ○), 2×10^{-6}M (●——●), control, i.e. no drug added (+——+). (B) Ouabain $0{\cdot}7\times10^{-7}$M (○——○), 7×10^{-7}M (■ - - - ■), and $1{\cdot}4\times10^{-6}$M (● - - - ●).

TABLE 3. *The effect of cardiac glycosides on the membrane potential and the amplitude of the action potential of isolated papillary muscles of guinea-pig*

Compound	Concentration of drug in the perfusion medium (M)	Membrane potential Control[1] (mV)	Membrane potential Glycoside[2] (mV)	Amplitude of action potential Control (mV)	Amplitude of action potential Glycoside (mV)
Ouabain	$0{\cdot}7\times10^{-7}$ (6)	95·1±1·1	92·5±0·5*	124·5±1·3	120·1±1·6*
	$2{\cdot}1\times10^{-7}$ (8)	92·9±0·8	91·8±1·4	123·1±1·2	117·1±2·1*
	$3{\cdot}5\times10^{-7}$ (8)	95·6±1·1	92·3±0·9*	125·9±1.8	114·3±1·2†
	$7{\cdot}0\times10^{-7}$ (7)	96·7±1·1	88·6±1·3†	127·0±1·8	102·8±1·5†
Digoxin	3×10^{-7} (7)	91·3±1·0	91·7±0·8	122·6±1·8	119·9±2·0
	1×10^{-6} (8)	93·2±1·1	82·1±0·9†	122·6±2·2	99·9±1·1†
Digoxigenin monodigitoxoside	3×10^{-7} (8)	94·2±0·8	95·6±1·1	126·9±1·7	125·1±2·1
	5×10^{-7} (7)	93·5±1·0	91·4±1·7	123·8±1·7	116·4±2·6*
	1×10^{-6} (8)	95·4±1·0	90·7±1·2**	121·7±1·5	110·6±2·1†
Digoxigenin bisdigitoxoside	1×10^{-6} (7)	95·3±1·4	90·6±1·8*	128·9±1·9	107·7±2·5†
Digitoxigenin bisdigitoxoside	1×10^{-7} (6)	98·0±0·9	95·0±1·4	130 ±1·1	121·8±2·1**

Results (mean±S.E.M.) were obtained from at least 3 measurements in each preparation. In parentheses: number of experiments in each group. [1], Control values measured from 90–120 min of the equilibration period before adding the glycoside; [2], Mean value obtained at the end of ouabain exposure (90–120 min). *$P<0{\cdot}05$; **$P<0{\cdot}01$; †$P<0{\cdot}001$.

mainly at the expense of the overshoot. Nevertheless, the membrane potential became depolarized only at advanced stages of glycoside action. It was fully reversible during wash-out and thus cannot be responsible for the inability of the action potential to recover fully.

Time-to-peak tension

The time-to-peak tension of isolated papillary muscles is influenced by cardiac glycosides in a biphasic manner. In Fig. 9A, the time-to-peak tension of the control group declined with time. At the non-toxic concentrations of digoxigenin monodigitoxoside (3×10^{-7}M, 5×10^{-7}M, 1×10^{-6}M) the time-to-peak tension was prolonged initially and was later shortened. This shortening is very pronounced at the toxic concentration of 2×10^{-6}M. For ouabain, similar findings are illustrated in Figure 9B.

Discussion

The results presented confirm the findings of Kassebaum (1963), Prasad *et al.* (1971) and Prasad (1972) that ouabain, as well as the other cardiac glycosides studied in the present paper, cause an immediate, dose-dependent shortening of the action potential. Furthermore, this effect has a different time course from that of the positive inotropic action. In fact, the rate of shortening of the action potential is always slower than the rate of increment of contractile force. The degree of dissimilarity as expressed by the ratio of the $T_{\frac{1}{2}}$ of either effect was surprisingly constant for each individual glycoside. This indicates that the positive inotropic effect is not necessarily linked to certain alterations in the action potential and that the two processes are probably independent of each other. The phenomenon of dissociation between the two rates and the particular degree of shortening at a given inotropic response for each individual cardiac glycoside makes it tempting to speculate that for further development of cardioactive drugs, a substance should be searched for, which does not only reduce

the action potential duration to a minimum degree, but also has a very slow time course of alteration.

As pointed out above we regard the shortening of the action potential as an unwanted side effect which is associated with a shortening of refractory period. This in turn is one requirement for re-excitation by ectopic impulses which from our findings do not originate in the myocardium of papillary muscles. Even at clearly ' toxic ' doses at which contracture developed we did not observe any extrasystoles with the exception of a few instances when microelectrodes with broken tips were used, thus mechanically irritating the muscle. Ventricular extrasystoles which are frequently seen in cardiac glycoside-induced arrhythmias probably originate in Purkinje fibres, because they are more sensitive to ouabain poisoning than papillary muscles (Hoffman & Singer, 1964). Using either preparation from the same heart, Vassalle *et al.* (1962) could demonstrate that Purkinje fibres do react to ouabain intoxication with extrasystoles before they become inexcitable. The papillary muscles in these experiments ceased to respond to stimulation after a much longer period of incubation during which the action potential duration shortened progressively, but no arrhythmias occcurred.

In an attempt to explain the observed changes in contractile force and shape of action potential, three well established effects of cardiac glycosides on ion metabolism should be considered:

(a) increase in Ca turnover,

(b) increase in passive membrane permeability for K^+ and Na^+,

(c) inhibition of Mg-dependent, Na^+ and K^+ activated membrane ATPase.

which are discussed in detail below.

Since the inotropic action of cardiac glycosides is thought to be produced in principle by an enhanced release of calcium ions per beat, the influence on myocardial calcium turnover has been studied extensively (Lüllmann & Holland, 1962; Klaus & Kuschinsky, 1962; Langer & Serena, 1970). Using radiocalcium Lüllmann & Holland (1962) and Klaus & Kuschinsky (1962) clearly showed that ' therapeutic ' concentrations of cardiac glycosides altered neither the efflux of Ca^{++} nor the total calcium content significantly, but markedly increased the exchangeable Ca^{++}-fraction, i.e. the calcium involved in release and rebinding during the contractile cycle. This increase in exchangeable fraction can only be demonstrated in contracting preparations and is therefore dependent on excitation. However, from the Ca^{++} uptake experiments it seems unlikely that more Ca^{++} enters the cell during excitation, but excitation rather facilitates the release of Ca^{++} from within the cell, for example from membrane structures. Also in voltage-clamp experiments, no increase in slow inward calcium current, which is the main charge carrier during the plateau phase of the action potential (Beeler & Reuter, 1970a), could be measured when exposing the preparations to cardiac glycosides (Schobe, 1971).

With the voltage-clamp method, an ion current through the plasma membrane is measured, and therefore, no effect of cardiac glycoside upon the calcium current should be expected, because these drugs enhance the release of Ca^{++} from binding sites, and not the amount of calcium passing through the cell membrane.

As demonstrated by Klaus, Kuschinsky & Lüllman (1962) by means of ^{42}K, and by Dudel & Trautwein (1958) and Kassebaum (1963) using microelectrodes, cardiac glycosides directly increase the passive membrane permeability for K^+. The lower resistance to potassium ions measured should cause an enhanced K^+

efflux from the cell and thus shorten the time needed for repolarization (Weidmann, 1956). The shortening of the action potential duration induced by cardiac glycosides can be thought to reflect an increase of the ion permeability during excitation. Whether or not K^+ ions are the only ion species involved cannot be decided at the moment, because there is some evidence, that the different ions do not only act as charge carriers, but may also influence the movement of other ions though the cell membrane (Beeler & Reuter, 1970b).

In contrast to other authors we do not think the simple inhibition of Na^+ and K^+-activated membrane ATPase is responsible for the shortening of the action potential (Hecht, 1970 ; Lee, Shin, Kang & Chien, 1970), since too many additional assumptions have to be made, for example compartmentation of ions inside as well as outside the cell membrane. However, with high concentrations of cardiac glycosides the inhibition of the Na^+- and K^+-activated ATPase certainly will reduce the effectiveness of the ion pump during the resting phase of the cardiac cycle leading to diminished ionic gradients and to depolarization. The suggestion of a causal relationship between the positive inotropic effect of cardiac glycosides and the inhibitory action on Na^+- and K^+-activated ATPase is based on the good correlation between these two parameters (Machova, 1960 ; Repke & Portius, 1963 ; Akera, Larson & Brody, 1970 ; Besch, Allen, Glick & Schwartz, 1970 ; Lee *et al.*, 1970). Here, too, additional assumptions are necessary in order to explain why an increase in the cellular Na-concentration and a decrease of the cellular K-concentration should lead to an inotropic response, since many compounds and conditions produce identical alterations of the Na- and K-distribution but fail to augment the force of contraction.

The biphasic effect of cardiac glycosides on the time-to-peak tension might be explained as follows: The positive inotropic effect develops not only by means of an increase in the maximal rate of tension but also through an increase of time-to-peak tension. The time-to-peak tension, however, is not independent of the duration of the action potential which is shortened by cardiac glycosides. Thus one effect prevails depending on the concentration and on the stage of drug action. At the beginning of exposure, the time-to-peak tension is prolonged, but as the action potential exerts an increasingly depressing influence, the time-to-peak tension is markedly reduced.

The two effects observed with non-toxic concentrations of cardiac glycosides probably reflect an enhanced release of Ca per beat and an increase in passive permeability to K^+. Whether these two processes are based on a common mechanism is questionable. The different time courses and the different ratios for individual glycosides rather suggest that there is more than one site of action of cardiac glycosides. At the present stage of knowledge a relationship between them cannot be established with certainty.

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