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# Potassium changes the relationship between receptor occupancy and the inotropic effect of cardiac glycosides in guinea-pig myocardium

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**1** $K^+$ (2.4–15.6 mmol $l^{-1}$) antagonized the positive inotropic effect of dihydro-ouabain. The concentration-effect curves became steeper with the shift to higher concentrations of the glycoside. At 1.2 mmol $l^{-1}$ $Ca^{2+}$, an increase in $K^+$ from 2.4 to 12 mmol $l^{-1}$ required tenfold higher concentrations of dihydro-ouabain to produce equal inotropic effects. This factor was reduced to four at 3.2 mmol $l^{-1}$ $Ca^{2+}$. The same change in $K^+$ concentration, at 1.2 mmol $l^{-1}$ $Ca^{2+}$, diminished the inotropic effect of ouabain on rested-state contractions by a factor of six.

**2** The positive inotropic effect of $Ca^{2+}$ was also antagonized by $K^+$ (1.2–12 mmol $l^{-1}$). Reduction of $Na^+$ from 140 to 70 mmol $l^{-1}$ abolished the antagonistic action of $K^+$ (1.2–8.0 mmol $l^{-1}$). Moreover the inotropic effect of $Ca^{2+}$ was enhanced.

**3** Reduction of $Na^+$, from 140 to 70 mmol $l^{-1}$, antagonized the positive inotropic effect of dihydro-ouabain more at low (2.4 mmol $l^{-1}$) than at high (8.0 mmol $l^{-1}$) $K^+$. Accordingly, the extent of the dihydro-ouabain-$K^+$ antagonism was reduced.

**4** When the $K^+$ concentration was increased from 2.4 to 12 mmol $l^{-1}$, [$^3$H]-ouabain binding was reduced by a factor of three. This is less than the reduction in the inotropic effectiveness of ouabain or dihydro-ouabain.

**5** Reduction of stimulation frequency from 1 to 0.1215 Hz did not significantly alter the antagonistic effect of $K^+$. Diminution of $\dot{V}_{max}$ of the action potential was observed only at $K^+$ concentrations greater than 5.9 mmol $l^{-1}$, whereas the resting membrane potential was continuously depolarized over the entire range of $K^+$ concentrations.

**6** The results support the view that the reduction in receptor affinity cannot be the sole cause of the antagonism between the glycoside and $K^+$. Impairment of passive $Na^+$ influx during diastole, due to the $K^+$-dependent depolarization of the resting membrane potential, contributed to about one half of the glycoside-$K^+$ antagonism.

## Introduction

Studies with ion-selective microelectrodes have provided convincing evidence that cardiac glycosides increase intracellular $Na^+$ activity in mammalian myocardium, probably by inhibition of active $Na^+$ extrusion from the cell via the ATP-driven $Na^+$ pump (Ellis, 1977; Deitmer & Ellis, 1978; Lee *et al.*, 1980; Cohen *et al.*, 1982; Daut, 1982; Sheu & Fozzard, 1982; Wasserstrom *et al.*, 1983). This effect correlates with an increase in intracellular $Ca^{2+}$ (Sheu & Fozzard, 1982; Allen *et al.*, 1984) and the development of a positive inotropic effect (Lee *et al.*, 1980; Wasserstrom *et al.*, 1983).

Increases in $K^+$ concentration inhibit the binding of cardiac glycosides (e.g., Gardner & Conlon, 1972), stimulate the $Na^+$ pump (for review see Glitsch, 1979), reduce $Na^+$ influx (Ellis, 1977; Eisner *et al.*, 1981), and possibly alter electrogenic $Na^+$–$Ca^{2+}$ exchange (Reeves & Sutko, 1980; Mullins, 1981). Thus, changes in $K^+$ may also alter the positive inotropic effect of cardiac glycosides. However, the reports on the influence of $K^+$ on the positive inotropic effect of cardiac glycosides are conflicting. Reiter *et al.* (1966) observed an antagonism, whilst Prindle *et al.* (1971)

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detected only a retardation in the development of the inotropic effect of digoxin and no change in the maximum response. When summarizing the then available evidence, Lee & Klaus (1971) concluded 'the toxic effect of CG (= cardiac glycosides) is always antagonized by K but the inotropic effect is not necessarily influenced by K'. Akera *et al.* (1978) reached a similar conclusion, that 'KCL delays the development of the positive inotropic action of glycosides, but has a relatively minor action on the magnitude of the inotropic action at steady state'.

We have, therefore, evaluated the influence of $K^+$, over a wide range of concentrations, on the positive inotropic effect of dihydro-ouabain. The aim of the study was to differentiate between the contribution of $K^+$-induced changes in digitalis-receptor affinity and in $Na^+$ fluxes in the $K^+$-glycoside antagonism. Since a dependence of $Na^+$ fluxes on $K^+$ may interfere with the effects of various inotropic agents, the cardiotonic effects of $Ca^{2+}$ in the presence of different $K^+$ concentrations were also investigated. A preliminary account of some of the results has appeared previously (Ebner *et al.*, 1983).

## Methods

### *Mechanical and electrical experiments*

Papillary muscles (diameter $< 1$ mm) from the right ventricle of guinea-pigs (250–350 g) of either sex were mounted in a two-chambered vessel with internal circulation of the bath medium. Isometric contractions were elicited (resting force 3.96 mN) by electrical square-wave pulses of 3 ms duration and at an intensity slightly above stimulation threshold. Unless stated otherwise the stimulation frequency was maintained at 1 Hz, temperature at 35°C, and pH at 7.5. Transmembrane potentials were recorded by means of conventional glass microelectrodes filled with 3 mol $l^{-1}$ KCl, resistance 10–20 MΩ. The signals and their first derivatives were displayed on an oscilloscope. The twitch shape characteristics were evaluated from the tracings of a pen recorder at a chart speed of 250 mm $s^{-1}$. The bath solution had, for standard conditions, the following composition (in mmol $l^{-1}$): NaCl 114.9, $NaHCO_3$ 24.9, KCl 4.7, $KH_2PO_4$ 1.2, $CaCl_2$ 3.2, $MgSO_4$ 1.2 and glucose 10; changes in the concentrations of $K^+$, $Na^+$, and $Ca^{2+}$ were achieved by adjustment with KCl, NaCl, or $CaCl_2$, respectively. The small changes in osmolarity remained uncorrected since addition of sucrose in comparable concentrations had no influence on force of contraction. When $Na^+$ was reduced from 140 to 70 mmol $l^{-1}$, osmotic adjustment was obtained by addition of 140 mmol $l^{-1}$ sucrose. The results were similar, when 70 mmol $l^{-1}$ choline chloride in the presence of 1 μmol $l^{-1}$ atropine sulphate was used instead of sucrose. The medium was gassed and circulated with 5% $CO_2$ in $O_2$.

To avoid the release of endogenous noradrenaline, all inotropic effects of $Ca^{2+}$ and those of dihydro-ouabain at $K^+ > 9.6$ mmol $l^{-1}$ were determined on muscles from animals pretreated with reserpine (5 mg $kg^{-1}$ body weight, injected intraperitoneally 24 h before the guinea-pigs were killed). At the lower concentrations of $K^+$, pretreatment with reserpine was without influence on the inotropic effect of dihydro-ouabain (Ebner & Reitner, 1979). The muscles were equilibrated for 1 h at a stimulation frequency of 1 Hz in the standard solution. Only a single cumulative dihydro-ouabain concentration-effect curve was obtained from each muscle. The inotropic effect of ouabain on rested-state contractions of reserpinized papillary muscles was evaluated after the stimulation frequency was reduced to 0.001 Hz. Only one ouabain concentration was applied to each muscle.

The possibility that deviation in $K^+$ concentration in the extracellular space from the bath concentration could interfere with the antagonism between $K^+$ and the glycoside effect was studied by relating the inotropic effect to muscle diameter. After the initial equilibration period, $K^+$ concentration and stimulation frequency were reduced to 2.4 mmol $l^{-1}$ and 0.5 Hz, respectively. When the inotropic response to dihydro-ouabain ($10^{-5}$ mol $l^{-1}$) had peaked, $K^+$ was raised to 9.6 mmol $l^{-1}$. The muscle diameter at both ends was then determined using a microscope (Zeiss, Oberkochen; Stereotubus I B with Okularmikrometer 10.100) attached to the experimental set-up. The antagonistic effect of $K^+$, i.e., the ratio of force values at 2.4 over 9.6 mmol $l^{-1}$ $K^+$, was found to be independent of the muscle diameter.

In experiments examining the effects of stimulation frequency on dihydro-ouabain-induced positive inotropism, the muscles were driven at 0.5 Hz. Once the response to dihydro-ouabain had reached a steady state, stimulation frequency was reduced to 0.125 Hz, then set back at 0.5 Hz and after the previous steady state had been regained, the next concentration of the drug was added in a cumulative manner.

The increase in peak force of contraction ($\Delta F_c$) over the predrug force was expressed as a percentage of the maximal increase induced by dihydro-ouabain. The data for the effects of $Ca^{2+}$ or stimulation frequency are presented as a percentage of the steady-state value of the initial equilibration period. Half-maximally effective concentrations ($EC_{50}$) and slope (P′) of individual concentration-effect curves were computed by fitting the data to $E = 100\,A^{P'}\,(A^{P'} + EC_{50}{}^{P'})^{-1}$, (Parker & Waud, 1971); where E = effect, 100 = maximal effect, and A = concentration of dihydro-ouabain. P′ is directly proportional to the maximal slope of semi-logarithmically plotted concentration-

effect curves. Linear regressions were calculated according to the method of least squares. The data are presented as the individual values or as arithmetic means ± s.e.mean.

*Binding experiments*

Quiescent papillary muscles from reserpinized guinea-pigs were incubated under conditions similar to the mechanical experiments. After a 30–45 min equilibration period, [$^3$H]-ouabain (18.5 kBq 15 ml$^{-1}$ medium) and [$^{14}$C]-sorbitol (18.5 kBq 15 ml$^{-1}$ medium) were added to the muscles. [$^{14}$C]-sorbitol was always left in contact with the tissues for 30 min since the sorbitol space remained unchanged during longer incubation periods. [$^3$H]-ouabain was added for variable periods of time. At the end of the incubation the muscles were cut from the apparatus, rinsed, gently blotted and weighed. After digestion in 1 ml Protosol for 2–4 h at 55°C and after the addition of 100 µl acetic acid followed by 10 ml of the scintillation mixture Econofluor, the samples were immediately counted in a Beckman 7500 liquid scintillation counter. Quench corrections were made by the external standard method. Radioactivity of samples (500 µl) of the bath solution was determined in 10 ml Biofluor. Since, at a high concentration of unlabelled ouabain (2.5 µmol l$^{-1}$) at 12 mmol l$^{-1}$ K$^+$, [$^{14}$C]-sorbitol space and [$^3$H]-ouabain binding were identical after 1 and 2 h of incubation, the 'unspecific' binding of ouabain corresponds to the sorbitol space. Therefore, the 'specifically' bound ouabain was determined by subtracting from the total amount of [$^3$H]-ouabain recovered in the tissue, the amount corresponding to the individual sorbitol space. The data were shown as arithmetic means ± s.e.mean of the tissue/medium ratios (d.p.m. mg$^{-1}$ wet weight of tissue) divided by (d.p.m. µl$^{-1}$ bath solution), i.e., µl mg$^{-1}$ wet weight.

*Drugs and materials*

Dihydro-ouabain (Hommel AG, Adliswil, Switzerland), ouabain (Serva, Heidelberg, Germany), $CaCl_2$, and KCl were added as aqueous solutions. Reserpine (Serva, Heidelberg, Germany) was dissolved in 5% ascorbic acid. [$^3$H]-ouabain (specific

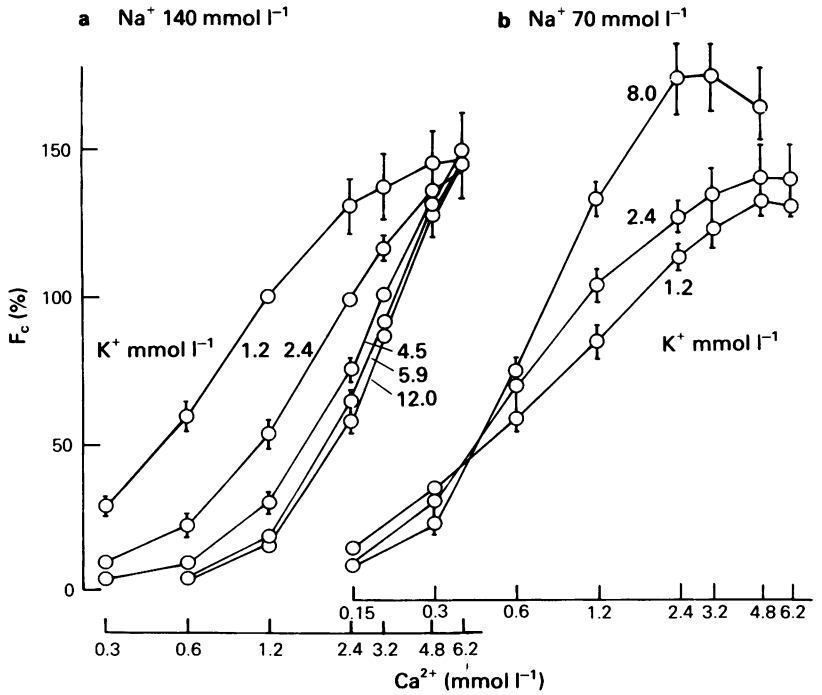


**Figure 1** The dual action of $K^+$ on the positive inotropic effect of $Ca^{2+}$: the influence of $Ca^{2+}$ on force of contraction ($F_c$) is shown at different $K^+$ concentrations at 140 mmol l$^{-1}$ $Na^+$ in (a) and 70 mmol l$^{-1}$ $Na^+$ in (b). Cumulative concentration-effect curves were constructed in reserpinized papillary muscles which were stimulated at a frequency of 1 Hz. Shown are arithmetic means; some s.e.mean are inserted in the graph as an example ($n$ = 5–6). Ordinates: force of contraction ($F_c$) as percentage of the value after the initial equilibration period at standard conditions (100% $F_c$ = 15.0 ± 0.65 mN) Abscissae: $Ca^{2+}$ concentration, mmol l$^{-1}$, (log scale).

activity 666 GBq mmol $l^{-1}$), [$^{14}$C]-sorbitol (specific activity 12.802 GBq $mmol^{-1}$), the tissue solubilizer Protosol (which, according to the manufacturer, contains quaternary hydroxides <50%, methanol <25%, methylate aromatics >25%), and scintillation mixtures Econofluor and Biofluor were purchased from New England Nuclear (Dreieich, Germany).

## Results

### *The positive inotropic effect of $Ca^{2+}$ and its dependence on $K^+$*

At a low concentration (1.2 mmol $l^{-1}$) of $K^+$ (140 mmol $l^{-1}$ $Na^+$; Figure 1a) $Ca^{2+}$ had a mean $EC_{50}$ value of 0.9 ± 0.08 mmol $l^{-1}$ for the positive inotropic effect. Increasing the concentration of $K^+$, impeded the development of the inotropic effects of $Ca^{2+}$. This antagonism was saturable, since $K^+$ concentrations higher than 4.5–5.9 mmol $l^{-1}$ did not produce further rightward shifts. The $Ca^{2+}$ concentration-effect curves at a high $K^+$ concentration failed to reach a maximum at a calcium concentration of 6.2 mmol $l^{-1}$. In agreement with previous findings (Reiter *et al.*, 1971) the inhibitory effects of $K^+$ were abolished by lowering $Na^+$ (Figure 1b). Low concentrations of $K^+$ augmented the positive inotropic effect of $Ca^{2+}$ without shifting the position of the concentration-effect curves, i.e., $K^+$ had a positive inotropic effect. Analysis of the shape of the isometric contraction, at 2.4 mmol $l^{-1}$ $Ca^{2+}$, showed that the positive inotropic effect of $K^+$ was accompanied by a retardation of relaxation (relaxation times, determined at 10% of peak force, were: 137 ± 2 ms at 1.2 mmol $l^{-1}$ $K^+$ vs. 164 ± 9 ms at 8 mmol $l^{-1}$ $K^+$; $P<0.02$), whereas the time to peak force was not changed (118 ± 5 ms at 8 mmol $l^{-1}$ $K^+$ vs. 123 ± 2 ms at 1.2 mmol $l^{-1}$ $K^+$). The influence of $K^+$ on the shape of the isometric contraction was similar at other concentrations of $Ca^{2+}$.

### *The positive inotropic effect of dihydro-ouabain and its dependence on $K^+$*

The positive inotropic effect of dihydro-ouabain was dependent on $K^+$ (Figure 2). The antagonism was surmountable, since the maximal effects could still be obtained. At low $K^+$ (2.4 mmol $l^{-1}$) dihydro-ouabain had a mean $EC_{50}$ value of 8.8 ± 0.9 μmol $l^{-1}$. When $K^+$ was raised to 12.0 mmol $l^{-1}$, the inotropic potency of dihydro-ouabain decreased tenfold. Furthermore, $K^+$ was more potent as an antagonist against small, rather than large inotropic effects as indicated in Figure 2 by arrows of identical length. Consequently the shape of the concentration-effect curves changed under the influence of $K^+$. This observation was

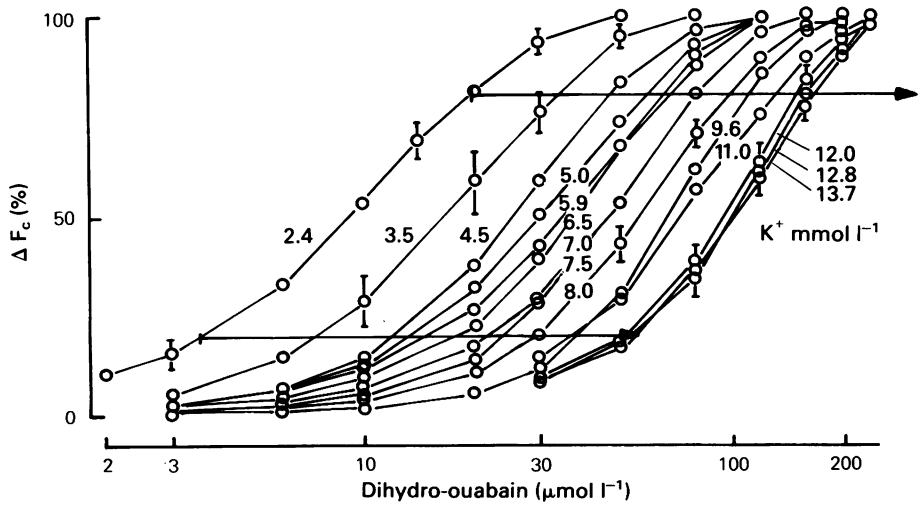


**Figure 2** The effect of $K^+$ on the positive inotropic effect of dihydro-ouabain: the positive inotropic effect of dihydro-ouabain (Δ $F_c$) is shown at different $K^+$ concentrations. Papillary muscles from non-pretreated guinea-pigs were used in the experiments at $K^+$ concentrations <9.6 mmol $l^{-1}$; for higher $K^+$ concentrations the animals were pretreated with reserpine. The muscles were stimulated at a frequency of 1 Hz, $Na^+$ 140 mmol $l^{-1}$, $Ca^{2+}$ 1.2 mmol $l^{-1}$. Arithmetic means and some s.e.means are presented ($n$ = 5–8). Ordinate scale: positive inotropic effect of dihydro-ouabain (Δ $F_c$) as a percentage of the maximal response (100% Δ $F_c$ = 18.84 ± 0.52 mN). Abscissa scale: dihydro-ouabain concentration, μmol $l^{-1}$ (log scale).

analysed more quantitatively by correlating the logarithms of slope P′ with the $EC_{50}$ of the individual concentration-effect curves. The correlation (Figure 3) shows that the curves became steeper with the shift to the right. As a further criterion of the antagonism the relationship between the $EC_{50}$ values for dihydro-ouabain and $K^+$ concentration was determined (Figure 4). At 1.2 mmol $l^{-1}$ $Ca^{2+}$ (data from the experiments of Figure 2) the relationship appeared to be linear, as one would expect if dihydro-ouabain and $K^+$ were to compete for a common binding site. However, statistical analysis of the data revealed that linearity must be rejected ($P < 0.001$). The nonlinear

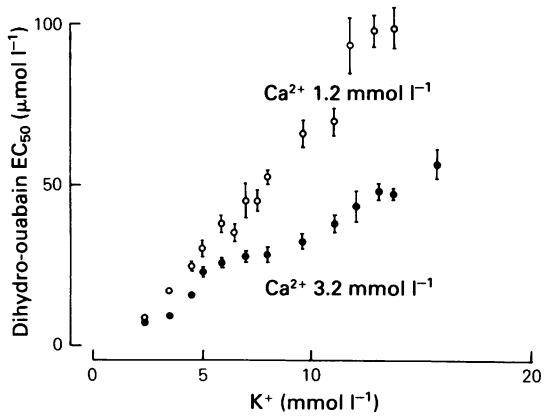


**Figure 4** The relationship between half-maximally effective concentrations ($EC_{50}$) of dihydro-ouabain and $K^+$ concentration: the data at 1.2 mmol $l^{-1}$ $Ca^{2+}$ (○) were obtained from the experiments of Figure 2, the values at 3.2 mmol $l^{-1}$ $Ca^{2+}$ (●) from separate experiments, otherwise under identical experimental conditions. Shown are arithmetic means of 5–12 muscles; s.e.means shown by vertical lines. Ordinate scale: $EC_{50}$ of dihydro-ouabain concentration-effect curves (in μmol $l^{-1}$) (linear scale). Abscissa scale: $K^+$ concentration, mmol $l^{-1}$ (linear scale).

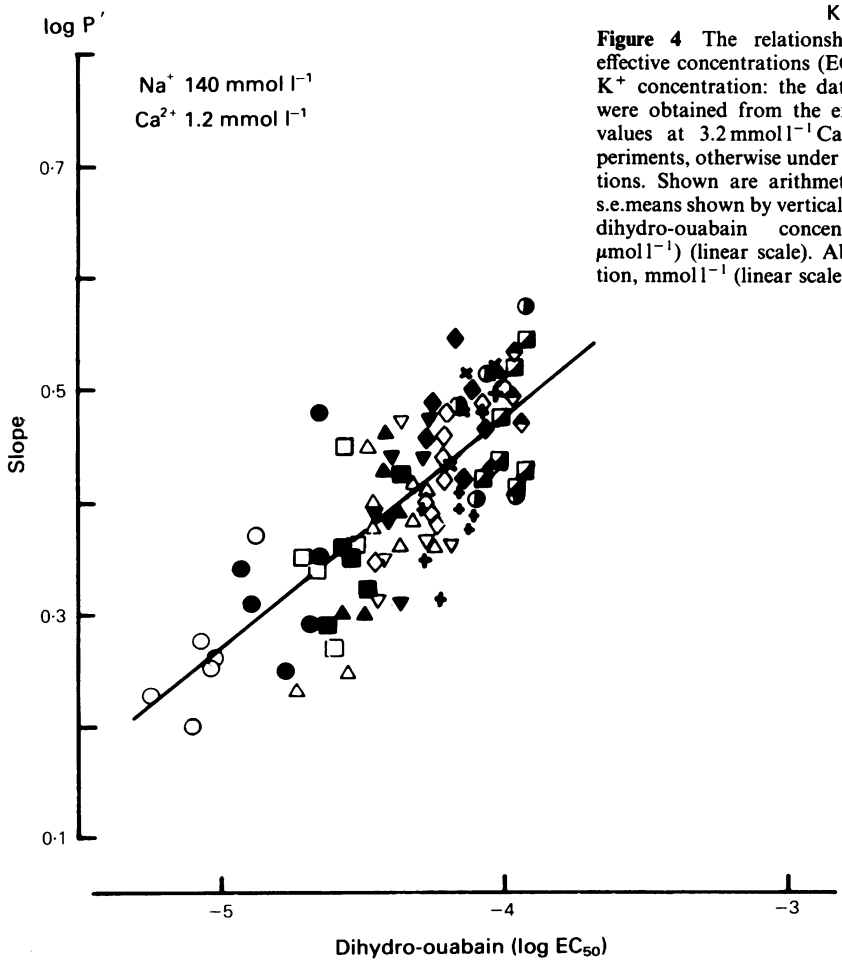


**Figure 3** The relationship between slope and half-maximally effective concentrations ($EC_{50}$) of dihydro-ouabain concentration-effect curves at different $K^+$ concentrations: the slopes (P′) of individual concentration-effect curves were correlated with the corresponding $EC_{50}$ values. $K^+$ (in mmol $l^{-1}$) (○) 2.4, (●) 3.5, (□) 4.5, (■) 5.0, (△) 5.9, (▲) 6.5, (▽) 7.0, (▼) 7.5, (◇) 8.0, (◆) 9.6, (+) 11.0, (◑) 11.6, (×) 12.0, (◈) 12.8, (◪). 13.7 Data are from the experiments of Figure 2. Regression: log P′ = (1.27 ± 0.005) + (0.20 ± 0.02) log $EC_{50}$; $r = 0.74$, $P < 0.001$. Ordinate scale: slopes, log P′. Abscissa scale: log $EC_{50}$ (mol $l^{-1}$ dihydro-ouabain).

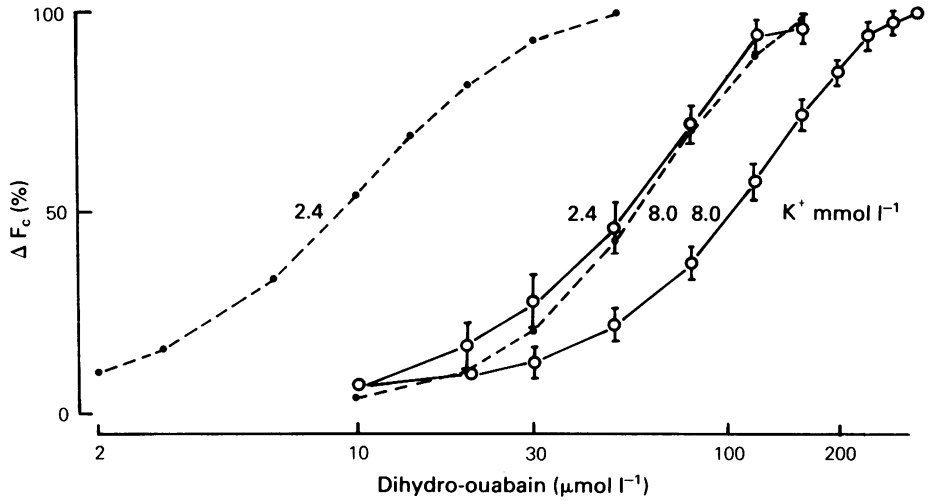


**Figure 5** The dihydro-ouabain-$K^+$ antagonism on force of contraction at reduced $Na^+$ (70 mmol $l^{-1}$): the dihydro-ouabain concentration-effect curves (O, continuous lines) were obtained under experimental conditions identical to those of Figure 2, except that $Na^+$ and $Ca^{2+}$ concentrations were reduced to (in mmol $l^{-1}$) $Na^+$, 70; $Ca^{2+}$, 0.6; osmotic adjustment was by 140 mmol $l^{-1}$ sucrose. For the sake of comparison the corresponding curves from Figure 2 at 140 mmol $l^{-1}$ $Na^+$ have also been inserted (●, broken lines). The concentration-effect curves were obtained cumulatively with different groups of muscles ($n$ = 5–8) from animals pretreated with reserpine. They were stimulated with a frequency of 1 Hz. Shown are arithmetic means; s.e.means shown by vertical lines. Ordinate scale: positive inotropic effect of dihydro-ouabain (Δ $F_c$) as a percentage of the maximal response (100% Δ $F_c$ = 11.02 ± 1.03 mN). Abscissa scale: dihydro-ouabain concentration, μmol $l^{-1}$ (log scale).

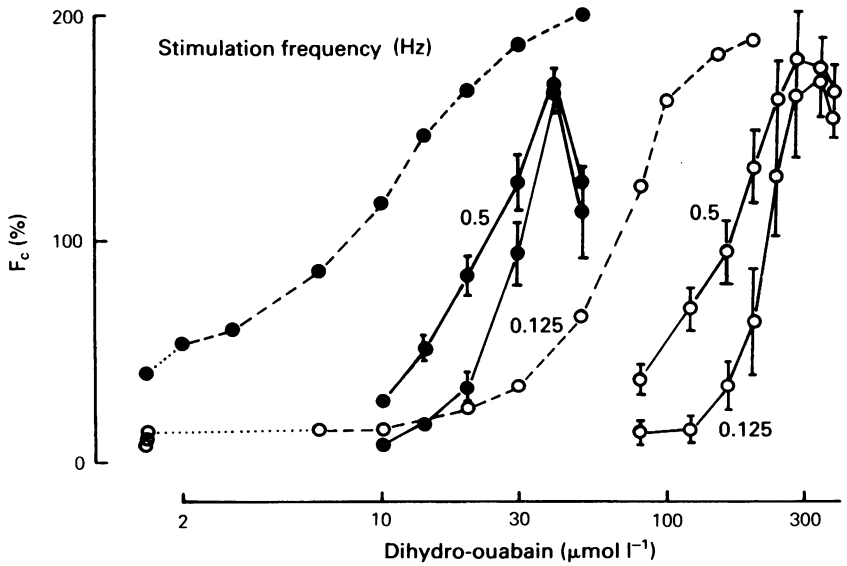


**Figure 6** The dihydro-ouabain-$K^+$ antagonism, on force of contraction, at reduced stimulation frequency: the curves at reduced stimulation frequencies (0.5–0.125 Hz; through lines) were obtained as described in Methods. The curves with broken lines at 1 Hz, are from the experiments described in Figure 2. $Na^+$, 140 (mmol $l^{-1}$); $Ca^{2+}$, 1.2 (mmol $l^{-1}$); $K^+$, (●) 2.4, (O) 9.6 (mmol $l^{-1}$). Arithmetic means of 7–8 preparations are presented; s.e.means shown by vertical lines. Ordinate scale: force of contraction ($F_c$) as percentage of the value after the initial equilibration period at standard conditions (100% $F_c$ = 10.56 ± 0.94 mN). Abscissa scale: dihydro-ouabain concentration, μmol $l^{-1}$ (log scale).

relationship between the $EC_{50}$ value and $K^+$ became more obvious when the antagonism was determined at 3.2 mmol $l^{-1}$ $Ca^{2+}$ (Figure 4). Despite the higher basal force of contraction, the potency of dihydro-ouabain at low $K^+$ concentrations remained nearly unchanged. In contrast to the situation at 1.2 mmol $l^{-1}$ $Ca^{2+}$, the slopes of the concentration-effect curves at 3.2 mmol $l^{-1}$ $Ca^{2+}$ did not change significantly until $K^+$ concentrations greater than 7 mmol $l^{-1}$ were used. Then the concentration-effect curves of dihydro-ouabain become steeper (log $P' = (1.53 \pm 0.008) + (0.286 \pm 0.06) \log EC_{50}$, $r = 0.60$, $P < 0.001$). Furthermore, the antagonistic effect of $K^+$ was also diminished.

*Dihydro-ouabain-$K^+$* antagonism in reduced $Na^+$

In these experiments (Figure 5), simultaneously with the reduction of $Na^+$ (to 70 mmol $l^{-1}$), $Ca^{2+}$ was also reduced to 0.6 mmol $l^{-1}$. This was done to balance the $Ca^{2+}$-antagonistic effect of $Na^+$ and to yield comparable values of basal force of contraction. Lowering of $Ca^{2+}$ concentration did not significantly alter the $Na^+$ dependence of the dihydro-ouabain effect. In accordance with the findings of Reiter (1963) lowering $Na^+$ concentration reduced the potency of dihydro-ouabain. Lowering of the $Na^+$ concentration also reduced the antagonistic effectiveness of $K^+$ (compare curves with broken lines, taken from Figure 2 with the corresponding curves with full lines). In addition to the shift in the position, the slopes of the concentration-effect curves were also changed by $K^+$ at reduced sodium (log $P' = (2.22 \pm 0.01) + (0.45 \pm 0.07)$ log $EC_{50}$, $r = 0.67$, $P < 0.001$). The regression coefficient of the slope to $EC_{50}$ plot indicates a significant difference as compared with the situation in normal $Na^+$ ($0.20 \pm 0.02$ in Figure 3 vs. $0.45 \pm 0.07$ in reduced $Na^+$).

*The role of excitation-dependent $Na^+$ influx in the dihydro-ouabain-$K^+$ antagonism*

In spite of the considerable negative inotropic effect induced by reduction in stimulation frequency from 1 to 0.125 Hz, the antagonistic effectiveness of $K^+$ remained unchanged (Figure 6). In studies of the transmembrane potential the maximal upstroke velocity ($\dot{V}_{max}$) of the action potential was used as an indirect measure of the fast $Na^+$ inward current. As shown in Figure 7a only high concentrations ($> 5.9$ mmol $l^{-1}$) of $K^+$ reduced $\dot{V}_{max}$, although the resting membrane potential was clearly dependent on $K^+$ (Figure 7b).

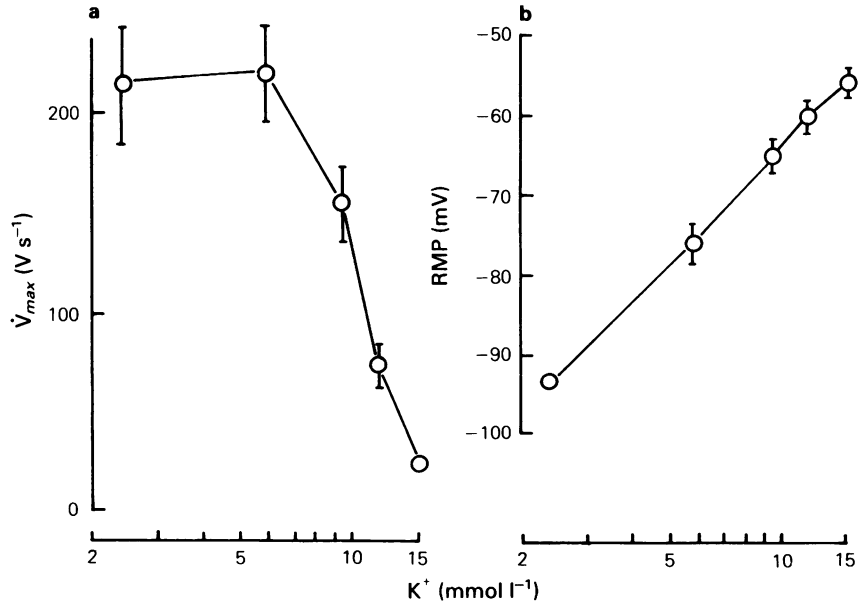


**Figure 7** The effects of $K^+$ on the maximal upstroke velocity ($\dot{V}_{max}$) of the action potential (a) and the resting membrane potential (RMP) (b). The electrical parameters ($\dot{V}_{max}$, V $s^{-1}$, in (a); RMP, in mV in (b), ordinates) in relation to $K^+$ concentration (abscissae, in mmol $l^{-1}$, log scale) were obtained under experimental conditions identical to those of Figure 2 ($Ca^{2+}$ 1.2, $Na^+$ 140 mmol $l^{-1}$; 1 Hz stimulation frequency). The data are derived from 5 preparations by continuous impalements. Arithmetic means are presented; s.e.means shown by vertical lines.

*The influence of $K^+$ on the inotropic effect and binding of ouabain*

The potency of dihydro-ouabain was reduced by a factor of 10 with a rise in $K^+$ from 2.4 to 12 mmol $l^{-1}$ (Figure 2). When analysing the effects of the parent compound, ouabain, on rested-state contractions (stimulation frequency 0.001 Hz), it was found that a 6 fold higher concentration of ouabain was necessary to produce equal responses at 12.0 mmol $l^{-1}$ $K^+$, compared to 2.4 mmol $l^{-1}$ $K^+$ (95% confidence limits 4.20–6.92; $Ca^{2+}$ 1.2 mmol $l^{-1}$). The slow development of the ouabain effect (3–7 h) necessitated the non-cumulative application of ouabain in different groups of muscles ($n = 6-8$). The similar influence of $K^+$ confirms previous work where we had found that dihydro-ouabain and ouabain act identically in guinea-pig papillary muscles (Reiter, 1967; Bachmaier *et al.*, 1982), and differ only in their affinities for the binding sites.

In the binding studies precise measurements of the accumulation of dihydro-ouabain in the tissue were not feasible because of its low affinity. Since the amount of [$^3$H]-ouabain bound to the tissue is also low at high $K^+$, the determination of the dissociation constant and maximal binding capacity were also precluded. Consequently an approach, assuming simple Michaelis-Menten kinetics, was chosen. At steady state, the ratio of tissue-bound ouabain is derived as $AR\,AR'^{-1} = (A + K_D')\,(A + K_D)^{-1}$ at identical concentrations of ouabain, A. The affinity of the receptor for A (dissociation constant $K_D$) is changed from $K_D$ to $K_D'$ by the presence of a high concentration of $K^+$. If the concentration of A is chosen so that $A \ll K_D$, the ratio of the specifically bound amounts of ouabain in the tissue, at different $K^+$ concentrations, equals that of the affinities. At a concentration of 1.85 nmol $l^{-1}$, the tissue/medium ratios of [$^3$H]-ouabain obtained after 6 h of incubation were compared at 2.4 and 12.0 mmol $l^{-1}$ K (Figure 8). It is evident that the accumulation of [$^3$H]-ouabain was reduced by a factor of 3 (95% confidence limits 1.76–4.06). At a higher [$^3$H]-ouabain concentration, 18.5 nmol $l^{-1}$, this factor was not significantly different from 3 (data now shown). If the condition $A \ll K_D$ had not been fulfilled, reduction in ouabain concentration should have increased the affinities ratio.

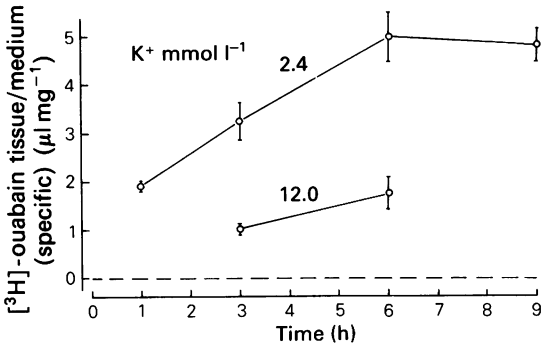


**Figure 8** The binding of [$^3$H]-ouabain to guinea-pig papillary muscles and its dependence on $K^+$: the uptake of [$^3$H]-ouabain (medium concentration 1.85 nmol $l^{-1}$) in quiescent papillary muscles from guinea-pigs pretreated with reserpine is shown in relation to incubation time at 2.4 and 12 mmol $l^{-1}$ $K^+$. Each arithmetic mean was obtained from 6 muscles, s.e.means shown by vertical lines. The horizontal, broken line reflects the size of the sorbitol space. Experimental conditions were the same as in Figure 2, except that the papillary muscles were not stimulated; $Ca^{2+}$ 1.2 mmol $l^{-1}$. Ordinate scale: tissue/medium ratios of specific [$^3$H]-ouabain binding (µl $mg^{-1}$ wet weight), i.e., corrected for that amount of [$^3$H]-ouabain corresponding to the individual [$^{14}$C]-sorbitol space. Abscissa scale: incubation time in h.

## Discussion

The results demonstrate that the positive inotropic effect of cardiac glycosides is antagonized by $K^+$, in a variety of experimental situations, thus corroborating previous results (Reiter *et al.*, 1966). The inhibition of [$^3$H]-ouabain binding by $K^+$ in quiescent guinea-pig papillary muscle (Figure 8) is consistent with other reports (Erdmann & Schoner, 1973; Lindenmayer & Schwartz, 1973; Inagaki *et al.*, 1974; Hansen, 1976; Akera *et al.*, 1978). In these studies it was shown that $K^+$ inhibited the binding of cardiac glycosides in various membrane preparations exhibiting $Na^+$, $K^+$ ATPase activity. The relationship between ouabain receptor affinity and $K^+$ was found to be hyperbolic by Gardner & Conlon (1972) which indicates a saturable, partially competitive antagonism. If the antagonism between $K^+$ and the positive inotropic effect of the glycoside were caused solely by a reduction of receptor affinity, the positive inotropic effect of dihydro-ouabain should be altered by $K^+$ according to the following criteria: (1) The concentration-effect curves should be shifted in parallel to the right. (2) The relationship between $EC_{50}$ and $K^+$ (cf. Vierling *et al.*, 1978) should be linear or hyperbolic for a fully or partially competitive antagonism, respectively. (3) The ratio of receptor affinities should equal that of the inotropically equieffective concentrations. In this study, however, none of these criteria could be verified. The concentration-effect curves, instead of being shifted in a parallel manner, became steeper under the influence of $K^+$ (Figures 2 and 3). The relationship between $EC_{50}$ and $K^+$ was neither linear nor hyperbolic (Figure 4). Moreover, the antagonistic effect of $K^+$ on glycoside binding was dissociated from its effect on the inotropic actions, even though it was

shown (Ebner, 1984) that the time curves of binding and effect paralleled each other. An increase in $K^+$ from 2.4 to 12 mmol $l^{-1}$ reduced [$^3$H]-ouabain binding by a factor of 3 (Figure 8). However, the inotropic action was, in the case of dihydro-ouabain, shifted by a factor of 10 (Figure 2), and by a factor of 6 for ouabain. This dissociation is further corroborated by the effect of $Ca^{2+}$ on the dihydro-ouabain-$K^+$ antagonism (Figure 4). At low $K^+$, the inotropic effect of dihydro-ouabain was not significantly altered by a change in extracellular $Ca^{2+}$. But at high $K^+$, the effects of dihydro-ouabain were enhanced when the $Ca^{2+}$ concentration was increased. Obviously, other effects, besides the influence on glycoside binding, were involved in the antagonism between $K^+$ and the positive inotropic effect of the glycoside. Previous reports on the retardation by $K^+$ of the positive inotropic effect of *p*-chloromercuriphenylsulphonic acid (PCMBS; Halbach *et al.*, 1981), have also stressed the complex nature of the $K^+$ antagonism. PCMBS inhibits $Na^+$, $K^+$ATPase, although inhibition of PCMBS binding by $K^+$ is unlikely.

In regard to the mechanisms which are responsible for the antagonism between $K^+$ and cardiac glycosides, besides an inhibition of receptor binding, it is necessary to consider the influence of $K^+$ on the inotropic effect of $Ca^{2+}$. At 140 mmol $l^{-1}$ $Na^+$, the inotropic effect of $Ca^{2+}$ was reduced with increasing $K^+$, whereas, at 70 mmol $l^{-1}$ $Na^+$, $K^+$ augmented it (Figure 1). The two opposing actions of $K^+$ are in accordance with its previously described negative and positive inotropic effects (Kavaler *et al.*, 1972). Reiter *et al.* (1971) have reported a $Na^+$ dependence of the positive inotropic effect of $K^+$ withdrawal. Their observation corresponds to the ineffectiveness of $K^+$ at 70 mmol $l^{-1}$ $Na^+$ and low $Ca^{2+}$. The concentrations of $K^+$ (1.2–4.5 mmol $l^{-1}$) which antagonized the inotropic effect of $Ca^{2+}$ at 140 mmol $l^{-1}$ $Na^+$, coincide with the concentrations found to activate the $Na^+$ pump. A concentration of about 2 mmol $l^{-1}$ $K^+$ produced a half-maximal shift of the $Ca^{2+}$ concentration-effect curves (Figure 1). According to Glitsch and coworkers (1976, 1978, 1979), $K^+$ concentrations between 0.2 and 1.5 mmol $l^{-1}$ caused, in guinea-pig atria, half-maximal activation of the $Na^+$ pump. Upon removal of external $K^+$, Deitmer & Ellis (1978) found an increase in intracellular $Na^+$ activity ($a^i_{Na}$), probably due to the inhibition of the $Na^+$ pump. Concentrations of 2–4.5 mmol $l^{-1}$ $K^+$, in guinea-pig ventricular muscle, did not appreciably alter the current associated with $Na^+$ pump activity, thus indicating its saturation (Daut, 1983). A positive correlation between pump inhibition, by withdrawal of various $Na^+$ pump-activating cations, and the magnitude of twitch tension has been described in sheep cardiac Purkinje fibres (Eisner & Lederer, 1980a,b). It is conceivable, therefore, that $Na^+_i$, as determined by $Na^+$ pump activity, regulates the inotropic effect of a rise in extracellular $Ca^{2+}$.

The inotropic effects of $Ca^{2+}$ were augmented considerably when $K^+$ was raised at reduced $Na^+$ concentration (Figure 1). It is likely that a reduction in $Na^+$ concentration, would diminish $Na^+$ influx into the myocytes via the $Na^+$–$Ca^{2+}$ exchanger and consequently also reduce $Ca^{2+}$ efflux during diastole. Further reductions in the degree of $Na^+$ influx through partial depolarization of the membrane potential, by a rise in $K^+$ concentration, would also impede $Ca^{2+}$ elimination. Conversely, the fluxes in the opposite direction, i.e., $Na^+$ efflux coupled to $Ca^{2+}$ influx, would be enhanced with depolarization. In accordance with several reports on the electrogenicity of the $Na^+$–$Ca^{2+}$ exchanger (Mullins, 1979; 1981; Reeves & Sutko, 1980), a $Na^+$-dependent $Ca^{2+}$ efflux was found to be inhibited by high external $K^+$ in guinea-pig (Busselen, 1982) as well as in frog atria (Chapman *et al.*, 1981). In frog myocardium (Chapman & Tunstall, 1980), the enhancement of $Na^+$ – withdrawal contractures by $K^+$ (3, 15 and 30 mmol $l^{-1}$) pointed to the role of $Na^+$–$Ca^{2+}$ exchange in the development of contracture.

Whereas the positive inotropic effect of $Ca^{2+}$ is considerably increased at reduced external $Na^+$ concentration, the inotropic effect of cardiac glycosides is diminished (Reiter, 1963). In various preparations the binding of cardiac glycosides was found to be proportional to the $Na^+$ concentration (Baker & Willis, 1972; Gardner & Conlon, 1972; Lindenmayer *et al.*, 1973; Erdmann & Schoner, 1973; Inagaki *et al.*, 1974; Hobbs & Dunham, 1978; Furukawa *et al.*, 1980). However, the reduction of ouabain receptor affinity cannot be the sole mechanism by which $Na^+$ withdrawal reduces the inotropic effect of cardiac glycosides. The lower antagonistic effect of $K^+$ after $Na^+$ reduction, in both the $Ca^{2+}$ (Figure 1) and the dihydro-ouabain (Figure 5) experiments, indicates the contribution of a $Na^+$- and $K^+$-dependent mechanism distinct from the change in receptor affinity. This is also indicated by the steeper slopes of the individual dihydro-ouabain concentration-effect curves, at reduced $Na^+$ or increased $K^+$, as well as by the dissociation between binding and inotropic effect.

The dependence of $Na^+$ influx and efflux on resting membrane potential, as influenced by $K^+$, probably represents the postulated $Na^+$- and $K^+$-dependent mechanism. This, in addition to the ion-induced change in glycoside receptor affinity, could explain the experimental results. The intracellular $Na^+$ activities which result from the magnitudes of $Na^+$ influx and efflux are within the range of $Na^+$ pump saturation, since the activation of dihydro-ouabain-sensitive $Na^+$ efflux was half-maximal at an $a^i_{Na}$ of 12 mmol $l^{-1}$ (Glitsch *et al.*, 1984). Therefore, increased $Na^+$ influx or insufficient activation of $Na^+$ efflux by low $K^+$

raises $a^i_{Na}$, further saturates the $Na^+$ pump, and diminishes the pump reserve. Equal degrees of pump inhibition by a glycoside will be, consequently, much less effective on $a^i_{Na}$ when $Na^+$ influx is reduced or efflux becomes highly stimulated by $K^+$.

Since $\dot{V}_{max}$ of the action potential was reduced by $K^+$ only in concentrations higher than 5.9 mmol $l^{-1}$ (Figure 7), whereas the antagonistic effect of $K^+$ was preserved at the lower stimulation frequencies (Figure 6), the influence on $Na^+$ influx during diastole seems to be relevant for the antagonistic effect of $K^+$. The role of the $Na^+$ influx during rest ('background') is supported by its magnitude which was similar to the excitation-induced $Na^+$ influx at 1 Hz stimulation frequency (Cohen *et al.*, 1982). A decrease in $a^i_{Na}$ in dependence on $K^+$ (1, 3, 10, and 30 mmol $l^{-1}$) has been observed by Ellis (1977) in resting sheep heart Purkinje fibres, and a reduced passive $Na^+$ influx with voltage-clamp-induced depolarization by Eisner *et al.* (1981). The antagonistic effect of $K^+$ could be partially reversed by a rise in extracellular $Ca^{2+}$ concentration (Figure 4). This can be explained by the facilitation of $Ca^{2+}$ uptake through $Na^+$–$Ca^{2+}$ exchange, when, at less negative membrane potentials, $a^i_{Na}$ is increased by a glycoside-induced inhibition of the $Na^+$ pump.

In conclusion the antagonism of $K^+$ against the positive inotropic effect of cardiac glycosides may involve modification of several processes; binding of the glycoside to the receptor, increase in $Na^+$ efflux/influx and interference with $Ca^{2+}$ elimination. The experimental evidence indicated that inhibition of drug binding by $K^+$ caused about one half of the antagonistic effect of $K^+$.

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