Influence of Extracellular Potassium Concentration on Myocardial Uptake and Inotropic Effect of Tritiated Digoxin

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ABSTRACT
Because of conflicting reports on the influence of extracellular potassium concentration ([K]o) on the isotropic action of cardiac glycosides, we compared the effects of varying [K]o (1.5, 4.5, and 7.5 mM) on the isotropic action and myocardial concentration of tritiated digoxin (5 × 10^-7 M) using the isolated cat papillary muscle. The time course of the inotropic response to digoxin was significantly altered by [K]o in an inverse manner; the rate of change of the active tension response to digoxin (g/mm² • min^{-1}) was 0.048 ± 0.004 with low [K]o, 0.025 ± 0.003 with normal [K]o, and 0.014 ± 0.002 with high [K]o. There were no differences, however, in the peak inotropic responses achieved at each [K]o. Muscles were analyzed for 3H-digoxin content after 45 minutes, and at the time of the peak inotropic response. The relationships found between [K]o and 3H-digoxin content were similar to those found between [K]o and active tension. Moreover, a significant positive correlation between digoxin content and the increment in active tension was observed. Thus, an inverse relationship exists between [K]o and the rate of development of the inotropic response to digoxin, changes that may be due to a [K]o-induced alteration in myocardial digoxin content.

KEY WORDS: force-velocity relations, isometric tension, contractility, maximal velocity of shortening, cardiac glycosides, cat papillary muscle

The efficacy of potassium administration in the treatment of digitalis-induced arrhythmias is well recognized (1, 2). However, the influence of the potassium ion on the positive inotropic effects exerted by the cardiac glycosides has not been well defined. Clarification of the relationships between the actions of digitalis and potassium would not only be of considerable clinical importance but might also provide insight into the basic biochemical mechanisms by which digitalis enhances myocardial contractility. It has been hypothesized that digitalis exerts its positive inotropic effects by interacting with Na⁺, K⁺-dependent adenosine triphosphatase (Na⁺, K⁺-ATPase), a membrane-bound enzyme thought to control active cation transport across membranes (3, 4). Such a hypothesis was suggested by the in vitro findings that glycosides consistently inhibited Na⁺, K⁺-ATPase activity in cardiac and other tissues (3, 6), that the inhibition was specific for cardioactive glycosides (7-9), and that the concentrations causing inhibition were estimated to be within the range causing positive inotropic effects (3-9). More recently it was demonstrated that when dog hearts were perfused with digoxin in situ, inhibition of Na⁺, K⁺-ATPase occurred concomitantly with an augmentation in myocardial contractility (10).

The inhibitory effects of digitalis on Na⁺, K⁺-ATPase activity, and the tendency of a myocardial Na⁺, K⁺-ATPase preparation to bind digoxin are inversely related to the potassium concentration of the incubation media (8). Thus, if the hypothesis that

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glycoside-induced augmentation of myocardial contractility is related to an interaction of digitalis with Na+, K+-ATPase is correct, alterations in extracellular potassium concentration would be expected to bear an inverse relationship both to the intensity of the inotropic response resulting from digitalis administration, and to the amount of digitalis bound to the myocardium.

To test this hypothesis and to determine if potassium does interfere with the inotropic effects of digitalis glycosides, we examined the effects of different extracellular potassium concentrations on the inotropic effects and myocardial uptake of tritiated digoxin using the isolated right ventricular papillary muscle of the cat.

Methods
Normal cats, weighing 1.5 to 3.0 kg, were anesthetized with intraperitoneal pentobarbital (35 mg/kg). The hearts were rapidly extirpated, and a suitable right ventricular papillary muscle was removed and placed in a myograph. The lever system used allowed the examination of either isometric or afterloaded isotonic contractions. The details of this preparation have been previously described (11, 12). The muscle bath was filled with one of three Krebs-bicarbonate buffered solutions (Table 1) aerated with 95% O2 and 5% CO2 at a constant temperature of 37°C. These solutions differed only in their potassium concentrations. Muscles were stimulated through platinum field electrodes 25 mm long and 0.5 mm in diameter placed in parallel with the muscle. A constant stimulus frequency of 12/min with a square wave pulse of 5 msec duration at 10 to 20 times above threshold was used. Under these conditions we found that propranolol (10^-6 M) had no effect on the contractile function of papillary muscles at varying [K+]o, indicating that the results obtained in the present study were not influenced by field stimulation-induced release of adrenergic transmitter from the autonomic nerves present in the papillary muscles. All muscles were allowed to equilibrate for at least 45 minutes before any measurements of mechanical function were performed. The average cross-sectional area for all muscles was 0.96 mm², and no papillary muscle was included with a cross-sectional area of more than 1.5 mm². There was no statistically significant difference in cross-sectional area between any of the muscle groups compared, the values averaging 0.93 ± 0.11, 1.03 ± 0.13, and 1.01 ± 0.07 mm² in the low, normal, and high [K+]o groups, respectively. No relationship between muscle size and its response to digoxin was observed when the results from the four largest and four smallest muscles within each experimental group were compared.

Fifteen muscles in the Krebs solution with low [K+]o, 12 in the solution with a normal [K+]o, and 14 in the solution with a high [K+]o were studied under isotonic conditions. The velocity of muscle shortening (muscle lengths/second) at preload weight and at successive 0.4-g increments in afterload was determined, and mean force-velocity curves were constructed for each group by plotting the velocity of shortening against total load (g/mm²).

The influence of extracellular potassium on isometric contractile function was determined in 32 muscles in the low [K+]o Krebs solution, 29 in normal [K+]o, and 31 in high [K+]o. Isometric contractions at the peak of the length-tension curve, Lmax, were analyzed in terms of active tension, the rate of tension development (dT/dt), and time required to reach peak tension. After control measurements of isometric function in the three muscle groups were obtained, the muscles were exposed to digoxin or tritiated digoxin at a concentration of 5 x 10^-7 M; isometric active tension and rate of tension development were monitored continuously after addition of digoxin to the muscle bath. No significant differences were found in the contractile responses to digoxin and 3H-digoxin. In one set of experiments in which 3H-digoxin was employed, each study was terminated after 45 minutes and the muscles from the low, normal, and high [K+]o Krebs solution were analyzed for digoxin content. In another set of experiments, each study was

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digoxin and extracellular potassium

Mean force-velocity curves derived from afterloaded isotonic contractions under conditions determined only after the peak isotropic response to digoxin was achieved. This was identified by at least 15-minutes of stability during which no further rise in active tension or dT/dt occurred. Having determined the time required for peak contractile response to be achieved, separate groups of muscles were then exposed to 3H-digoxin for periods approximating the time required to reach peak contractile response in the low, normal, and high [K] Krebs solution. The muscles analyzed for 3H-digoxin content were removed from the bath at the appropriate time, rinsed with 10 to 15 drops of distilled water, and blotted on fine gauze. Each muscle was then transferred to a cellophane bag, weighed, dried under a heat lamp, and burned by the modified Schoniger technique (13, 14). Tritium content was determined by analysis in a liquid scintillation spectrometer and expressed as micrograms tritiated digoxin per gram wet weight (15).

Statistical analysis was performed using Student's t-test for paired and unpaired data where appropriate. The influence of extracellular potassium concentration on isometric tension and tritiated digoxin content were also analyzed through standard linear regression analyses. Any significant correlation was proved to be linear and did not fit a higher order polynomial equation. The rate of development of the isotropic responses to digoxin at the three different potassium concentrations was compared by determination of the slopes of each response (dT/time). The mean rate of response of each group, determined by the method of least squares, was compared by analysis of variance. All results are expressed as the mean ± se.

**Results**

**Influence of Potassium Concentration on Myocardial Contractility**

Mean force-velocity curves derived from afterloaded isotonic contractions under condi-
tions of different extracellular potassium concentration are illustrated in Figure 1. Maximum velocity of shortening at the lightest load studied (Vmax) was found to bear a significant inverse relationship to extracellular potassium concentration (correlation coefficient 0.42 P < 0.01). At the lightest average load (0.4 g/mm²) Vmax was 1.26 ± 0.05 muscle lengths/sec in 15 muscles in the low [K] Krebs solution, 1.10 ± 0.10 muscle lengths/sec in 12 muscles in the normal [K] solution, and 0.90 ± 0.07 muscle lengths/sec in 14 muscles studied in the high [K] solution.

The influence of extracellular potassium concentration on isometric contractile function is shown in Figure 2. Active tension and dT/dt were inversely related to [K]. The correlation coefficient for active tension in the three groups was -0.21 (P < 0.05) while that for dT/dt was -0.45 (P < 0.001). When compared using Student's t-test, active tension and dT/dt were significantly greater than normal in the low [K] group (P < 0.05) while these values in the high [K] group were not significantly different from normal.

Time to peak tension was not altered by changes in [K].

INFLUENCE OF POTASSIUM CONCENTRATION ON THE INOTROPIC EFFECTS OF DIGOXIN

A marked alteration of the time course of the inotropic response to digoxin was produced by changing [K] (Fig. 3). After 45 minutes of exposure to digoxin the increment in active tension (ΔT) in 13 muscles in the low [K] Krebs solution averaged 2.06 ± 0.19 g/mm² compared with 1.12 ± 0.14 g/mm² in 13 muscles from the normal [K] solution (P < 0.01) and 0.69 ± 0.10 g/mm² in 12 muscles studied in the high [K] solution (P < 0.01). Similar differences were observed when the percent increment in active tension after 45 minute-exposure to digoxin were compared in each group, the values averaging 49 ± 5%, 34 ± 3%, and 22 ± 4% in the low, normal, and high [K] Krebs solutions, respectively. When the mean rates of change (ΔT/time) of the linear portion of each digoxin response were compared by standard analysis of variance, a highly significant
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Although the time course of the inotropic response to digoxin was significantly altered by [K]o, no significant differences were observed in the peak increments or absolute levels of tension and dT/dt finally reached by the three groups of muscles (Fig. 4).

INFLUENCE OF EXTRACELLULAR POTASSIUM ON 3H-DIGOXIN CONTENT

When papillary muscles were exposed to 3H-digoxin at low, normal, and high potassium concentrations for 45 minutes, a highly significant inverse relationship between digoxin content and [K]o was observed (Fig. 3). Digoxin content in the muscles exposed to low [K]o was 0.68 ± 0.04 (µg/g wet weight) compared with 0.49 ± 0.05 in those at normal [K]o and 0.39 ± 0.02 in muscles at a high [K]o (r = -0.71, P < 0.001). When a correlation of the increment in active tension (ΔT) and digoxin content was performed on those muscles exposed to 3H-digoxin, a highly significant positive correlation between digoxin content and ΔT was noted (r = +0.70, P < 0.01).

When 3H-digoxin concentration of papillary muscles was measured at or near the time of the peak inotropic response, it was found to be similar in the three different [K] solutions.

Discussion

The major purpose of this investigation was to examine the influence of extracellular potassium concentration on the inotropic effects and myocardial concentration of digoxin. We found that the rate of development of the inotropic response to digoxin was inversely related to [K]o whereas the peak contractile response to digoxin was unaltered by [K]o. Similarly, the rate of uptake of 3H-digoxin was inversely related to [K]o whereas the concentration at peak contractile response was unaltered by [K]o. In addition, the myocardial concentration of 3H-digoxin under varying [K]o, correlated with the degree of the digoxin-induced augmentation of myocardial contractility.

Several investigators have previously shown that the time of onset of electrocardiographic toxicity produced by digitalis was inversely
related to \([K]\) \((16-19)\). However, the results of attempts to define the effects of \([K]\) on the inotropic response to digitalis glycosides have been conflicting. Carb and Venturi \((20)\) reported that \([K]\), ranging between 3.5 to 8.5 mEq/liter did not alter the inotropic effects of ouabain in the failing cat papillary muscle. Leonard and Hadja \((21)\) found no change in the inotropic effects of strophanthidin when \([K]\) varied between 2.5 to 5.0 mEq/liter in the frog heart or from 4.7 to 7.5 mEq/liter in guinea pig or rabbit hearts. Leight and coworkers \((22)\) also reported that the digitalis-induced increase in right ventricular force in the open-chest dog was not affected by \([K]\). In contrast, Lee et al. \((23)\) showed that in the cat papillary muscle a \([K]\) of 24 mEq/liter delayed the onset of the inotropic and toxic effects of ouabain, and diminished the peak inotropic response. Studies by Nayler \((16)\) in isolated toad hearts demonstrated that a \([K]\) below 3.2 mEq/liter was associated with a more rapid onset of the inotropic effects of G-strophanthin, but the magnitude of the peak response was decreased. Caprio and Farah \((19)\) concluded that either high or low \([K]\) reduced the positive inotropic response in rabbit right ventricular strips. More recently, Cohn et al. \((24)\) found that the increment in isometric tension and rate of tension development produced by ouabain in right ventricular guinea pig strips was not altered when \([K]\) was reduced from 5 to 2 mEq/liter but was decreased when \([K]\) was increased to 10 mEq/liter.

The reasons for these conflicting results are unclear, but as a rule the time course of the inotropic response was not analyzed, and myocardial preparations were often used which did not permit adequate control of the many variables that can alter contractile function. These objections were overcome in the present investigation \((1)\) by determining the time course necessary for development of a stable peak inotropic response to digoxin under varying concentrations of potassium and comparing the effects of \([K]\) on the inotropic activity and myocardial concentration of digoxin as a function of time, and \((2)\) by using the cat right ventricular papillary muscle, a stable preparation that allows careful control of the factors that alter contractile force development.

In addition to influencing the inotropic effects of digoxin, potassium also tended to alter base-line contractile function in an inverse manner. Maximal velocity of shortening at preload weight was significantly greater at low \([K]\), and less at high \([K]\). When isometric active tension and myocardial tension development were compared, only the values for low \([K]\), were significantly different from normal \([K]\). Although these results agree with those of several previous investigations \((23-25)\), they are at variance with the results of other studies \((21, 28, 27)\). It is possible that the frequent failure to demonstrate the inverse relationship between \([K]\) and myocardial contractile state may be related to the use of cardiac preparations insensitive to small changes in contractility, to the use of a small number of experiments for each \([K]\) studied, or to differences in species responsiveness.

In several studies, attempts have been made to relate the therapeutic or toxic effects of digitalis to the amount of the glycoside taken up by the myocardium \((28-30)\). Although Kuschinsky and coworkers \((29, 30)\) reported a positive correlation between the inotropic effects and tissue binding of various cardiac glycosides, they believed that a large portion of the uptake of digoxin and digitoxin by isolated guinea pig atria was nonspecific and of no importance in mediating the inotropic actions of these drugs. Although such a possibility cannot be ruled out as an explanation for our results, it has been shown that the nonspecific binding of digoxin to a particulate preparation of cardiac Na+,K+-ATPase is not altered by changes in \([K]\) \((8)\). Therefore, the observation in the present investigation that there is a close correlation between the inotropic effects and tissue concentration of digoxin despite different concentrations of potassium suggests that under the experimental conditions employed, the amount of digoxin taken up by the myocardium is an
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important determinant of myocardial contractility.

Our data relating to the myocardial uptake of tritiated digoxin in the cat papillary muscle may be compared with those obtained after administration of various digitalis preparations to perfused hearts or to anesthetized animals following alterations in external potassium concentrations. Dutta and Marks (31) documented that there was an inverse relation between the concentration of potassium in the extracellular medium and the myocardial uptake of tritiated ouabain in isolated guinea pig hearts perfused for 64 minutes. Lefler et al. (32) also observed the same relation between serum potassium concentration and the myocardial concentration of tritiated convallatoxin and tritiated cymarol when guinea pigs were killed after 1 hour and 3 hours, respectively. Marcus et al. (15), as well as Morgan and Binnion (33), found a decrease in the myocardial uptake of tritiated digoxin in the hyperkalemic dog killed 1 hour after administration of this glycoside. Marcus et al. (15) attempted to determine the effect of hyperkalemia on the myocardial concentration of tritiated digoxin at equilibrium by killing dogs 4 hours after intravenous injection of tritiated digoxin. They found that the myocardial digoxin concentration in the hyperkalemic dog was still less than that of the normokalemic control. This is different from the findings in the present experiment, in which the rate of myocardial uptake of tritiated digoxin was inhibited by a potassium concentration of 7.5 mEq/liter, but the myocardial concentration at equilibrium was no different from that of muscles bathed in a medium with a potassium concentration of 4.5 mEq/liter. A possible explanation for this discrepancy may be the following. In the anesthetized dog preparation, the inhibition of digoxin uptake by the heart and possibly other tissues (33) resulted in an elevated serum concentration of digoxin. In turn, this was accompanied by a significant increase in the urinary excretion of tritiated digoxin, leaving less glycoside available for continued uptake by the heart. In contrast, the papillary muscle was bathed by a uniform concentration of tritiated digoxin.

A second major goal of this investigation was to gain more information regarding the possible basic biochemical mechanisms by which digoxin enhances contractility. A large body of evidence has accumulated implicating Na⁺, K⁺-ATPase as the possible receptor responsible for mediating the positive inotropic effects of digitalis (3-10). However, it is still not clear whether the interaction of digitalis and Na⁺, K⁺-ATPase is causally related to the inotropic effects of digitalis or merely represents a coincident event. If this interaction were in some way responsible for the digitalis-induced augmentation of myocardial contractility, it would be expected that any intervention altering the inhibitory effects of digitalis on Na⁺, K⁺-ATPase would produce analogous changes in the binding and the inotropic effects of the glycoside. Schwartz and coworkers have demonstrated that increasing [K]o reduced both the rate at which digitalis is bound to a particulate preparation of cardiac Na⁺, K⁺-ATPase, and the rate at which it produced its inhibitory effect on this enzyme (8, 9). However, the maximal capacity of Na⁺, K⁺-ATPase to bind digitalis was unaltered by [K]o (34). The results of the present investigation demonstrated analogous findings in an intact tissue preparation in that increasing [K]o decreased both the rate of uptake of ²H-digoxin and the rate of development of the positive inotropic response; however, at the peak levels of contractile state eventually achieved, [K]o altered neither the magnitude of the inotropic effects of digoxin nor the myocardial concentration of ²H-digoxin. These findings, although indirect, are consistent with the hypothesis that an interaction between digitalis and Na⁺, K⁺-ATPase plays an essential role in the augmentation of myocardial contractility produced by digitalis.

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