Effects of Inotropic and Arrhythmogenic Digoxin Doses and of Digoxin-Specific Antibody on Myocardial Monovalent Cation Transport in the Dog

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SUMMARY The effects of digoxin on monovalent cation active transport were determined in cardiac tissue obtained from dogs given inotropic, toxic, or lethal doses of digoxin. In hemodynamically monitored dogs, active uptake of the K+ analogue Rb+ was determined in vitro in a control myocardial biopsy, and then in serial biopsies from the same dog after the infusion of [3H]digoxin in doses sufficient to cause a sustained positive inotropic effect in the absence of toxicity, and finally after additional doses to induce overt toxicity. Nontoxic digoxin doses producing a mean increase of 20% in left ventricular (LV) dP/dt significantly reduced Rb+ active transport by 25% below control values. At the onset of digoxin-induced arrhythmias, maximal LV dP/dt was 53% above control whereas active Rb+ transport was reduced by 60% below baseline values (P < 0.001). Control dogs given vehicle alone showed no significant change in contractility or in monovalent cation active transport. In another group of dogs given a lethal dose of digoxin, Rb+ active transport was reduced 55% below control levels at the onset of overt toxicity and was further reduced 80% below control at the time of onset of a fatal rhythm disturbance. When dogs were given high affinity digoxin-specific IgG or Fab fragments at the onset of overt toxicity, toxicity was rapidly reversed, and monovalent cation active transport increased to 51% of control at the time of restoration of sinus rhythm. Twenty-four hours after antibody reversal of arrhythmias, monovalent cation transport values approximated normal control levels. These data provide quantitative estimates of the extent of inhibition of monovalent cation transport by digoxin at inotropic, toxic, and lethal endpoints. Similar degrees of transport inhibition were present at the time of onset of digoxin-induced arrhythmias and at the time of arrhythmia reversal by digoxin-specific antibodies. Circ Res 44: 23-31, 1979

SCHATZMANN in 1953 first demonstrated that cardiac glycosides inhibit the active transport of monovalent cations across cell membranes. In the decades since this observation, numerous studies have shown changes in potassium (and less commonly in sodium) concentrations in cardiac tissue exposed to cardiac glycosides (Grupp and Charles, 1964; Müller 1965; Akera and Brody, 1978; Langer, 1977; Ettinger et al., 1977; Ku et al., 1977), presumably brought about by the inhibitory effect of cardiac glycosides on monovalent cation transport. Ten years after Schatzmann’s observation, Repke (1963) advanced the hypothesis that cardiac glycoside inhibition of the monovalent cation transport enzyme complex sodium- and potassium-activated adenosine triphosphatase (Na+,K+-ATPase) is responsible for the therapeutic and toxic effects of digitalis. However, the relationship of cardiac gly-
coside inhibition of Na⁺,K⁺-ATPase and of monovalent cation active transport to changes in myocardial contractility remains unsettled (Akera and Brody, 1978; Akera et al., 1970; Besch et al., 1970; Cohen et al., 1976; Rhee et al., 1976; Schwartz et al., 1975; Hougen and Smith, 1978). Although substantial circumstantial evidence suggesting a causal relationship exists, direct proof of the validity of the hypothesis has not been forthcoming (for reviews, see Schwartz et al., 1975, and Akera and Brody, 1978).

Whereas inhibition of myocardial monovalent cation transport by subtoxic but positively inotropic doses of ouabain in intact dogs has been demonstrated recently (Hougen and Smith, 1978), the quantitative relationship between induced arrhythmias (toxic or fatal) and myocardial monovalent cation transport has not been established in an intact animal model. In addition, it is not known whether or to what extent transport function is restored when toxic manifestations are reversed by digoxin-specific antibody treatment.

Cardiac glycoside-specific antibody preparations are able to reverse many of the actions of cardiac glycosides. Previous studies have shown reversal of digitalis-induced biochemical, electrophysiological, and hemodynamic alterations (see review by Smith et al., 1977). However, the effect of digoxin-specific antibody on myocardial monovalent cation transport has not been studied. In particular, its effect has not been investigated in an intact animal model of digitalis toxicity.

To examine myocardial monovalent cation active transport during digoxin-induced inotropy and toxicity, we studied the active uptake of the K⁺ analogue Rb⁺ in serial left ventricular biopsies from dogs given inotropic and toxic doses of digoxin. Additionally, to test the hypothesis that digoxin-induced arrhythmias would be associated with a certain critical level of inhibition of myocardial monovalent cation transport, and that this inhibition would be reversed (together with the rhythm disturbance) by digoxin-specific antibodies or their Fab fragments, we studied the interrelationships among myocardial active transport of Rb⁺ and the development and subsequent reversal by specific antibody of digoxin-induced cardiac arrhythmias.

The present studies, therefore, had three objectives: to determine whether myocardial transport inhibition could be documented at subtoxic digoxin doses in the intact dog; to quantitate myocardial monovalent cation transport inhibition soon after the onset of inotropy, at the onset of overt toxicity, and at the onset of lethal arrhythmia; and to determine whether and to what extent monovalent cation transport inhibition is reversed when electrophysiological toxicity is reversed by digoxin-specific IgG or Fab fragments.

Methods

Animal Instrumentation

Twelve healthy adult mongrel dogs (from 13.6 to 33 kg, mean 21.8 kg) of either sex were anesthetized with intravenous pentobarbital (30 mg/kg), intubated, and ventilated with 100% oxygen using a Harvard respirator. The chest of each dog was opened by a midline sternotomy. Instrumentation was introduced as described previously (Hougen and Smith, 1978) to measure left ventricular (LV) pressure and maximum rate of change in LV pressure (dP/dt), to maintain constant heart rate by atrial pacing, to record intra-arterial pressure, and to monitor the electrocardiogram. A femoral artery cannula was placed to obtain blood samples for measurement of pH, PO₂, PCO₂, hematocrit, potassium concentration, and plasma digoxin concentration as indicated in experimental protocols.

Another 33 healthy adult mongrel dogs of either sex (from 11.4 to 24.1 kg, mean 19.5 kg) were anesthetized with intravenous pentobarbital (30 mg/kg), intubated with a cuffed endotracheal tube, and ventilated with 50% oxygen using a Harvard respirator, with rate and tidal volume adjusted to maintain normal levels of pH, PO₂, and PCO₂. Standard limb leads were attached and the electrocardiogram continuously monitored. One polyethylene catheter was inserted into a forelimb vein for administration of drugs and another placed in a femoral vein for blood sampling.

Myocardial Biopsy Technique

After introduction of the appropriate instrumentation, and when the dogs were hemodynamically stable (usually within 30 minutes), transmural left ventricular biopsies were obtained using a 4-mm inside diameter corkborer attacked to a handheld power drill as previously described (Hougen and Smith, 1978). In other experiments, the biopsies were obtained at designated times by rapidly performing a left lateral thoracotomy, opening the pericardium, and drilling several biopsies from the left ventricular free wall of the beating heart. The elapsed time from skin incision to biopsy did not exceed 20 seconds. Biopsy tissue was rinsed briefly in incubation medium and then sliced lengthwise into strips less than 1 mm² in cross-sectional area weighing between 3 and 15 mg each.

Rubidium Transport Measurements

The techniques used to measure myocardial uptake of Rb⁺ using ⁸⁶Rb⁺ as tracer have been described in detail recently (Hougen and Smith, 1978). Briefly, the samples of myocardium were incubated for 5 minutes at 30°C in medium containing, in mM concentrations: KC1, 4.0; NaCl, 120; NaHCO₃, 24; MgCl₂, 2.0; CaCl₂, 2.5; glucose, 5.6; Na phosphate buffer, 1.1, adjusted to pH 7.4, and equilibrated with 95% O₂, 5% CO₂. After this equilibration period, samples were transferred to flasks containing the same medium except that the KCl concentration was 2.0 mM, and l⁸⁶RbCl (New England Nuclear) was added together with unlabeled RbCl to a final concentration of 0.1 mM. Samples were incubated for 15 minutes at 30°C in this uptake medium in the presence or absence of 1 mM ouabain. Samples were then rinsed, counted, and weighed as previ-
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ously described (Hougen and Smith, 1978). Active transport of Rb\(^+\) in nmol/mg wet weight/15 min was calculated as the difference between uptake in both the presence and absence of 1 mM ouabain.

To validate and characterize further the previously reported method of Rb\(^+\) uptake measurement, we compared the relative uptake of K\(^+\) to Rb\(^+\) in the same myocardial tissue sample using \(^{86}\)Rb\(^+\) and K\(^+\) as tracers. After a 15-minute equilibration period in physiologic medium, samples were transferred to medium containing \(^{42}\)K\(^+\) (specific activity 10\(^5\) dpm/\(\mu\)mol K\(^+\), or 2 \times 10\(^6\) dpm/ml of medium) together with the usual K\(^+\) concentration of 2.0 mM used in all media. \(^{86}\)Rb\(^+\) was present at a specific activity of 10\(^5\) dpm/\(\mu\)mol (10\(^6\) dpm/ml incubation medium), together with 0.1 mM unlabeled Rb\(^+\). Measurements were made both in the presence and in the absence of 1 mM ouabain to allow estimation of passive as well as active uptakes. Samples were removed after 15 minutes, rinsed, counted to determine total \(^{42}\)K\(^+\) plus \(^{86}\)Rb\(^+\) uptake, and weighed. To determine \(^{86}\)Rb counts in the absence of any appreciable contribution from \(^{42}\)K\(^+\) (radioactive \(t_i\), 12.4 hours), myocardial samples were then returned to their counting vials, stored at 4°C for at least 7 days, and then recounted. Uptake values for \(^{86}\)Rb\(^+\) and K\(^+\) were then calculated after correction for radioactive decay.

The response of the biopsy preparation to graded in vitro digoxin concentration was determined to assess the sensitivity and precision of the method. Three healthy dogs were anesthetized with pentobarbital, intubated, and ventilated as described above. Thirty minutes later the chest was rapidly opened and multiple LV biopsies were quickly obtained from the beating heart. Samples prepared as described above were incubated for 15 minutes at 30°C in physiologic medium as previously described (Hougen and Smith, 1978) in the presence of digoxin in concentrations ranging from zero to 10\(^{-5}\) M. They were then transferred to flasks containing the identical medium and digoxin concentration except that \(^{86}\)Rb\(^+\) was present for an additional 15-minute incubation period. Paired slices were identically incubated in the presence of 1 mM ouabain. Passive uptake was defined as that uptake measured in the presence of 1 mM ouabain; active transport was defined as the difference between Rb\(^+\) taken up in the presence and absence of 1 mM ouabain.

Plasma and Myocardial Digoxin Determinations

Five-milliliter samples of heparinized arterial blood were withdrawn prior to and 5, 10, 15, and 30 minutes after infusion of digoxin (Lanoxin, Burroughs Wellcome) to which had been added suitable amounts of randomly labeled \([\text{H}]\)digoxin (New England Nuclear) for measurement of plasma digoxin concentrations. Blood samples were also taken at the time of biopsies obtained soon after the development of inotropy and at the onset of overt toxicity. Plasma and myocardial digoxin contents were measured by quantitation of tritium counts as described elsewhere (Hougen and Smith, 1978). Quenching correction was by the use of internal standards for both plasma and myocardial samples. Samples were counted until stable count rates were obtained, using a Packard model 3500 scintillation counter with appropriate window settings and correction for \(^{86}\)Rb\(^+\) spill into the \(^{3}\)H channel.

Antibody Production and Characterization

Sheep were immunized with digoxin-serum albumin conjugates as previously described (Smith et al., 1970; Curd et al., 1971). Blood was collected by venipuncture, and plasma separated by centrifugation. IgG was prepared from crude antiserum by ammonium sulfate precipitation (Curd et al., 1971). Fab fragments were prepared by papain digestion as reported elsewhere (Curd et al., 1971; Nisonoff, 1964). Fab and IgG preparations were passed through a sterile 0.22-\(\mu\)m Millipore filter (Millipore Corp.) and stored at -20°C. Concentrations of specific antibody were determined by \([\text{H}]\)digoxin binding studies as previously described (Smith et al., 1970). Preparations used in the present study had average intrinsic affinity constants of 5 \times 10\(^6\) M\(^{-1}\) to 1.5 \times 10\(^7\) M\(^{-1}\). Nonspecific IgG and Fab were identically prepared from sheep not specifically immunized. Total protein concentrations of antibody preparations were determined by the method of Lowry et al. (1951).

Protocols

Measurement of Monovalent Cation Transport after Infusion of Inotropic and Toxic Doses of Digoxin

After a control biopsy was obtained and hemodynamic conditions were stable, \([\text{H}]\)digoxin in vehicle (12% ethanol in saline) was administered as an intravenous injection of 0.05 mg/kg to six open-chest dogs.

After the development of a sustained positive inotropic response, a second LV biopsy was obtained. Sequential doses of digoxin (0.05 mg/kg) were then given intravenously at 30-minute intervals until the emergence of overt digitalis toxicity, defined as the electrocardiographic appearance of atrioventricular junctional tachycardia or ventricular tachycardia. A final LV biopsy was obtained at the onset of toxicity. Control dogs were given similar doses of vehicle alone and were biopsied at comparable times.

Measurement of Monovalent Cation Transport at the Onset of Toxic and Lethal Arrhythmias and after Antibody Reversal of Arrhythmias

Digoxin, 0.3 mg/kg, or an equivalent volume of vehicle (40% propylene glycol, 10% ethanol in saline), was administered intravenously over 1 minute to 33 pentobarbital-anesthetized closed-chest dogs. Sinus rhythm was present in all animals prior to digoxin administration. The dogs were divided into five groups, as follows:

A. Control Group \((n = 9)\). Vehicle alone was
given to these dogs and myocardial biopsies were obtained at intervals from 15 to 100 minutes later (average, 53 minutes). A wide range of biopsy times was selected to determine whether the interval between vehicle infusion and biopsy affected Rb⁺ transport.

B. Toxicity Onset Group (n = 5). These dogs received digoxin, 0.3 mg/kg, and cardiac rhythm was monitored until development of toxicity (defined above). The chest was then rapidly opened and left ventricular biopsies obtained.

C. Lethal Toxicity Group (n = 5). The dogs in this group were given digoxin, 0.3 mg/kg. At the onset of toxicity, nonspecific antibody was infused. The toxic arrhythmia progressed to a fatal arrhythmia in all instances (ventricular fibrillation in four dogs and asystole in one), at which time the left ventricle was immediately biopsied.

D. Antibody Reversal Group (n = 5). After administration of digoxin, 0.3 mg/kg, ventricular tachycardia occurred in all dogs, at which point digoxin-specific antibody (IgG) was administered intravenously over 3 minutes in a dose equimolar with that of administered digoxin. A further antibody infusion equal to one-third of the amount of antibody initially given was then infused over 30 minutes with a constant infusion pump. The electrocardiogram was continuously monitored. After restoration of 30 seconds of continuous sinus rhythm without ectopic beats, the chest was quickly opened and the left ventricle biopsied.

E. Twenty-four Hour Postreversal Group (n = 9). These dogs were treated identically to those in the antibody reversal group (D) except that five received digoxin-specific IgG and four were given an equivalent number of binding sites as digoxin-specific Fab fragments at the onset of toxicity. After return of normal sinus rhythm, the dogs were allowed to recover from anesthesia. Twenty-four hours later, they were again anesthetized with intravenous pentobarbital (30 mg/kg), intubated, and ventilated as described above. An electrocardiogram was recorded. The chest was then opened and the left ventricle was biopsied.

**Electrically Induced Ventricular Fibrillation**

To assess the possible effect of ventricular fibrillation on myocardial monovalent cation transport, ventricular fibrillation was induced by electrical stimulation in three of the nine dogs in the control group. The right external jugular vein was cannulated, and a 6F bipolar catheter (Elecath 6F, Electro-catheter Corp.) was passed into the right ventricle. Sustained ventricular fibrillation was induced by delivering a brief (3-second) train of 60 Hz alternating current impulses of 1 volt RMS employing a standard laboratory fibrillator (Mansfield, 1962). After an interval of ventricular fibrillation of about 15 seconds, equal to that in group C (lethal toxicity group) dogs, the chest was rapidly opened and Rb⁺ active transport measured in left ventricular biopsy tissue as described above.

**Data Analysis**

Data from the open-chest dogs were analyzed by paired t-test, each dog serving as its own control. Data from the closed-chest groups were analyzed by unpaired t-test. Differences in values were considered significant when P values were less than 0.05.

**Results**

**Validation of Rb⁺ Uptake Studies**

Comparison of the simultaneous uptake of K⁺ and Rb⁺ by myocardial slices in the presence and absence of ouabain is summarized in Table 1. The ratio of K⁺ to Rb⁺ uptake was 19.5 ± 0.4 (SE) in the absence of ouabain, essentially identical to the 20:1 ratio of K⁺ to Rb⁺ present in the medium. This ratio did not vary with the 10⁻⁵ M ouabain, a second set of slices yielding a mean ratio of 20.3 ± 0.4 in its presence. Thus, both active and passive uptake of Rb⁺ are interchangeable with K⁺ under the conditions of these experiments.

The in vitro concentration-effect curve for digoxin inhibition of Rb⁺ transport in myocardial samples is shown in Figure 1. Concentrations of digoxin greater than 10⁻⁶ M could not be dissolved reliably, and ouabain was substituted for the 10⁻⁵ M concentration point. As shown, digoxin at 10⁻⁸ M caused a 21 ± 8% (P < 0.05) reduction in Rb⁺ active uptake compared to active uptake in the presence of vehicle alone. Half-maximal inhibition occurred at 8 × 10⁻⁹ M digoxin.

Previous work from this laboratory has shown that active uptake of Rb⁺ by these myocardial samples is linear for at least 30 minutes, under the incubation conditions used in the present set of experiments (Hougen and Smith, 1978).

**Plasma and Myocardial [³H]Digoxin Concentrations**

Plasma and myocardial digoxin concentrations are shown in Figure 2 for the open-chest dogs receiving an initial positive inotropic but subtoxic dose, followed by additional doses of digoxin to onset of overt toxicity. The mean plasma digoxin

**Table 1** Comparison of Simultaneous Rb⁺ and K⁺ Uptake by Canine Myocardial Biopsy Slices

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rb⁺ uptake (nmol/mg/15 min)</th>
<th>K⁺ uptake (nmol/mg/15 min)</th>
<th>K⁺:Rb⁺ uptake ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain absent</td>
<td>0.170 ± 0.006</td>
<td>3.325 ± 0.158</td>
<td>19.5 ± 0.4</td>
</tr>
<tr>
<td>(n = 9)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Ouabain present</td>
<td>0.047 ± 0.003</td>
<td>0.965 ± 0.061</td>
<td>20.3 ± 0.4</td>
</tr>
<tr>
<td>(10⁻⁵ M)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td></td>
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* Values expressed as mean ± SE of uptake in nmol/mg wet weight/15 min
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FIGURE 1 In vitro digoxin concentration-effect curve for inhibition of Rb⁺ active transport. Left ventricular biopsy tissue obtained from three pentobarbital-anesthetized dogs was preincubated for 15 minutes at 30°C in the presence of the digoxin concentrations shown (horizontal axis). Ouabain was substituted for the 10⁻⁶ M concentration point. The tissues were incubated an additional 15 minutes in the presence of 86Rb⁺. Complete inhibition of active transport was defined as that observed in the presence of 10⁻⁶ M ouabain. Statistically significant (P < 0.01) transport inhibition was observed at concentrations of 10⁻⁷ M digoxin or greater. Half maximal inhibition occurred at 8 x 10⁻⁸ M. Numbers in parentheses indicate numbers of individual tissue samples assayed; brackets indicate one SE above and below the mean.

concentration at the time of LV biopsy during inotropy was 27 ± 3 ng/ml (3.5 x 10⁻⁸ M) and at the onset of arrhythmia 113 ± 6 ng/ml (1.5 x 10⁻⁷ M), an increase of 4-fold. The mean left ventricular concentration of digoxin in the specimen obtained soon after the onset of inotropy was 272 ± 33 ng/g wet weight. At toxicity, the myocardial content increased to 873 ± 79 ng/g wet weight. The larger ratio of myocardial to plasma digoxin content at toxicity would be expected on the basis of the later sampling time.

Effects of Inotropic and Toxic Doses of Digoxin

As summarized in Table 2, there was no significant difference in mean body weight, hematocrit, serum potassium concentration, arterial blood Po₂, PCO₂, or pH between control dogs and those given digoxin. Mean arterial pressure and heart rate did not vary significantly from the initial values at any point during the course of the experiments prior to onset of digoxin-induced arrhythmias.

The initial digoxin dose of 0.05 mg/kg was selected to produce a demonstrable positive inotropic effect without toxicity. Following this dose, no elec-

![Figure 2](http://circres.ahajournals.org/) Plasma (vertical axis to left) and myocardial (vertical axis to right) digoxin content after inotropic (open bar) and toxic (cross-hatched bar) doses of [³H]digoxin iv. Plasma digoxin concentrations at onset of sustained inotropy (open bar) and toxicity (cross-hatched bar) represent the mean ± se for six dogs. Myocardial digoxin content (ng/g wet weight) in corresponding biopsy samples is also shown.

![Figure 3](http://circres.ahajournals.org/) DIGOXIN CONCENTRATION (M)

**FIGURE 2** Plasma (vertical axis to left) and myocardial (vertical axis to right) digoxin content after inotropic (0.05 mg/kg) and toxic (mean 0.17 mg/kg) doses of [³H]digoxin iv. Plasma digoxin concentrations at onset of sustained inotropy (open bar) and toxicity (cross-hatched bar) represent the mean ± se for six dogs. Myocardial digoxin content (ng/g wet weight) in corresponding biopsy samples is also shown.

**TABLE 2** Initial Measurements from Control and Digoxin-Treated Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 6)</th>
<th>Digoxin-treated group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>19.5 ± 1.9</td>
<td>21.8 ± 2.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>38 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Serum potassium (mEq/liter)</td>
<td>3.5 ± 0.2</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.46 ± 0.03</td>
<td>7.48 ± 0.03</td>
</tr>
<tr>
<td>Arterial Po₂ (mm Hg)</td>
<td>500 ± 29</td>
<td>452 ± 21</td>
</tr>
<tr>
<td>Arterial PCO₂ (mm Hg)</td>
<td>27 ± 4</td>
<td>21 ± 3</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE.
trocardiographic evidence of digoxin toxicity appeared, consistent with our own previous experience (Beller and Smith, 1975; Beller et al., 1975) and that of others (Goldman et al., 1973). At the time of biopsy, shortly after the appearance of sustained positive inotropy, a mean of 26 ± 3 minutes after digoxin administration, maximal LV dP/dt had increased 20 ± 3% above baseline values (P < 0.001, paired t-test) as shown in Figure 3. Additional digoxin doses (to an average total of 0.17 mg/kg, iv) were given to the onset of arrhythmia, manifest as ventricular tachycardia in five dogs and atrioventricular dissociation in one. Immediately prior to the onset of arrhythmia, maximal LV dP/dt increased 53 ± 7% above baseline values (P < 0.001, paired t-test), an average of 88 ± 8 minutes after the initial dose of digoxin was infused. In contrast, control dogs given vehicle alone showed unchanged contractility at comparable times (Fig. 3).

**Effects of Digoxin on Myocardial Active Transport of Rb⁺ at Onset of Inotropy and Toxicity**

Active transport of Rb⁺ into myocardial samples obtained soon after the onset of digoxin-induced inotropy was significantly reduced by 25 ± 5% (P < 0.01) below control values (Fig. 4). At the onset of digoxin-induced rhythm disturbances, myocardial active uptake of Rb⁺ was markedly reduced by 60 ± 4% (P < 0.001) below baseline values. In contrast, dogs receiving vehicle (12% ethanol in saline) showed unchanged Rb% active transport compared to baseline values when measured at comparable times (Fig. 4). These data demonstrate significant inhibition of monovalent cation active transport by positively inotropic but nontoxic doses of digoxin. Onset of arrhythmia was associated with markedly reduced active transport to 60% below control levels.

**Time Course of Digoxin Toxicity and Reversal with Antibody**

There were no significant differences in mean weight, serum potassium, hematocrit, or initial arterial Po₂, PCO₂, and pH among the five groups of dogs used in these experiments. Figure 5 summarizes the time course of the development of toxic arrhythmias after digoxin administration in these five groups of dogs. The time of onset of toxicity was similar among the various digoxin-treated groups, with each developing overt toxicity at mean times of 14–19 minutes after digoxin infusion. Ventricular tachycardia was the arrhythmia in all group B dogs. The digoxin-induced rhythm disturbance progressed to ventricular fibrillation in four dogs and ventricular asystole in one dog in the lethal toxicity group (C), a mean of 54 ± 15 minutes after digoxin treatment. Overt toxicity developed in the dogs subsequently undergoing antibody reversal (group D), an average of 17 ± 4 minutes after digoxin administration. Ventricular tachycardia developed in all dogs in this group, at which point digoxin-specific antibody was infused. Sinus rhythm returned in all instances, a mean interval of 74 ± 9 minutes after antibody administration.

**Toxic arrhythmias (ventricular tachycardia in all cases) developed in group E (24-hour post-reversal group) 14 ± 2 minutes after digoxin administration. Antibody in the form of digoxin-specific IgG (n = 5) or Fab fragments (n = 4) was then infused. Sinus rhythm returned in all dogs, a mean of 69 ± 15 minutes after antibody infusion. All remained healthy and in sinus rhythm for the ensuing 24 hours. As shown in Figure 5, the time course of antibody reversal of toxicity in groups D and E was similar.**

**Monovalent Cation Active Transport at Onset and Reversal of Digoxin-Induced Arrhythmias**

Active transport of the K⁺ analogue Rb⁺ in myocardial biopsy samples from the five groups of dogs is summarized in Figure 6. Active Rb⁺ uptake in
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CONTROL GROUP

DIGOXIN-TREATED GROUP

FIGURE 4 Effect of digoxin on myocardial active transport of Rb⁺. Left ventricular biopsies after positive inotropic doses (0.05 mg/kg) and at toxicity (0.17 mg/kg, iv) were incubated at 30°C and Rb⁺ active uptake was determined as described in the text. Control dogs given vehicle alone were biopsied at comparable times. Short horizontal bars represent mean values.

biopsies obtained from control (group A) dogs was 0.118 ± 0.008 nmol Rb⁺/mg wet weight tissue/15 min. Rb⁺ transport was not influenced by the interval between control vehicle infusion and the time of biopsy. Three electrically fibrillated dogs had Rb⁺ transport values similar to other animals in the control group. Thus, ventricular fibrillation per se did not alter Rb⁺ active transport measurements.

Onset of digoxin-toxic arrhythmias (group B) was associated with a marked reduction in active transport to 0.048 ± 0.004 nmol Rb⁺/mg wet wt/15 min, 59 ± 4% below the control value (P < 0.001).

Left ventricular biopsies obtained at the time of fatal arrhythmia (group C) indicated a further decrease of active transport to 0.024 ± 0.005 nmol Rb⁺/mg wet wt/15 min. This represented an 80 ± 5% decrease of active transport compared to the control value (P < 0.001).

Those dogs given digoxin-specific antibody and biopsied immediately after restoration of sinus rhythm (group D) had partial recovery of active transport to a level that was significantly greater than that observed at the onset of overt toxicity (0.060 ± 0.005 compared to 0.048 ± 0.004, P < 0.05), but still substantially below control active transport values.

FIGURE 5 Time course of digoxin-induced toxic and lethal arrhythmias, and of reversal of toxicity with digoxin-specific antibody. Control dogs (group A) given vehicle remained in sinus rhythm while groups B, C, D, and E received digoxin, 0.3 mg/kg intravenously, and developed toxic and lethal rhythm disturbances at the times indicated. After the onset of arrhythmia, groups D and E received digoxin-specific antibody intravenously at the onset of toxicity (shown by arrows). Group E was allowed to recover and was biopsied the following day, indicated by *. Vertical lines indicate ± 1 se; numbers of dogs are indicated in parentheses. Bx, biopsy time.

Measurement of Rb⁺ active transport in nine 24-hour survivors in group E (five IgG, four Fab) demonstrated further recovery of pump activity to 0.107 ± 0.004 nmol Rb⁺/mg wet wt/15 min, not significantly different from control (Fig. 6). No difference was evident in mean Rb⁺ transport between IgG- and Fab-treated animals (IgG, 0.106 compared with Fab, 0.109 nmol Rb⁺/mg wet wt/15 min).

FIGURE 6 Digoxin-induced inhibition of Rb⁺ active transport and reversal of inhibition by digoxin-specific antibody. Control dogs received vehicle; other groups were given digoxin 0.3 mg/kg. Two groups (cross-hatched) were treated with digoxin-specific antibody at onset of toxicity. Rb⁺ active transport was measured in vitro in myocardial biopsy samples as described in the text. Vertical lines represent ± 1 se; numbers of dogs are indicated in parentheses.
These data demonstrate that, in intact dogs given a single lethal dose of digoxin, digoxin-specific antibody reversed advanced toxic arrhythmias, and sinus rhythm was reestablished in all instances. Monovalent cation active transport returned toward normal at the time of reversal of toxicity and was not significantly different from normal control values 24 hours after reversal.

**Discussion**

These experiments demonstrate that significant inhibition of active transport of the K⁺ analogue Rb⁺ is present at the time positive inotropy produced by a nontoxic dose of digoxin is first clearly evident in intact dogs. The impact of animal-to-animal biological variation on data analysis was minimized by using each dog as its own control. We also regard as important the assessment of transport function in intact cells by a method sensitive to low (10⁻⁸ m) concentrations of digoxin similar in magnitude to those present in plasma of treated patients. Although these data do not prove a causal relationship between digoxin-induced transport inhibition and positive inotropy, they do demonstrate that a necessary (but not sufficient) condition is met with regard to the Na⁺,K⁺-ATPase inhibition hypothesis of cardiac glycoside inotropic action. This relationship has been discussed in detail recently by a number of authors (Akera and Brody, 1978; Langer, 1977; Ettinger, 1977; Schwartz et al., 1975; Hougen and Smith, 1978) and will not be considered further here.

Our results further indicate that the progression from positive inotropy alone to arrhythmic manifestations of digoxin toxicity to fatality is associated with marked and progressive decreases in myocardial monovalent cation active transport (Figs. 5 and 6). These data are consistent with the recent findings of Ettinger and associates (1977), who detected significant decreases in myocardial K⁺ and increases in Na⁺ content in areas of dog hearts from which acetylstrophanthidin-induced arrhythmias appeared to originate.

Although the effects of toxic doses of digoxin on myocardial monovalent cation active transport in intact dogs have not been investigated previously, the relationship between the arrhythmic manifestations of digitalis toxicity and Na⁺,K⁺-ATPase inhibition has been explored. Besch et al. (1970) found 59% inhibition of enzyme activity at the time of onset of ventricular ectopic activity in response to ouabain infusion, while Akera et al. (1970) found 47% inhibition of Na⁺,K⁺-ATPase at a similar endpoint. Dutta et al. (1970) demonstrated 54% and 75% inhibition of myocardial Na⁺,K⁺-ATPase in dogs given ouabain in arrhythmogenic and lethal doses, respectively. Zavecz and Dutta (1977) studied the effect of a constant infusion of actodigin, a short-acting semisynthetic glycoside, on canine myocardial Na⁺,K⁺-ATPase at the onset of toxicity and after return of sinus rhythm following cessation of drug infusion. These workers showed that at the onset of arrhythmia, Na⁺,K⁺-ATPase was reduced by 55% but returned to control values at the time of recovery to normal sinus rhythm. Akera et al. (1977) found canine myocardial Na⁺,K⁺-ATPase to be reduced 73% below control levels by chronic administration of toxic doses of digoxin. Thus, enzyme inhibition at toxicity and lethal arrhythmia observed in these prior studies are similar to the degree of monovalent cation active transport inhibition reported here. We found that, at the onset of toxicity, monovalent cation active transport was 59% below control in one group and 60% in another; at the appearance of fatal arrhythmia, 80% inhibition of transport was present.

The transport activities measured in the present study represent pump activity in myocardium rather than Purkinje network or other parts of the specialized cardiac conduction system. Although one must be cautious in inferring that parallel declines in pump activity are present in myocardial cells and specialized conduction tissue, the similarity of monovalent cation active transport at onset of arrhythmia and at the time of arrhythmia reversal suggests that there may be a critical level of pump activity necessary to maintain electrophysiological stability. It is of interest that variation in time to reach toxicity did not appear to alter the degree of cation transport inhibition measured at the onset of toxicity, whether assessed in open- or closed-chest dogs. In dogs first receiving an inotropic dose followed by toxic doses of digoxin (total, 0.17 mg/kg), toxicity developed 88 ± 8 minutes after the initial digoxin was infused. In dogs given a single, rapidly fatal 0.3 mg/kg dose of digoxin, ventricular tachycardia appeared after 19 ± 3 minutes. However, at toxicity, Rb⁺ active transport was reduced by 60 ± 4% below control in the first group and 59 ± 4% in the latter group of dogs.

Also of interest in these experiments is the fact that the progressive electrocardiographic changes of digoxin toxicity followed a reversed pattern after administration of digoxin-specific antibody. In most instances, the large dose of digoxin given in these studies produced premature atrioventricular junctional and/or ventricular beats at an increasing rate until a sustained rapid junctional or ventricular tachycardia emerged. The rhythm sequence coming out of toxicity generally retraced the sequence of arrhythmias during development of toxicity. In parallel with this symmetry of rhythm disturbances was the degree of monovalent cation inhibition at onset (59–60%) and at reversal (49%) of rhythm disturbances. The delay between antibody infusion and reversal of arrhythmia presumably is due to factors including the time necessary for antibody to bind free digoxin in the plasma and interstitial space, to shift the equilibrium in favor of dissociation of digoxin from cardiac receptor sites, and to allow correction of cellular electrophysiological abnormalities.
We conclude that these results are consistent with (but do not prove) the hypothesis that binding to and inhibition of myocardial Na\(^+\)\(,\)K\(^+\)\(-\)ATPase are related to both inotropic and toxic effects of digoxin. Digoxin-induced arrhythmias develop in association with reduction in myocardial monovalent cation active transport to about 40% of levels observed in normal control myocardium, and lethal arrhythmias are associated with reduction to 20% of normal. The reversal of advanced digoxin toxicity by digoxin-specific antibodies or Fab fragments is associated with the restoration of myocardial monovalent cation transport function.

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