Differing Sensitivities of Purkinje Fibers and Myocardium to Inhibition of Monovalent Cation Transport by Digitalis

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ABSTRACT The extent of inhibition of monovalent cation active transport in Purkinje fibers and myocardium in response to toxic and inotropic doses of digitalis were studied in the dog to elucidate the factors underlying the different relative sensitivities of these tissues to the toxic arrhythmogenic effects of digitalis. Monovalent cation transport inhibition was assessed by measuring uptake of the K^+ analog Rb^+ in samples of myocardium and Purkinje fibers after in vitro ouabain exposure and after acute or chronic administration of digoxin in vivo. The active uptake of Rb+ was determined as the difference between total uptake and uptake in the presence of 1.0 mM ouabain. Mean active uptake of Rb^+ by Purkinje fibers from control hearts was 1.62±0.11 (SEM) nmol/mg wet wt per 15 min, significantly greater than the value of 0.49±0.05 for myocardium ($P < 0.001$). Concentration-effect curves for inhibition of monovalent cation active transport by in vitro exposure to graded concentrations of ouabain showed that the concentration for half-maximal inhibition of monovalent cation transport, IC_{50}, for Purkinje fiber transport was 0.4 μM, significantly less than the value of 1.4 μM for myocardial samples. Dogs were given toxic doses of digoxin (0.3 mg/kg i.v.). At onset of sustained ventricular tachycardia, they were killed and monovalent cation transport measured in myocardial and Purkinje fiber samples. Active Rb^+ uptake was inhibited by 44% in myocardial samples, whereas a significantly greater inhibition of 76% was noted in Purkinje fibers ($P < 0.01$). Similar data were obtained for both transmural myocardial biopsy samples and subendocardial trabecular samples obtained from regions adjacent to Purkinje fibers. Another group of dogs received a nontoxic dose of 0.02 mg/kg i.v. daily for 6 d. Myocardium showed a 17% reduction in Rb^+ active uptake compared to control animals receiving no drug, whereas Purkinje fiber transport was reduced in these dogs to a significantly greater extent averaging 44% ($P < 0.001$). Thus, both toxic and inotropic (nontoxic) doses of digoxin inhibited monovalent cation transport in Purkinje fibers to a greater extent than in myocardium. This difference in apparent sensitivity of monovalent cation transport to digoxin may contribute to the previously reported tendency of digitalis toxic arrhythmias to arise in Purkinje fibers.

INTRODUCTION

The basis for the apparently greater sensitivity of Purkinje fibers than of ventricular myocardium to the toxic arrhythmogenic effects of digitalis is not well understood. Electrophysiologic approaches to this problem have included the studies of Moe and Mendez (1), who demonstrated that conduction, while depressed by digitalis in specialized conducting tissue, was well maintained in muscle. Related results were reported by Swain and Weidner (2), suggesting that ouabain-induced ventricular fibrillation developed from increased Purkinje automaticity with marked depression of conduction in Purkinje tissue. Studies by Vassalle et al. (3) and by Nowak and Haustein (4) have shown that ouabain produces toxicity sooner in the conduction tissue than in myocardium. Hollander (5) demonstrated that a threefold greater concentration of ouabain is necessary to precipitate toxicity in ventricular muscle than Purkinje fibers.

Cardiac glycosides are known to increase the rate of discharge of spontaneously firing Purkinje fibers (6, 7). In a system in which isolated canine Purkinje fibers were superfused with arterial blood from a donor dog receiving digitalis, Rosen and colleagues demonstrated overt toxic changes in cardiac rhythm in the donor animal that were coincident with abnormalities in action potential amplitude, resting membrane po-

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tential, maximal slope of phase-zero depolarization, and shortening of action potential duration in the isolated Purkinje preparation (8, 9). Thus, the sensitivity of specialized conducting fibers to digitalis has been implicated in the genesis of cardiac arrhythmias. However, this view is not unanimous in that Ettinger et al. (10) reported findings in a dog model suggesting that acetyl strophanthidin-induced ventricular arrhythmias arise within epicardial myocardium.

Despite the considerable body of detailed information on electrophysiologic responses of Purkinje fibers to cardiac glycosides in vitro, relatively few studies have dealt with underlying mechanisms whereby Purkinje fibers may be more sensitive than working myocardial cells to the toxic electrophysiologic effects of digitalis. The only well-defined cellular effects of cardiac glycosides at pharmacologic doses and plasma levels are the highly specific inhibition of sodium and potassium-dependent adenosine triphosphatase [(Na^+,K^+)ATPase][1] and associated changes in monovalent cation active transport (11). Accordingly, we studied the relative sensitivity to digitalis-induced inhibition of monovalent cation transport in Purkinje fibers and myocardium of dogs given digoxin intravenously. The technique used employs the potassium analog Rb^+, which is similar to K^+ in its transport properties and has been used as an indicator of (Na^+,K^+)ATPase-dependent sodium and potassium transport in erythrocytes (12–14), rat brain (12), guinea pig left atrium (15), guinea pig ventricular slices (16), canine left ventricular samples (17), and cat ventricular myocardium (18). Using ouabain-inhibitable Rb^+ uptake as an index of (Na^+,K^+)ATPase-dependent monovalent cation transport in samples of canine myocardium and Purkinje fibers, we studied the effects of ouabain exposure in vitro as well as the effects of acutely toxic doses and chronically administered subtoxic but positively inotropic doses of digoxin to test the hypothesis that the increased sensitivity of Purkinje fibers to digitalis is accompanied by more pronounced inhibition of monovalent cation transport, and that this relative sensitivity is manifest both at acutely toxic and at chronically administered subtoxic doses of digitalis.

METHODS

Animal procedures

Dogs (20–30 kg) of either sex were anesthetized with pentobarbital (30 mg/kg) given intravenously, and their temperatures maintained at 37°C with radiant heat. The animals were intubated with a cuffed endotracheal tube and ventilated with room air adjusted to maintain arterial blood gases within normal limits for the dog. A lead II electrocardiogram was monitored continuously, together with femoral arterial blood pressure. Arterial blood gases (P_{O_2}, P_{CO_2}, and pH) and serum electrolytes (Na^+, K^+, and Cl^-) were determined together with measurement of hematocrit.

A midline thoracotomy was performed. Transmural left ventricular myocardial biopsy samples were obtained using a high speed drill driven by compressed nitrogen (3M Co., St. Paul, Minn.) with a modified biopsy bit (5 mm I.D.). The tissue was removed from the biopsy bit, carefully divided into strips ~1 × 2 × 5 mm, average wt 10 mg, preserving epicardial to endocardial architecture, and placed immediately in oxygenated physiologic medium (see below). Biopsies were taken from the free wall of the left ventricle with care taken not to injure visible coronary vessels. The biopsy site was closed using 3-0 silk purse-string sutures. At least five biopsies could be taken in this way without eliciting sustained arrhythmias or measurable alterations in hemodynamic state.

Purkinje tissue and additional myocardial samples were obtained immediately upon sacrifice of the animal. The left and right ventricular cavities were opened quickly, avoiding stretch of the Purkinje fibers. Free running fibers were excised, avoiding inclusion of myocardial tissue and were immediately placed in oxygenated physiologic medium. Only those samples with a distinct margin between fiber and myocardium and weighing between 2 and 8 mg were studied. Purkinje fiber and myocardial samples were obtained in random order to avoid systematic bias in time of tissue sampling.

In additional experiments, samples of trabeculated myocardium lying adjacent to Purkinje tissue being sampled were removed in a similar way to Purkinje tissue and transport properties were studied as described below. Thus, samples of endocardium obtained at the same time and exposed to the same blood pool as Purkinje fibers were also studied.

Monovalent cation transport measurements

Monovalent cation active transport by myocardial and Purkinje fiber samples were assessed by measurement of Rb^+ uptake. Samples were placed in physiologic medium containing (mM) 4.0 KCl, 120 NaCl, 24 $NaHCO_3$, 2.0 $MgCl_2$, 2.5 $CaCl_2$, 5.6 glucose, and 1.1 NaH_2PO_4. The pH was adjusted to 7.4 with HCl, the medium oxygenated with 95% O_2 and 5% CO_2, and the temperature maintained at 30°C. After a 5-min equilibration period, samples were transferred to individual vessels containing 1 ml of the medium just described except that 0.1 mM RbCl was added containing tracer ^{86}Rb as $^{86}RbCl$ (New England Nuclear, Boston, Mass.) to give count rates of 10^5 cpm/ml. The tubes were gassed with 95% O_2 and 5% CO_2. The tissue samples were then incubated in the presence or absence of ouabain (1.0 mM) for periods varying from 0 to 30 min at 30°C. At the completion of the incubation, slices were rinsed briefly in physiologic medium without radioactive tracer and Cerenkov radiation from individual samples determined using a scintillation counter. The samples were immediately blotted and weighed after counting. Active uptake of Rb^+ was determined (nanomoles Rb^+/milligram wet weight per 15 min) as the difference between uptake in the presence and absence of 1.0 mM ouabain. It should be noted that the tissue sampling technique used in the present experiments yields greater absolute transport rates than the method previously described (17), probably because of reduced tissue injury.

The time-course of uptake of Rb^+ was determined at 15 and 30 min of incubation. Samples were incubated with Rb^+ with and without 1.0 mM ouabain and removed at 15 and 30 min to determine active and passive Rb^+ uptake.

[1] *Abbreviations used in this paper*: dP/dt, first derivative of pressure with respect to time; IC_{50}, concentration for half maximal inhibition of monovalent cation transport; (Na^+,K^+)ATPase, sodium and potassium-dependent ATPase.

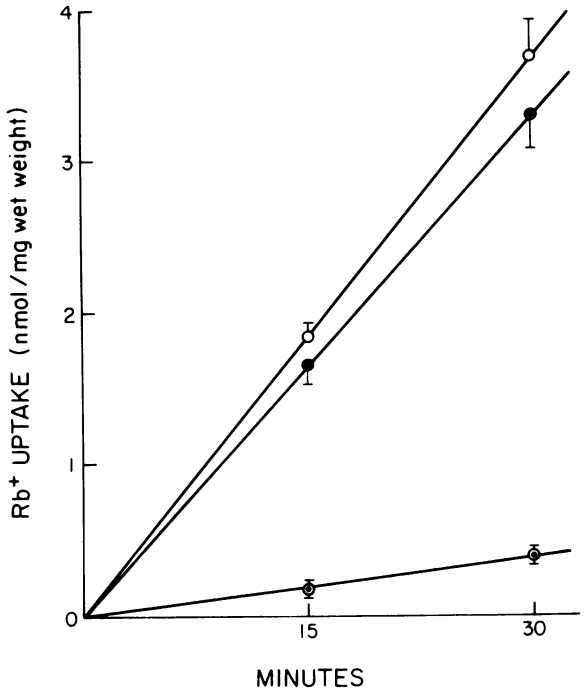

FIGURE 1 Time-course of Rb^+ uptake by canine Purkinje fibers in vitro. Values are mean±SEM. 10 Purkinje fibers (2–8 mg wet wt) were studied at each time point, half of which were incubated in the presence of 0.1 mM Rb^+ including tracer $^{86}Rb^+$ and the other half with both Rb^+ and 1 μM ouabain. Upper curve (○) shows total Rb^+ uptake in the absence of ouabain; the lower curve (⊙) shows uptake in the presence of 1 μM ouabain. The difference is plotted (●) as active Rb^+ transport.

To validate the use of Rb^+ uptake as a marker for the active inward transport of K^+ across the Purkinje cell membrane, experiments comparing transport of Rb^+ (using $^{86}Rb^+$ as tracer) with that of K^+ ($^{42}K^+$ tracer) were performed as described for myocardial samples (18). Briefly, tracer quantities of $^{42}K^+$ were added to the incubation medium together with the standard amount of $^{86}Rb^+$. After incubation, as described above, myocardial samples were individually counted and then recounted 7 d later when the $^{42}K^+$ activity ($t_{1/2}$, 12.4 h) had decayed to <0.006% of the activity present at the time of initial counting. In this way, total and ouabain-inhibitable uptakes of K^+ and Rb^+ could be determined independently in each Purkinje fiber sample.

To determine relative sensitivity of monovalent cation active transport to ouabain added in vitro, concentration-effect curves for inhibition of Rb^+ uptake by Purkinje and myocardial tissue were determined by incubating samples in the presence of ouabain concentrations varying from 10 nM to 1 mM. Rb^+ uptake was determined for 15-min uptake periods after exposure for 30 min to the stated cardiac glycoside concentration.

Experimental protocols

Rb^+ transport at onset of digoxin-induced ventricular tachycardia. Studies were performed comparing monovalent cation transport in myocardium and Purkinje tissue obtained from eight dogs before cardiac glycoside administration and from eight dogs at onset of digoxin-induced ventricular tachycardia. After stable hemodynamic conditions were established and control biopsy samples taken, 0.3 mg/kg i.v. of digoxin (Burroughs Wellcome Co., Research Triangle Park, N. C.) was administered. At onset of sustained ventricular tachycardia, which occurred in all animals, samples of myocardium and Purkinje tissue were taken for transport measurements. Myocardial samples could thus be compared for extent of transport inhibition in each dog before and after digoxin administration.

Rb^+ transport during chronic administration of subtoxic digoxin doses. Eight dogs were given 0.02 mg/kg digoxin i.v. each day for 6 consecutive d. 24 h after the last dose of digoxin, blood samples were taken and serum digoxin concentration determined by radioimmunoassay as previously described (19, 20). Animals were then sacrificed and myocardial and Purkinje samples obtained in random order. Transport studies were performed as described above.

Statistical analysis

The significance of differences between mean values was determined by Student's *t* test, using the method of paired comparisons when appropriate. Experimental results are reported as mean±SEM. Analysis of the concentration-effect curves for ouabain inhibition of Rb^+ transport was performed using a log-logit plot [formally similar to the method of Brown and Hill (21)] to determine concentration for half-maximal inhibition of cation transport (IC_{50}).

RESULTS

Rb^+ transport properties and ouabain sensitivity of canine Purkinje fibers in vitro. Active uptake of Rb^+ by Purkinje fibers remained linear between 0 and 30 min as summarized in Fig. 1. Thus, at 15 min, the uptake duration chosen for the remainder of the study, active uptake was linear with time as we have shown previously for myocardial samples (17). Active uptake was also found to be linear with size of sample incubated over the range of 2–8 mg wet wt. Data in Table I demonstrate that the passive uptake of Rb^+ in the presence of 1.0 mM ouabain constituted similar percentages of total uptake (13 and 11%, respectively) in Purkinje and myocardial samples.

Experiments were also performed to validate the use of Rb^+ as a marker for K^+ transport. If transport of both ionic species were identical, the predicted ratio of K^+ uptake to Rb^+ uptake would be 40:1. Experiments on 40 myocardial samples and 20 Purkinje samples from three dogs, summarized in Table I, yielded mean ratios of 38:1 for active transport by myocardium and 43:1 for Purkinje fibers. Neither ratio differs significantly from the theoretical value of 40:1.

Rb^+ uptake by Purkinje fibers and myocardium incubated in the presence of graded concentrations of ouabain in vitro is shown in Fig. 2. For Purkinje fibers, the curve for ouabain inhibition of monovalent cation transport was shifted to the left of that observed for myocardium, representing greater sensitivity of Pur-

TABLE I

Comparison of K^+ and Rb^+ Transport in Myocardium and Purkinje Fibers

	Myocardium*		Purkinje fibers*	
	Rb^+	K^+	Rb^+	K^+
	nmol/mg wet wt/15 min			
Total‡	0.54±0.06	19.82±2.20	1.87±0.11	79.27±3.75
Passive§	0.06±0.01	1.80±0.23	0.25±0.03	9.21±0.48
Active	0.48±0.06	18.02±2.21	1.62±0.11	70.06±3.78

* Values are given as mean±SEM; conditions described in Methods.

‡ Uptake in the absence of ouabain.

§ Uptake in presence of 1.0 mM ouabain.

kinje fibers to the inhibitory effects of in vitro ouabain exposure. Analyzed using a log-logit plot, the IC_{50} value for Purkinje fibers was found to be 0.4 compared to 1.4 μM for myocardial samples ($P < 0.05$).

Effects of digoxin administered in vivo on Rb^+ transport by myocardial and Purkinje tissue. Rb^+ uptake by ventricular myocardium from eight dogs (10 samples from each animal) at time of sacrifice without digoxin administration showed active uptake to be 0.48±0.06 (SEM) nmol/mg wet wt per 15 min. Purkinje fibers taken in an alternating sequence with myocardial samples from these same hearts showed active Rb^+ uptake to be 1.62±0.11 nmol/mg wet wt per 15 min. Thus, active Rb^+ uptake by Purkinje fibers in vitro in the absence of cardiac glycosides was more than threefold greater than uptake by myocardium under similar conditions.

Myocardial biopsies taken from a second set of eight dogs before digoxin administration yielded Rb^+ transport data indistinguishable from the dogs just described (0.49±0.05 nmol/mg wet wt per 15 min). Digoxin (0.3 mg/kg) was then given intravenously to these dogs, resulting in development of ventricular tachycardia after a mean time of 16±3 min. At onset of ventricular tachycardia, heart rate increased an average of 20%, whereas hematocrit, serum electrolytes, and arterial blood gases were not significantly changed. In samples from dogs sacrificed after 1 min of sustained digoxin-induced ventricular tachycardia, myocardial active Rb^+ uptake was 0.27±0.04 and Purkinje uptake was 0.39±0.04 nmol/mg wet wt per 15 min. As summarized in Fig. 3, comparing control transport values (stippled bars) to those observed at onset of overt toxicity (horizontally hatched bars) in myocardial tissue, we found a mean inhibition of monovalent cation transport of 44±10% ($P < 0.01$ by the method of paired comparisons). However, a significantly greater inhibition of 76±3% was noted for Purkinje fibers ($P < 0.01$) at the same endpoint of ventricular tachycardia compared to samples from animals unexposed to digitalis (Fig. 3).

Rb^+ transport by myocardial and Purkinje tissue during chronic digoxin administration. Digoxin, 0.02 mg/kg i.v., was administered daily to eight dogs for 6 d to achieve steady-state conditions (22). Serum digoxin concentrations in the steady state, 24 h after the last dose, averaged 2.1±0.3 ng/ml. After pentobarbital anes-

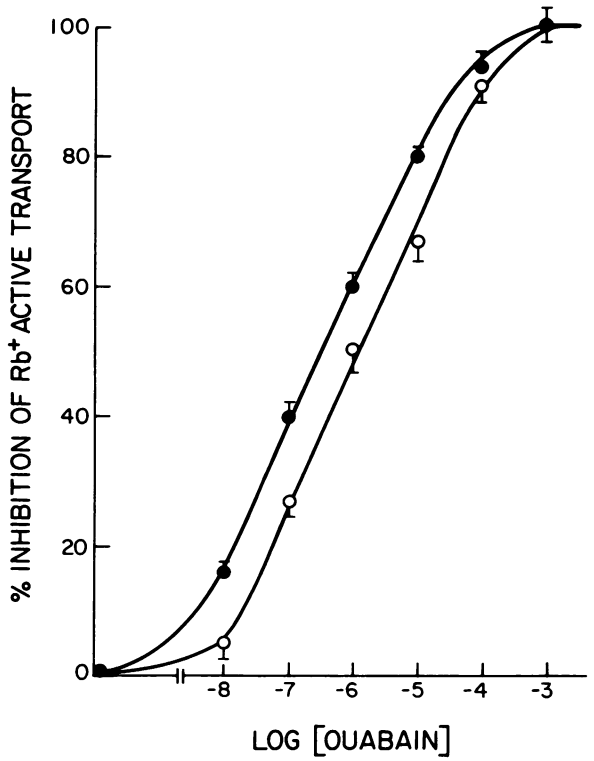

FIGURE 2 Effect of ouabain concentration on inhibition of Rb^+ active transport by myocardium (○) and by Purkinje fibers (●). Tissue samples were incubated in vitro for 30 min in the presence of the stated concentration of ouabain, and active transport of Rb^+ was then measured (in the presence of the same ouabain concentration) as described in Methods. Significantly greater inhibition of Purkinje fiber transport ($P < 0.05$) was found at 10 nM (−8), 0.1 μM (−7), 1 μM (−6), and 10 μM (−5) ouabain concentrations. Vertical lines indicate 1 SEM; $n = 20$ for each point.

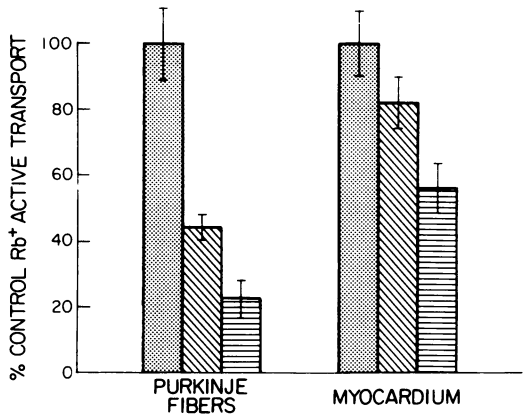

FIGURE 3 Comparison of Rb^+ active transport in Purkinje fibers and myocardium from animals not exposed to digoxin (n = 8; stippled bars), chronically dosed animals receiving 0.02 mg/kg digoxin daily (n = 8; diagonally hatched bars), and animals at onset of ventricular tachycardia after acute administration of 0.3 mg/kg digoxin (n = 8; horizontally hatched bars). Values are reported as percentage change compared to active transport in animals not receiving digoxin. The percent inhibition is significantly greater in the Purkinje fibers than in the myocardium for both acute and chronic dosage regimens ($P < 0.01$).

thesia, animals were instrumented and found to have a heart rate of 105±8 beats/min and a mean arterial pressure of 72±4 mm Hg, not significantly different from values in the acutely studied animals of 105±10 and 81±7, respectively.

Rb^+ active transport in myocardial samples was 0.40 ±0.02 nmol/mg wet wt per 15 min, a mean 17% reduction compared to myocardium from animals unexposed to digitalis (Fig. 3, diagonally hatched bars). Rb^+ active uptake in Purkinje tissue was 0.91±0.04 nmol/mg wet wt per 15 min, a 44% mean reduction in transport compared to Purkinje tissue from control animals. This enhanced inhibition of transport in Purkinje tissue compared to myocardium was highly significant ($P < 0.001$). Passive myocardial uptake of Rb^+, that is, uptake in the presence of 1.0 mM ouabain, was unchanged from that observed in studies of samples from dogs not given digoxin (Fig. 1). In the chronically digitalized dogs, no change in serum Na^+, K^+, or Cl^- concentrations or of arterial blood P_{O_2}, P_{CO_2}, or pH values were observed compared to animals not given digoxin.

Myocardial samples of endocardial trabeculae were also studied in dogs unexposed to digoxin and in animals chronically exposed to digoxin. In two dogs (20 samples from each dog), Rb^+ active transport in endocardial trabeculae was 0.45±0.06 nmol/mg wet wt per 15 min. In two chronically digitalized animals, transport was 0.28±0.05 nmol/mg wet wt per 15 min. These values were not significantly different from those observed from the transmural myocardial biopsy sample technique in these same animals.

DISCUSSION

A substantial body of information, summarized in the introduction, indicates greater sensitivity of Purkinje fibers than of myocardium to the toxic effects of cardiac glycosides. von Kubler and associates (23), however, reported greater cardiac glycoside sensitivity in vitro of (Na^+,K^+)ATPase from calf myocardial homogenates compared to homogenates of Purkinje tissue. Palfi and associates (24) found that in vitro ouabain sensitivities of (Na^+,K^+)ATPase from detergent-treated homogenates of calf Purkinje tissue and papillary muscle from the same heart differed only slightly, with myocardium from papillary muscles showing 3–5% greater inhibition by ouabain concentrations bracketing the IC_{50} value. In any event, these previous studies leave open the possibility that Purkinje fiber monovalent cation transport in vivo may be more sensitive to cardiac glycosides than is the case for myocardium, possibly due to intrinsic differences in ion concentration and/or fluxes across the intact cell membrane (25, 26), or that differences in drug access may occur in vivo.

In the present studies, we have used an experimental model that permits relatively direct examination of the effects of cardiac glycosides on monovalent cation transport, rather than the more indirect assessment of transport by measurement of sodium- and potassium-sensitive ATP cleavage in broken-cell homogenates of cardiac tissue. The latter approach, although of considerable interest in a number of contexts, lends itself less well to the study of the extent of transport inhibition in vivo because of the possibility of altered cardiac glycoside binding to sodium pump sites during preparative procedures and the uncertainties introduced by disruption of normal transmembrane ion gradients and fluxes (11).

The methods developed for assessment of monovalent cation active transport by Purkinje fibers are closely similar to those previously used in our laboratory for myocardial transport studies (17). As was found for myocardial samples, active uptake of the K^+ analog Rb^+ was linear with tissue weight over the range employed in these studies, indicating that diffusion was not rate limiting. Rb^+ uptake was also linear with time for periods well beyond the 15 min uptake interval chosen for purposes of comparison of experimental groups (Fig. 3). Also analogous to previous (and present) findings for myocardial samples, Rb^+ at a final bath concentration of 0.1 mM was taken up by Purkinje fibers in a manner indistinguishable from K^+, with a ratio of 43:1 for active K^+:Rb^+ uptake, compared to a theoretical value of 40:1 in the presence of 4.0 mM K^+ and 0.1

mM Rb^+. The finding of greater Rb^+ and K^+ active transport rates by Purkinje fibers compared to myocardium implies a greater K^+ exchange rate under our experimental conditions, although normalization of transport rates to mg wet weight of tissue introduces an element of uncertainty because of the probability of different ratios of sarcolemmal membranes to tissue mass in Purkinje fibers and myocardium.

The present studies demonstrated significantly greater ouabain-induced transport inhibition in Purkinje fiber samples compared to myocardium after in vitro exposure of intact tissue samples to ouabain. The concentration-effect curves (Fig. 2) for ouabain-induced inhibition of transport are essentially parallel over a broad range of ouabain concentrations, with significantly greater inhibition of transport in Purkinje fibers at each ouabain concentration from 10 nM to 10 μM. It should be emphasized, however, that these findings do not distinguish with certainty between differences in affinity of Purkinje fiber and myocardial (Na^+,K^+)ATPase sites for cardiac glycosides and differences in drug access to these sites.

In addition to the differences observed with in vitro cardiac glycoside exposure, we also found substantially greater degrees of transport inhibition in Purkinje compared with myocardial tissue after in vivo administration of digoxin. The greater transport inhibition in Purkinje tissue was evident both under conditions of acute administration of large, toxic (0.3 mg/kg) doses of digoxin and under conditions of chronic administration of digoxin using a regimen that we have shown previously to produce a pharmacokinetic steady state characterized by an increase in myocardial contractile state as judged by left ventricular maximum first derivative of pressure with respect to time (dP/dt) and absence of any signs of toxicity (22). Steady-state plasma digoxin concentrations achieved in the latter study averaged 2.2 ng/ml, quite similar to the value of 2.1 ng/ml found in the present study. Although we did not measure inotropic responses in the present experiments, previous studies have demonstrated a mean increase in left ventricular maximum dP/dt of 23% under identical conditions (22), whereas in other intact animal models 21–25% inhibition of Rb^+ active transport was associated with 20–29% enhancement of left ventricular maximum dP/dt (17, 27).

At onset of ventricular tachycardia induced by toxic doses of digoxin, myocardial samples in the present study showed a mean 44% inhibition of Rb^+ active transport, somewhat less than the 60% reduction of transport at a similar endpoint after administration of digoxin to dogs by a different dosage schedule (27) but quite similar to the 46% inhibition of myocardial transport found in neurally intact cats at onset of ouabain-induced ventricular tachycardia (18). These values for inhibition of myocardial monovalent cation transport are, in general, similar to levels of inhibition of (Na^+,K^+)ATPase activity in particulate fractions from homogenates of hearts taken at onset of overt cardiac glycoside-induced arrhythmias in the studies of Besch et al. (28) [59% inhibition of (Na^+,K^+)ATPase], Akera et al. (29) [47% inhibition of (Na^+,K^+)ATPase], and Dutta et al. (30) [54% inhibition of (Na^+,K^+)ATPase]. The 76% mean inhibition of Rb^+ transport in Purkinje fibers at onset of ventricular tachycardia observed in the present study thus represents a substantially greater degree of transport inhibition than that observed at comparable endpoints in a number of studies of myocardial tissue.

A source of concern after the completion of the acute experiments was the possibility that differences in apparent sensitivity of Purkinje fiber monovalent cation transport compared to myocardium might represent a transient phenomenon related to differing kinetics of digoxin binding to tissue sodium pump sites rather than a true difference in tissue sensitivity when steady-state equilibrium conditions were attained. For this reason, the complementary experiments using chronic administration of subtoxic digoxin doses were performed. The greater digoxin-induced inhibition of transport in Purkinje fibers under these steady-state conditions, compared to either transmural myocardial samples or subendocardial trabecular samples taken from myocardium adjacent to and exposed to the same blood pool as the adjacent Purkinje fibers, confirms the findings from the acute experiments with toxic digoxin doses. Although these findings suggest greater intrinsic sensitivity (i.e., affinity) for cardiac glycosides in Purkinje tissue, we reiterate that differences in access to tissue (Na^+,K^+)ATPase sites cannot be excluded with certainty.

The cellular mechanism(s) by which cardiac glycosides cause cardiac arrhythmias remains an area of active investigative interest. The interesting possibility that sodium pump inhibition may lead to intracellular calcium overload accompanied by transient depolarizations and arrhythmic activity in Purkinje fibers has been raised by Tsien and colleagues (31, 32). Voltage clamp experiments consistent with oscillatory release of calcium from intracellular stores (31) suggest a sequence of increased $[Na]_i$ due to sodium pump inhibition leading to elevated $[Ca]_i$ through an alteration in sodium-calcium exchange (31, 33, 34). Oscillatory release of calcium may then give rise to the digitalis-induced transient inward current that appears to underlie the transient depolarizations associated with spontaneous impulse formation in digitalis-induced arrhythmias [35–37, reviewed by Ferrier (38)].

Our finding of pronounced (76%) inhibition of monovalent cation transport in Purkinje fibers at onset of cardiac glycoside-induced arrhythmias is consistent with the role ascribed to the Purkinje network in the genesis of digitalis-induced arrhythmias by a number of authors (1–5, 38). It seems reasonable to speculate that the relatively greater sensitivity of Purkinje fibers compared to myocardium to digitalis-induced transport inhibition may underlie, at least in part, the relatively low therapeutic ratio long known to be associated with the use of this class of drugs.

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