Preferential Sensitivity of the Left Canine Purkinje System to Cardiac Glycosides

JOSEPH REISER AND GARY J. ANDERSON

SUMMARY Previous studies have shown that the toxic effects of cardiac glycosides are not manifested uniformly throughout the myocardium. The purpose of our study was to determine whether cardiac glycosides exert different effects on the right vs. left peripheral Purkinje systems and to ascertain mechanisms involved. Control in vitro measurements of paired right and left canine Purkinje fibers showed higher spontaneous rates in left (24.2 ± 1.75 beats/min) than in right (11.6 ± 1.55 beats/min, P < 0.01, n = 81) Purkinje fiber bundles. Following overdrive stimulation, left Purkinje fiber bundles also showed earlier escape beats. After ouabain exposure (2 × 10^-7 M), left Purkinje fiber bundles showed earlier signs of toxicity in 20 of 28 experiments, as determined by changes in the maximum diastolic potential, the degree of diastolic depolarization, spontaneous escape intervals, and the magnitude of delayed after-depolarizations. The enhanced sensitivity of left Purkinje fiber bundles was independent of the extracellular potassium concentration and glycoside polarity, and was also observed in situ. We conclude that distal Purkinje fibers are functionally dissimilar and that the left Purkinje system shows greater sensitivity to cardiac glycosides than the right Purkinje system. These data also support the observation that digitalis-induced dysrhythmias arise in the left ventricle. Circ Res 49:1043-1054, 1981

THE electrophysiological property of automaticity is present in the specialized conduction system of the heart and is known to be affected by cardiac glycosides. The effect of cardiac glycosides on automaticity of the specialized conduction system is diverse, and different effects have been reported in both therapeutic and toxic doses (Lown and Levine, 1954; Vassalle et al., 1962; Hoffman and Singer, 1964; Scherlag et al., 1971). For instance, subsidiary or junctional pacemakers may be accelerated following digitalis exposure and overtake the normally dominant sinus pacemaker. Other studies have shown that pacemakers residing in the left ventricle predominate in digitalis-induced ventricular tachycardia (Rothberger and Winterberg, 1910; Damato et al., 1971; Kastor et al., 1972). These studies and our preliminary experiments (Reiser and Anderson, 1976, 1977) suggest that left ventricular Purkinje fibers may exhibit greater sensitivity to cardiac glycosides than Purkinje fibers from the right ventricle. We therefore initiated this study to determine the electrophysiological effects of cardiac glycosides on simultaneously impaled right and left Purkinje strands in vitro.

Methods

In Vitro Electrophysiological Measurements

Adult mongrel dogs weighing 10-20 kg were anesthetized with sodium pentobarbital (30 mg/kg), intravenously. Following a lateral thoracic incision, the heart was removed and immediately placed in oxygenated Tyrode's solution. Both right and left ventricles were opened, and single ("free-running") Purkinje strands between the free wall and the base of the papillary muscle on the septum were excised. In all experiments the selection of Purkinje strands was confined to this anatomic level. Purkinje strands from the left ventricle consisted of strands from the anterior and posterior divisions. Whereas, we did not evaluate the effects of ouabain between strands of the anterior and posterior division, strands obtained from either division were utilized in tandem with those obtained from the right ventricle. Purkinje strands from both ventricles were variable in length (5-16 mm) and width (0.5-2 mm) and showed no consistent differences in fiber dimension from either right or left ventricle. In a separate group of experiments, fibers were chosen from this anatomic level but closely matched for fiber length and width. Matching for fiber size was accomplished by utilizing existing fibers of equal dimensions or by selecting strands approximately equal in width and cutting to comparable lengths. Fiber dimensions were determined by means of a dissecting microscope with an ocular grid insert.

A single Purkinje strand from each ventricle was subsequently pinned (without stretch) to the bottom of the same 10-ml muscle chamber and was superfused continuously with oxygenated Tyrode's...
right and left Purkinje fibers showed statistically insignifi-
cant differences in action potential config-
uration (see Results).

The middle of each strand was selected as the
recording site. We further matched impalements by
recording only from surface cells showing typical
Purkinje fiber action potential configurations.
Transitional and muscle cells were excluded from
our study. By adhering to such criteria, cells from
right and left Purkinje fibers showed statistically
insignificant differences in action potential config-
uration (see Results).

Either ouabain or acetylstrophanthidin was ad-
ministered in concentrations from 1-2 × 10^{-7} M to
the perfusate; the potassium concentration was al-
tered by superfusing solutions containing different
potassium concentrations. Data were recorded from
the display of a Tektronix 5000 series storage oscil-
loscope with high-speed Polaroid film. For perma-
nent data storage, a Honeywell 1858 CRT Visi-
corder and a Honeywell 96 tape system were used.
Statistical analysis included Student's t-test for
grouped or paired data, where applicable (Colton,
1974).

In Vivo Electrophysiological Measurements

Mongrel dogs weighing between 15 and 30 kg
were anesthetized with sodium pentobarbital (30
mg/kg), intravenously. Respirations were maintained
with a Harvard respirator through a pharyngo-
tracheal tube. The chest was opened by a right tho-
racotomy and the heart suspended in a pericardial
sling. Since the heart rate may be accelerated fol-
lowing pentobarbital anesthesia, the sinus node was
crushed at times to achieve a slower spontaneous
heart rate and to allow for His bundle stimulation.

For purposes of stimulation and recording, bipo-
lar plunge electrodes with an interelectrode dis-
tance of 1-2 mm were placed into both the atrial
and the ventricular walls. His bundle stimulation
and recording were performed according to the
method of Scherlag et al. (1967). Activation of the
conduction system was measured by bipolar plunge
electrodes in the appropriate region. In all experi-
ments, electrophysiological measurements were
 correlated with the anatomic location of the plunge
electrodes, determined postmortem. Surface re-
cordings also were correlated in time with lead I
and II electrodes attached to the extremities. Re-
fractoriness was determined by introducing a pre-
mature beat of at least four times threshold follow-
ing a minimum of 12 paced beats.

Programmed basic and premature beats were
elicted through a Frederick Haer Pulsar 6i-i stim-
ulator. Digoxin (40 μg/kg) was administered in two
doses 20 minutes apart by means of a catheter
seured in the left femoral vein.

Histological Determination of Fiber Collagen

Right and left Purkinje fibers of variable size
were obtained from the mongrel dogs weighing 15-
20 kg. Upon excision, right and left strands were
fixated in 10% formalin and embedded in paraffin
with standard techniques. Fiber cross-sections, ob-
tained from the midsections of these strands, were
subsequently stained with either chromotrope-
line blue (CAB) or Gomori's trichrome, which are
specific stains for collagen. Slides containing fiber
cross-sections were projected onto a large screen by
means of a grid-containing microscope lens. The
total number of grids superimposed on collagen and
noncollagen areas was determined. Individual
squares, which covered both collagen and non-collagen sections of the fiber, were visually divided into four parts for added accuracy. In this manner, the ratio of collagen to whole fiber size was determined. Student’s t-test for grouped data was employed to determine statistical significance (Weiner, 1971).

Results

Action Potential Characteristics of Normal Right and Left Ventricular Purkinje Fibers

Both left and right Purkinje cells showed similar maximum diastolic potentials when stimulated [92.7 ± 1.0 vs. 91.3 ± 0.8 mV (mean ± SE), BCL = 900, respectively] and when spontaneously active [89.6 ± 1.1 vs. 88.8 ± 0.9 mV (mean ± SE), respectively; n = 32]. Likewise, no statistically significant difference in the action potential duration, measured at 80% repolarization, could be determined between right and left Purkinje strands, [324.5 ± 7.8 vs. 314.9 ± 10.3 msec (mean ± SE), BCL = 900, respectively; n = 32]. Action potential amplitudes also were comparable [127.2 ± 1.2 vs 125.6 ± 2.3 mV (mean ± SE), respectively]. Significant differences were found in the intrinsic rate and escape intervals between right and left Purkinje strands (Fig. 1, Table 1). The intrinsic rate of left Purkinje strands under control conditions was found to be higher (24.2 ± 1.75 beats/min (mean ± SE) than the spontaneous rate observed in right Purkinje strands (11.6 ± 1.66 beats/min, P < 0.01, n = 31 pairs). Cells of the left Purkinje network showed a faster intrinsic rate in 73 of 81 pairs of fibers (90%). Of these experiments in which left Purkinje strands exhibited a higher rate, the difference in the intrinsic rate exceeded 100% in 50 of 73 experiments (68%). In contrast, the intrinsic rate of the right Purkinje strands exceeded the intrinsic rate of the left Purkinje strands in only eight experiments; in three of these the difference was greater than 100%.

Escape intervals, measured simultaneously in both left and right Purkinje strands following overdrive stimulation, were prolonged with decreasing cycle lengths. Escape intervals of left Purkinje strands were, however, consistently shorter over the range of cycle lengths examined (P < 0.05), as shown in Figure 1. In each panel, the top trace represents the cell from the left Purkinje strand. In Figure 1A, the intrinsic rate of the left Purkinje

![Intrinsic rate and escape intervals of left (top trace) and right (bottom trace) ventricular Purkinje fibers in Tyrode's solution containing 2.7 mM K⁺. In Figure 1A, the intrinsic rate (IR) of the left Purkinje cell is 17 beats/min compared to the significantly slower rate of 3 beats/min observed in the right Purkinje cell. Figure 1, B–D, shows the spontaneous escape intervals after a 3-minute stimulation period at cycle lengths of 1000, 900, and 700 msec, respectively.](http://circres.ahajournals.org/)

**Figure 1.** Intrinsic rate and escape intervals of left (top trace) and right (bottom trace) ventricular Purkinje fibers in Tyrode’s solution containing 2.7 mM K⁺. In Figure 1A, the intrinsic rate (IR) of the left Purkinje cell is 17 beats/min compared to the significantly slower rate of 3 beats/min observed in the right Purkinje cell. Figure 1, B–D, shows the spontaneous escape intervals after a 3-minute stimulation period at cycle lengths of 1000, 900, and 700 msec, respectively.
Effect of Ouabain on Right and Left Ventricular Purkinje Fibers Perfused in Normal Tyrode’s Solution

In 28 experiments, a determination was made of the effects of ouabain on simultaneously impaled right and left Purkinje strands (unmatched for fiber size) stimulated at various basic cycle lengths. Ouabain was superfused at two different concentrations: 1 × 10^{-7} M (n = 5) and 2 × 10^{-7} M (n = 23). In each experiment, changes in the maximal diastolic potential, the slope of diastolic depolarization, the escape interval, the intrinsic rate, and the magnitude of delayed after-depolarizations were recorded as indices of the toxic effects of ouabain.

The toxic effects of glycoside exposure first were noted in the left Purkinje cells, manifest by changes in the action potential duration, the maximum diastolic potential, and the slope of the diastolic depolarization. These effects (Fig. 2) were usually established by 10–20 minutes of ouabain exposure. Continued superfusion induced after-depolarizations and spontaneous (triggered) firing in the left Purkinje cells. Evidence of glycoside toxicity in right Purkinje cells similar to the early changes observed in left Purkinje cells usually occurred after 40–50 minutes of ouabain superfusion (not shown).

Left Purkinje cells in ouabain concentrations of 2 × 10^{-7} M developed cellular toxicity before the right Purkinje cells in 17 of 21 experiments; in two experiments the right Purkinje cells showed earlier signs of toxicity. In concentrations of 1 × 10^{-7} M ouabain, the response was similar but delayed in onset. The differences in the onset of toxicity between right and left Purkinje cells were not dependent on the cycle length of stimulation.

The data describing the non-uniform effects of ouabain on right and left ventricular Purkinje fibers are quantified in Figure 3, A–E. These data were pooled from 11 of 23 experiments in which the cycle length of stimulation was 700 msec. In Figure 3, A and B, the effect of ouabain on the maximum diastolic potential (MDP) and on diastolic depolarization is shown. Diastolic depolarization (Fig. 3B) was determined as the difference between the maximum diastolic potential (MDP) and the take-off potential (TOP) of the subsequent action potential. In each parameter, ouabain produces both earlier and more pronounced effects in cells from the left Purkinje strand. Similarly, delayed after-depolarization (DAD’s) were manifest earlier and were of a higher amplitude in cells from the left Purkinje strand. Similarly, delayed after-depolarization (DAD’s) were manifest earlier and were of a higher amplitude in cells from the left Purkinje fibers (Fig. 3C). Escape intervals (EI), measured simultaneously in right and left Purkinje fibers, shortened in both strands after ouabain exposure (Fig. 3D). However, escape beats occurred earlier in cells from the left ventricular Purkinje fibers.

### Table 1: Mean Intrinsic Rates and Escape Intervals of Right and Left Purkinje Strands

<table>
<thead>
<tr>
<th>BCL (msec)</th>
<th>n</th>
<th>LBB</th>
<th>RBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>32</td>
<td>9.9 ± 1.7*</td>
<td>25.9 ± 3.3</td>
</tr>
<tr>
<td>900</td>
<td>18</td>
<td>12.0 ± 2.7*</td>
<td>26.8 ± 4.5</td>
</tr>
<tr>
<td>800</td>
<td>33</td>
<td>14.7 ± 2.8*</td>
<td>34.7 ± 4.6</td>
</tr>
<tr>
<td>700</td>
<td>16</td>
<td>18.0 ± 4.1*</td>
<td>36.2 ± 6.9</td>
</tr>
<tr>
<td>600</td>
<td>32</td>
<td>19.6 ± 3.8*</td>
<td>37.9 ± 3.6</td>
</tr>
<tr>
<td>500</td>
<td>17</td>
<td>23.0 ± 4.2*</td>
<td>44.1 ± 8.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LBB = left Purkinje fiber; RBB = right Purkinje fiber; n = number of experiments.

### Table 2: Control Parameters of Geometrically Matched Right and Left Purkinje Fibers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LBB</th>
<th>RBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDP (mV)</td>
<td>93.6 ± 1.1</td>
<td>94.0 ± 0.7</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>127.5 ± 1.8</td>
<td>125.7 ± 2.6</td>
</tr>
<tr>
<td>OS (mV)</td>
<td>31.9 ± 1.8</td>
<td>30.5 ± 2.2</td>
</tr>
<tr>
<td>APD_{90}</td>
<td>270.7 ± 7.9</td>
<td>287.3 ± 13.9</td>
</tr>
<tr>
<td>Ph (mV/sec)</td>
<td>6.8 ± 1.2</td>
<td>5.06 ± 1.3</td>
</tr>
<tr>
<td>EI (sec)</td>
<td>29.9 ± 8.2*</td>
<td>501 ± 10.7</td>
</tr>
<tr>
<td>IR (beats/min)</td>
<td>26.1 ± 4.8*</td>
<td>13.4 ± 3.9</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>8.02 ± 0.8</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>1.12 ± 0.2</td>
<td>1.33 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Total of nine experiments. LBB = left Purkinje fiber; RBB = right Purkinje fiber; BCL = 700 msec.

* P < 0.005 (paired Student’s t-test)
The intrinsic rate (IR) after ouabain exposure (Fig. 3E) showed no significant change in either preparation during the first 10 minutes of ouabain exposure, although an increase in the slope of phase 4 depolarization was observed in left Purkinje strands (Fig. 3B). Between 10 and 20 minutes of ouabain exposure, a pronounced increase in the intrinsic rate occurred in left Purkinje fibers, whereas only insignificant changes in the intrinsic rate were seen in right Purkinje strands. This abrupt increase in the IR parallels enhancement of the diastolic slope and the amplitude of the after-depolarizations in left Purkinje strands (Figure 3, B-C). The large deviation from the mean (i.e., large error bars) observed in the left strands reflects the mixture of extremely toxic cells as being either quiescent or as having a slow IR and cells approaching this endpoint which demonstrate very high IR's. By 30 minutes of ouabain superfusion, the intrinsic rate decreased significantly in the left Purkinje preparation and was associated with further reduction in the maximum diastolic potential (Fig. 3A).

As stated earlier, the experiments described in Figures 2 and 3 were carried out with Purkinje fiber bundles unmatched for fiber size. When fibers matched for fiber size were exposed to ouabain, the enhanced sensitivity of left Purkinje fiber bundles when compared to the fiber bundles from the right ventricle was identical to that observed in Figure 3 (not shown).
The Effect of Cardiac Glycosides on Spontaneously Beating Right and Left Ventricular Purkinje Fibers

Five experiments were conducted to evaluate the sensitivity to ouabain of non-paced Purkinje cells, allowed to discharge at their intrinsic rate. Ouabain exposure (2 × 10^{-7} M) caused a reduction in the maximum diastolic potential, an increase in the slope of diastolic depolarization, and a higher intrinsic rate. In each experiment, ouabain induced earlier effects in the Purkinje strand that showed a higher intrinsic rate and greater slope of diastolic depolarization during control. Left fibers in three experiments and right fibers in two experiments showed these latter characteristics at control. Figure 4 exemplifies the sequential membrane effects that occur after ouabain exposure in a pair of spontaneously beating right and left Purkinje fiber bundles. In this example, the left fiber bundle showed the higher intrinsic rate and diastolic slope at control and thus showed the earlier onset of toxicity. However, the electrophysiological changes denoting the differential onset of digitalis toxicity were the same in both right and left fiber bundles.

Effect of Ouabain on Right and Left Ventricular Fibers in Tyrode’s Solution Containing 4 mM K^+

We considered that the preferential effect of ouabain on left Purkinje fibers may be related to the potassium concentration of the perfusate (2.7 mmol). In four experiments with 4 mM K^+, the control intrinsic rate was significantly slower in both right and left Purkinje strands and the onset of membrane toxicity after ouabain superfusion was delayed (comparable membrane effects occurred 10-15 minutes later). However, Purkinje cells from the left ventricular conduction system showed higher intrinsic rates in three of four experiments and developed earlier membrane toxicity in all four experiments. The above-mentioned differences in the onset of ouabain toxicity in a 4 mM K^+ perfusate are demonstrated in Figure 5.
GLYCOSIDE TOXICITY IN CARDIAC PURKINJE FIBERS

In Vivo Electrophysiological Measurements

Using the results obtained from the in vitro electrophysiological studies, we attempted to determine whether cardiac glycosides preferentially involved the left infra-His conduction system in intact hearts of open-chest dogs. In eight experiments, electrograms were recorded from the His bundle, the bundle branch system, and ventricular myocardium. Figure 6 shows the changes in the activation sequence of the bundle branches after digoxin administration. In addition to lead I and II, three other electrode recording sites are shown in descending order: the proximal left bundle branch (near bundle of His), left Purkinje fibers near the septal wall (anterior division), and the right ventricle near the base of the anterior papillary muscle. In the control sequence, premature stimulation at a coupling interval of 190 msec conducts via the normal activation pattern to both ventricles as evidenced by the activation spikes of both the left bundle branch electrode (LBBE) and the left Purkinje system (LP). After 40 minutes of digoxin infusion (Fig. 6B), a premature beat delivered at the same coupling interval (190 msec) induced conduction block of the left bundle branch shown by the disappearance of the left bundle branch electrogram spike and prolongation of activation to the left ventricular electrogram site. The right ventricular activation time remained unchanged.

Further support for the preferential involvement of the left ventricular conduction system after digoxin infusion is provided by the normally conducted sinus beat shown in Figure 6B immediately after the premature beat. Both the LBBE and LVE electrodes show left bundle branch and left Purkinje

Figure 4 Effect of ouabain ($2 \times 10^{-7}$ M) on automaticity in right and left Purkinje strands. The intrinsic rate of the left Purkinje cell (top trace) was 9 beats/min compared with the intrinsic rate of the right Purkinje cell (bottom trace), which is 3 beats/min. The slope of diastolic depolarization was 1.8 mV/sec in the left Purkinje fiber and <0.5 mV/sec in the right Purkinje strand. By 20 minutes of ouabain superfusion, a transition toward a higher intrinsic rate and lower maximum diastolic potential occurs in the left Purkinje strand, while the right Purkinje cell is unchanged. Both the intrinsic rate (23 beats/min) and the diastolic slope (7.2 mV/sec) continued to increase in the left Purkinje strand (Fig. 4B) at 30 minutes of ouabain superfusion, compared to 4 beats/min and only a slight increase in diastolic slope in the right fiber. In addition, no significant change in the maximum diastolic potential of the right Purkinje cell has occurred, while the left cell had depolarized to $-45$ mV and had become quiescent. The right Purkinje cell continues to show membrane parameters virtually unchanged from control ($O_L$, $O_R$ = zero potential of respective Purkinje strands).
FIGURE 5  The effect of ouabain superfusion (2 × 10⁻⁷ M) in right (bottom trace) and left (top trace) Purkinje strands exposed to 4 mM K⁺. Both bundles were intermittently stimulated using trains of stimulus pulses (10 beats each, BCL 500 msec) followed by a 5-second pause. The pause was interposed to allow early recognition of the development of delayed after-depolarizations. The first indications of developing after-depolarizations can be observed 39 minutes after ouabain exposure in the left Purkinje cell. The change in the diastolic slope which occurs first in the left Purkinje cell is more clearly visualized by slowing the stimulation rate (BCL 700; 40 minutes ouabain superfusion). By 50 minutes ouabain exposure, spontaneous escape beats occur in the left strand along with after-depolarizations. In contrast, the right Purkinje cell maintains action potential parameters similar to control. The toxic effects of ouabain are further exaggerated by 65 and 76 minutes of ouabain exposure resulting in membrane depolarization (~42 mV), resistance to external stimulation, and the development of abnormal automaticity. Only at this time period of ouabain exposure are significant after-depolarizations seen in the right Purkinje cell (bottom trace, 76-minute picture).

activation before the corresponding ventricular activation. In three experiments in which the electrogram recordings were optimal or the premature stimulation did not elicit ventricular tachyarrhythmias following digoxin administration, left ventricular conduction blocked preferentially over right ventricular conduction.

Histological Determination of Collagen Content in Right and Left Purkinje Fibers

Cardiac Purkinje fibers are known to be sheathed by collagen to a varying extent (Thornell, 1975). Since the collagen sheath represents a barrier to the superfusing medium, differences in the collagen...
GLYCOSIDE TOXICITY IN CARDIAC PURKINJE FIBERS/Reiser & Anderson

CONTROL

•CL

LEAD I

LEAD II

LBBE

RVE

LVE

FIGURE 6 Determination of bundle branch refractoriness after digoxin administration (80 µg/kg) in the intact heart. Panel A shows control recording from the standard leads I and II, plunge electrodes in the left bundle branch (LBBE), the right ventricle near the base of the anterior papillary muscle (RVE), and the left ventricle in the Purkinje strand of the left anterior division (LVE). Stimulation was performed from the bundle of His. In control (panel A), a premature stimulus at a coupling interval of 190 msec conducts via both Purkinje strands to the ventricular myocardium. After digoxin infusion (panel B), a premature stimulus at the same coupling interval fails to conduct via the left conducting system, and the left Purkinje spike (LP) is no longer observed. The third beat in Figure 6B is a sinus beat.

surrounding Purkinje strands may influence drug accessibility to the cell surface and influence the interpretation of our results. We approached this question by selectively staining 10 pairs of right and left Purkinje strands for collagen and determining the ratio of collagen to total fiber size in each strand. The mean ratio in the left Purkinje strands was 0.702 ± 0.081 (mean ± se) as compared with 0.551 ± 0.093 in the right fibers. The difference between right and left fibers did not reach statistical significance (0.1 > P > 0.05).

Discussion

The findings reported in this study indicate the disparate effects that cardiac glycosides have on the ventricular specialized conducting system. However, unlike the differential effect of glycosides on Purkinje and muscle cells (Polimeni and Vassalle, 1971), the effect on the right and left ventricular conducting tissue was dissimilar only in the time of onset of toxicity and not in the final toxic manifestations. The differential effect of glycoside-induced toxicity of left Purkinje fibers was manifest as an earlier decline in the maximum diastolic potential, enhanced development of delayed after-depolarizations, earlier escape beats, and higher rates of automaticity. These disparate electrophysiological effects of right and left Purkinje strands occurred at both normal and below-normal extracellular potassium concentrations (Fig. 5) and were independent of glycoside polarity. In the latter studies, we compared the electrophysiological effects of a non-polar agent (acetylstrophanthidin) to those of ouabain, a polar glycoside. The response to acetylstrophanthidin, although faster in onset, was qualitatively similar to the effects observed in right and left strands after ouabain exposure (data not shown). Thus, our studies suggest that the mechanism of the disparate ouabain affects on right and left Purkinje fiber bundles involves intrinsic differences in the cellular or receptor properties of these fiber bundles.

Our study represents the first quantitative in vitro comparison of the differential effect of ouabain in right and left Purkinje fibers and supports the findings of previous in vivo studies (Rothberger and Winterberg, 1910; Damato et al., 1971; Kastor et al., 1972). Both the Damato and the Kastor groups have shown that the origin of ouabain-induced ventricular tachycardia was in the left rather than the right ventricle. Furthermore, both studies suggested that the focus of the tachycardia was within the terminal Purkinje fibers, although the evidence was mostly indirect. These unifocal ventricular tachycardias were thought to represent accelerated idioventricular rhythms and were seen relatively late during digitalis exposure.

The studies by Zipes et al., (1974) added further support to the theory that cardiac glycosides preferentially affect the left Purkinje system. In these studies, glycosides initiated accelerated left ventricular escape beats in dogs with experimentally induced AV block. These in vivo studies are consistent with our in vitro findings. The highest in-
trintrinsic rates and shortest escape intervals were observed in left Purkinje fibers and occurred once digitalis toxicity had developed (Fig. 2 and 3; Table 1). At this time, toxic membrane effects due to digitalis exposure are manifested by a decreasing membrane potential, enhanced automaticity, and after-depolarizations, which may reach threshold (Fig. 3, A–D). Since spontaneous beating was associated with after-depolarizations reaching threshold, such automatic activity appeared to represent "triggered," rather than true, automaticity (Ferrier et al., 1973). This is further supported by the finding that a single premature beat may initiate a more rapid response by increasing the height of the delayed after-depolarization to reach threshold (Davis, 1973). Although reentry cannot be excluded, Figure 4 (20 minutes) may illustrate this mechanism. These findings, which occur earlier in left Purkinje fibers, may thus account for accelerated idioventricular rhythms of left ventricular origin following digitalis exposure.

Added support for the preferential involvement of the left ventricular conducting system after glycoside exposure is provided by the measurements obtained in situ (Fig. 6). The results obtained are consistent with our in vitro findings and with those by Damato et al. (1971) and Kastor et al. (1972). The left ventricular conduction block observed after premature stimulation suggests altered refractoriness specific to the left ventricular conduction system. Since our in vitro studies show significant depolarization (P < 0.05) of the membrane potential during the later stage of digitalis exposure, the altered refractoriness may reflect a shift toward time dependence which is known to exist at lower membrane potentials. Although the actual site of block cannot be accurately deduced from our recordings, the preferential effect of digoxin on the left infra-His-conduction system may be of added significance in view of the prolonged refractoriness in the right bundle branch known to exist under physiological conditions (Bailey et al., 1978).

The enhanced intrinsic rate in left Purkinje fibers under control conditions was not an expected finding. This observation is unlikely to be explained by the potential trauma of dissection, since the tissue was allowed ample time to recover prior to recording of the intrinsic rate. Furthermore, preparations that were unable to maintain an acceptable membrane potential in the normal range after external stimulation stopped were not included in the averaging of results. However, the 2-fold higher intrinsic rate determined in left Purkinje cells is consistent, in part, with several prior studies. Kastor et al. (1972) noted that the intrinsic rate of left Purkinje fibers in four of five in vitro preparations was higher than in right Purkinje strands before digitalis exposure. Hope et al. (1976) also showed the dominance of left ventricular pacemakers. In 15 of 22 in vivo experiments, the pacemaker site under control conditions was localized to the left Purkinje system.

In the in vitro experiments, however, those investigators showed no significant differences in the intrinsic rate between the right and left conducting systems. This latter finding is not inconsistent with our data. In their study, all of the proximal left or right bundle branches and most of their Purkinje networks were included in the preparation; hence the peripheral Purkinje system was not systematically divided as in our studies. Therefore, since the more proximal pacemakers of the bundle branch system have a more rapid intrinsic rate, this would inhibit the expression of slower but dissimilar rates in the distal Purkinje system. However, the relationship of distance to the His bundle and pacemaker hierarchy may also contribute to the observed differences in the Hope (1976) study. Since our tissue samples are not matched in this regard, comparison between our studies is limited.

Although the specific mechanism underlying the enhanced effect of cardiac glycosides on the distal left ventricular conduction system remains unknown, several possibilities may be considered in view of our findings. Since differences in the mechanism of action of cardiac glycosides must ultimately involve the cell membrane and its properties, the mechanism may involve differences in the affinity of the glycoside to the Na⁺, K⁺ ATPase. If the number of glycoside receptors in right and left fibers were comparable, more rapid ouabain binding by left fibers would result in the earlier onset of toxicity in left fibers. Alternatively, qualitative differences in ventricular sarcolemmal binding sites for ouabain (Wellsmith and Lindenmayer, 1980) or differences in the number of binding sites could also account for the differential onset of membrane toxicity.

One factor that may influence the relative degree of glycoside binding to the membrane ATPase is the known competitive interaction of potassium ions and cardiac glycosides (Anderson et al., 1976; Choi and Akera, 1977). Although we have demonstrated that changes in the bulk potassium concentration do not alter the disparate effects of ouabain on right and left Purkinje strands, the concentration of potassium ions in the cleft spaces surrounding Purkinje strands may, however, be dissimilar to the bulk phase as reported by Cohen et al. (1976). A proportionally lower membrane-associated cleft potassium concentration in left Purkinje strands would thus afford less competition to binding by the glycoside and allow expression of earlier toxicity. Differences in the membrane-associated potassium concentration could, in turn, be the result of varying the rate of ion pumping. Other studies support the hypothesis that differences in active transport may influence the corresponding effect of ouabain (Polimeni and Vassalle, 1971). By measuring both the potassium flux and membrane potential of Purkinje and muscle fibers, they concluded...
that Purkinje fibers are more sensitive to ouabain than are muscle fibers, primarily because of the proportionally greater active ion transport existing in Purkinje fibers during stimulation. Presumably, this difference in active ion transport could be related to variation in the potassium transport or to the relative degree of inhibition by ouabain in each tissue.

To what extent differences in the control parameters relate to the earlier onset of membrane toxicity in left Purkinje strands remains speculative. It is known that the degree of toxicity is related to the number of action potentials per unit time (Vassalle et al., 1962). Therefore, if Purkinje strands were allowed to beat spontaneously, the left Purkinje strands, having a more rapid intrinsic rate, would become toxic earlier (see Fig. 4). However, if both fibers were paced at a rate sufficient to overdrive their respective intrinsic rates, then equal numbers of action potentials would occur in both left and right Purkinje fibers, as in the in situ condition. Under these conditions, as in our experiments, phase 4 depolarization could be more carefully assessed and correlated with the onset of toxicity. The slope of phase 4 depolarization was almost always greater in left Purkinje fibers, as was the intrinsic rate (23 of 31 experiments; 74%). In those fibers of left ventricular origin that demonstrated a greater degree of phase 4 depolarization at control, 21 of 23 showed earlier signs of ouabain toxicity. It is not unlikely that digitalis exaggerated these changes in phase 4 depolarization. Therefore, toxic changes would appear first in left Purkinje fibers. Consistent with this hypothesis is the finding of earlier toxicity in two right Purkinje strands having a greater degree of phase 4 depolarization at control than their respective left Purkinje fibers.

Histological variations between right and left Purkinje strands must also be considered as a source for the disparate responses to cardiac glycosides. On a comparative basis, however, histological studies to date have been confined mainly to the anatomic level of the proximal bundle branches, where similar qualitative and quantitative structural differences were found in both human and canine hearts (James et al., 1974). In these studies, Purkinje cells in the left bundle branch had a greater diameter and length and also a greater cell population. This quantitative difference in the number of Purkinje cells observed in the left Purkinje strand has been suggested as the mechanism for the higher left ventricular automaticity observed in the presence of complete AV block (Truex, 1974). Presumably, a larger cell population of latent pacemaking cells in the left strand would also greatly enhance the possibility of spontaneous escape beats in that strand. To what extent these histological differences exist at more distal anatomic sites, as is the case with the tissue in our study, is not known. Our results also suggest that differences in the extent of the collagen sheath between right and left fibers do not account for the observed differences in the ouabain effect. Although in our experience left Purkinje fibers are often more difficult to impale, possibly because of a thicker fibrous sheath, these apparent differences are not supported by our histological studies. Furthermore, a thicker collagen sheath in the left Purkinje system is contrary to the earlier ouabain effects manifested in these fibers, since the collagen sheath should present an added barrier to ouabain binding, which would theoretically delay its action.

These studies demonstrate functional differences between false tendons from the peripheral Purkinje system, whether under physiological conditions or after cardiac glycoside exposure. The increased sensitivity of the left Purkinje system to cardiac glycosides is consistent with our clinical observations in patients as well as acute toxicity induced in animals. Since these studies utilized healthy tissue which was subsequently exposed to cardiac glycosides, the observed differences may ultimately be exaggerated further because of the enhanced sensitivity of cardiac glycosides in cardiac disease states.

We recognize the limitation of these studies, since they were based on acute toxicity. We cannot exclude the possibility that such differences may not exist in chronic toxicity. However, our observation that digitalis toxicity in humans is associated with predominantly left ventricular extrasystoles is supportive of our experimental findings and underscores the validity of the proposed mechanism of action.

Acknowledgments

We wish to thank Andrea Saxon for her diligent assistance in completing the histological studies.

References

Choi YR, Akera T (1977) Kinetic studies on the interaction between ouabain and (Na+K+) ATPase. Biochim Biophys Acta 481: 648-659
Hoffman BF, Singer DH (1964) Effects of digitalis on the electrical activity of cardiac fibers. Prog Cardiovasc Dis 7: 225-250


Moe GK, Mendez R (1951) The action of several glycosides on conduction velocity and ventricular excitability in the dog heart. Circulation 4: 729-734


Thornell LE (1975) Morphological characteristics of Purkinje fiber bundles separated from their connective tissue sheath. J Mol Cell Cardiol 7: 191-194


Preferential sensitivity of the left canine purkinje system to cardiac glycosides.
J Reiser and G J Anderson

doi: 10.1161/01.RES.49.4.1043

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/49/4/1043