Inhibition of Binding of Tritiated Digoxin to Myocardium by Sodium Depletion in Dogs

By Carlos E. Harrison, Jr., M.D., Ph.D., and Khalil G. Wakim, M.D., Ph.D.

ABSTRACT

Ten intact dogs were subjected to hemodialysis against a solution of low-sodium content to determine the influence of sodium depletion on myocardial binding of digoxin-3H. Serial determinations of serum sodium, potassium, chloride, bicarbonate, pH, and osmolality, of cardiac output, and of cardiac rhythm were obtained in these and in 10 control dogs dialyzed against the standard solution. A dose of digoxin-3H (0.05 mg/kg) was given intravenously after start of the dialysis and the animals were killed 1 hour afterward for measurement of myocardial radioactivity, potassium, and sodium. Significant depletion of serum sodium and chloride and myocardial sodium (P<0.001) occurred in all animals dialyzed against the low-sodium solution. No significant changes occurred in other serum electrolytes or pH; osmolality was maintained constant by a slow intravenous drip of 25% urea or mannitol. Myocardial radioactivity was reduced in sodium-depleted animals by 50% (P<0.001). The Na-K ratio was 0.40 in the myocardium of control animals and 0.26 in sodium-depleted animals. It was concluded that depletion of body sodium inhibits binding of digitalis glycosides to the myocardium during the first hour after injection of the dose.

ADDITIONAL KEY WORDS digitalis glycosides cardiac glycoside content dialysis cation deficiency

Depression of the early myocardial binding of digoxin-3H has recently been demonstrated in rats depleted of potassium (1). In the presence of ATP and other nucleotides, sodium stimulates digoxin binding in vitro to ATPase isolated from beef cardiac vesicles (2, 3); in the presence of magnesium, manganese, and inorganic phosphate, sodium inhibits binding. The purpose of this investigation was to determine whether sodium depletion influenced myocardial binding of cardiac glycosides in intact dogs.

Materials and Methods

Mongrel dogs weighing 12 to 16 kg were used in this study. Topical, local, or regional anesthesia of the areas needed was achieved with lidocaine and procaine hydrochloride whenever intubation, catheterization, or cannulation was performed. A fine polyethylene catheter was introduced into the external saphenous vein and anchored for intravenous drip of succinylcholine (Anectine); then the trachea was intubated with a soft catheter in preparation for artificial respiration with 100% oxygen. Areas on the neck, inguinal region, and extremities were shaved for electrocardiographic electrodes and cannulation (for introduction of catheters for recording blood pressure, for arterial and venous sampling, and for connections to the artificial kidney for hemodialysis). Blood pressure was recorded through strain-gauge pressure transducers on moving photosensitive paper. Control values for all variables were established before hemodialysis was started. Recordings and samples were taken at 15-minute intervals during dialysis and at the end of the 1-hour hemodialysis period. The direct-writing Sanborn electrocardiograph was used for recording standard leads I, II, and III, and the esophageal lead. Ten dogs were dialyzed against a low-sodium solution1 and 10 control animals with lidocaine and procaine hydrochloride whenever intubation, catheterization, or cannulation was performed. A fine polyethylene catheter was introduced into the external saphenous vein and anchored for intravenous drip of succinylcholine (Anectine); then the trachea was intubated with a soft catheter in preparation for artificial respiration with 100% oxygen. Areas on the neck, inguinal region, and extremities were shaved for electrocardiographic electrodes and cannulation (for introduction of catheters for recording blood pressure, for arterial and venous sampling, and for connections to the artificial kidney for hemodialysis). Blood pressure was recorded through strain-gauge pressure transducers on moving photosensitive paper. Control values for all variables were established before hemodialysis was started. Recordings and samples were taken at 15-minute intervals during dialysis and at the end of the 1-hour hemodialysis period. The direct-writing Sanborn electrocardiograph was used for recording standard leads I, II, and III, and the esophageal lead. Ten dogs were dialyzed against a low-sodium solution1 and 10 control animals

1Low-sodium solution: dextrose, 220 mm; calcium, 60 mg/liter; magnesium, 18 mg/liter; sodium, 30 mEq/liter; potassium, 2.0 mEq/liter; chloride, 5 mEq/liter; acetate, 33 mEq/liter; pH 7.1.
matched for weight were dialyzed against a standard dialysate.²

Five minutes after start of dialysis, ³H-labeled digoxin³ (0.05 mg/kg of body weight) was injected intravenously. At 1 hour after injection of digoxin-³H, the dogs were killed after an anesthetic dose of pentobarbital, and the hearts were excised for measurement of total radioactivity, sodium, and potassium.

The blood samples were analyzed for sodium, potassium, calcium, magnesium, chloride, bicarbonate, urea, glucose, pH, and osmolality by standard methods. Osmolality was maintained near the control value of about 300 mOsm/liter by a constant infusion of 25% mannitol or 25% urea.

Analysis of Tissue for Sodium and Potassium.—

The hearts were rinsed once in triple-distilled water after they were excised; they were weighed immediately and again after drying for 5 hours at 100°C. The tissues were ashed at 1,090°C overnight in platinum dishes; the ash was dissolved in 4N hydrochloric acid and diluted for determining the potassium and sodium in a flame photometer (Coleman) with appropriate standards.

Liquid Scintillation Counting.—These techniques have been previously described (1, 4). Samples (0.5 ml) of whole blood were diluted 1:10 with absolute alcohol, and tissues were homogenized in 10 ml of 50% ethanol, both in the presence of 2 mg of nonradioactive digoxin (carrier). Quadruplicate specimens of myocardium were obtained from the free walls of right and left ventricles. The proteins were precipitated with 10 volumes of absolute alcohol. After centrifugation at 3,000 rpm for 20 minutes, the supernatant fluid, which contained all the radioactivity, was transferred to a flask and brought to dryness in a vacuum. The inside of the flask was rinsed with a measured quantity of a solution containing (in parts per 1,000) xylene (410), 1,4-dioxane (410), 2,5-diphenyloxazole (4.1), 2-(1-naphthyl)-5-phenyloxazole (0.04), naphthalamine (65), and 95% ethanol (110). Samples (15 ml) were transferred to 25-ml polyethylene vials and the radioactivity was counted in a Packard Tri-Carb liquid scintillation counter utilizing external standards to correct for quenching.

Chromatography.—Thin layer chromatography was performed to verify the purity of the digoxin-³H used in this study and to determine whether the tritium was attached to digoxin in the myocardial tissue. Carrier (marker) digoxin was added to the digoxin-³H and the mixture was spotted on Eastman Chromagram sheets (type K 301R) which had been previously saturated with formamide. The chromatograms were developed (ascending technique) for 1 to 3 hours at room temperature with methyl butyl ketone-isopropyl ether-butanol (8:2:1) saturated with formamide. The carrier digoxin was identified by the Zimmerman reaction. This system separates more rapidly moving digoxin from its primary metabolite, digoxigenin-di-digitoxoside (4). The chromatograms were cut into 2-cm segments, the thin layer was scraped off, and the radioactivity was measured in the liquid scintillation system. Only single peaks of radioactivity coinciding with nonradioactive digoxin markers were found.

Cardiac Output.—This was determined by the Stewart-Hamilton dye dilution technique with rapid injection of indocyanine green into the right atrium. Femoral arterial blood was sampled through the densitometer. The dye dilution curves were recorded on moving photosensitive paper.

Dialysis.—The two-layer Kiil artificial kidney was used throughout this study. Complete mixing of the dialysate was always achieved before the dialysis was started. Blood was transferred from the femoral artery to the Kiil artificial kidney and back to the femoral vein by cardiac action alone, without the use of a subsidiary pump. This eliminated the risk of excess hemolysis. The Kiil artificial kidney is a low-resistance countercurrent-flow dialyzer in which the entering blood is divided into two parallel layers. We found that this arrangement eliminates uneven distribution of blood flow and is more efficient for transfer of solutes (5). The whole artificial kidney apparatus, including tank and tubes, was sterilized by formaldehyde whenever the equipment was not in use.

Results

Dialysis for 1 hour against the low-sodium solution decreased the serum sodium (Table 1) to half its predialysis value. There was a comparable loss of chloride from the vascular space. In both control and sodium-depleted animals, a slight acidosis occurred. There were no significant changes in serum potassium, calcium, magnesium, bicarbonate, or osmolality during the 1-hour dialysis. Blood radioactivity per 100 ml at 1 hour was 0.17% of dose (se = 0.06, n = 6) in con-

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²Standard solution: dextrose, 6 nm; calcium, 60 mg/liter; magnesium, 18 mg/liter; sodium, 132 mEq/liter; potassium, 2.0 mEq/liter; chloride, 105 mEq/liter; acetate, 33 mEq/liter; pH 7.1.

³Digoxin-³H was kindly supplied by Burroughs Wellcome & Co.; specific activity was 120 μc/mg.
TABLE 1

Effect of 1-Hour Hemodialysis on Blood Chemistry Values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard solution*</th>
<th>Low-sodium solution†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/L)</td>
<td>Before dialysis</td>
<td>After dialysis</td>
</tr>
<tr>
<td></td>
<td>143 ± 2</td>
<td>140 ± 4</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>3.3 ± .3</td>
<td>3.5 ± .3</td>
</tr>
<tr>
<td>Cl (mEq/L)</td>
<td>113 ± 5</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>Ca (mg/100 ml)</td>
<td>10.6 ± .8</td>
<td>10.1 ± .5</td>
</tr>
<tr>
<td>Mg (mg/100 ml)</td>
<td>1.45 ± .62</td>
<td>1.50 ± .34</td>
</tr>
<tr>
<td>pH†</td>
<td>7.38 ± .07</td>
<td>7.40 ± .03</td>
</tr>
<tr>
<td>Pco₂ (mm Hg)⁺</td>
<td>33.7 ± 5.6</td>
<td>34.3 ± 8.1</td>
</tr>
<tr>
<td>HCO₃ (mEq/liter)‡</td>
<td>19.6 ± 3.4</td>
<td>20.8 ± 4.9</td>
</tr>
<tr>
<td>Osmolality (mOsm/liter)</td>
<td>289 ± 8</td>
<td>296 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± sd.
*Ten dogs, mean weight 13.2 ± 1.4 (sd) kg.
†Ten dogs, mean weight 13.3 ± 1.2 (sd) kg.
‡By Astrup technique.

TABLE 2

Effect of Sodium Depletion on Myocardium

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (10 dogs)</th>
<th>Sodium-depleted (10 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/kg)</td>
<td>34.0 ± 1.3</td>
<td>20.1 ± 1.1*</td>
</tr>
<tr>
<td>K (mEq/kg)</td>
<td>83.5 ± 1.8</td>
<td>77.6 ± 3.5</td>
</tr>
<tr>
<td>Digoxin-²H (% dose/100 g)</td>
<td>3.37 ± .36</td>
<td>1.78 ± .06*</td>
</tr>
</tbody>
</table>

Values are means ± se.
*For difference from control, P < 0.001.

FIGURE 1

Average cardiac output (± se) at 15-minute intervals for 1 hour in 13 dogs given digoxin-²H (0.05 mg/kg, i.v.). The groups were: three dogs, digoxin but no dialysis (solid triangles); four dogs, digoxin + dialysis with low-sodium solution (solid circles); six dogs, digoxin + dialysis with standard solution (open circles).
Table 3

Serial Determinations of Mean Arterial Pressure

<table>
<thead>
<tr>
<th>Time (min)*</th>
<th>Control Arterial pressure (mm Hg)</th>
<th>Sodium-depleted Arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>134 ± 19</td>
<td>122 ± 13</td>
</tr>
<tr>
<td>15</td>
<td>103 ± 29</td>
<td>124 ± 22</td>
</tr>
<tr>
<td>30</td>
<td>93 ± 25</td>
<td>117 ± 13</td>
</tr>
<tr>
<td>45</td>
<td>88 ± 25</td>
<td>111 ± 25</td>
</tr>
<tr>
<td>60</td>
<td>79 ± 29</td>
<td>101 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SD. There were 10 control and 10 sodium-depleted dogs.

*Measured from start of dialysis.

trols and 0.22% of dose (se = 0.01, n = 9) in sodium-depleted animals.

The content of digoxin-3H in the myocardium (Table 2) of the sodium-depleted animals at 1 hour after the injection was approximately 50% of that found in animals with normal serum sodium ($P < 0.001$). In four animals in which the tritium content was measured in dry tissue, the same ratio was found. The mean (±SD) heart weight of the sodium-depleted animals was $92 ± 24$ g, and in the control animals it was $83 ± 15$ g. The radioactivity in the heart probably represents tritium attached to digoxin because the tritium could not be separated from non-radioactive digoxin in the chromatographic system. On analysis of the tissue, significant decrease of the sodium content was found.

Cardiac outputs were determined by the Stewart-Hamilton technique in 13 dogs given digoxin (Fig. 1). No significant differences occurred in the cardiac outputs or cardiac indices of these animals from 10 minutes after the injection of the glycoside until the termination of the experiment at 60 minutes. Serial determinations of mean arterial pressure (Table 3) at 15-minute intervals revealed no statistically significant differences of pressures in the two groups. From these data we concluded that changes in the cardiac output and arterial pressure induced by anesthesia or dialysis were not responsible for depression of myocardial glycoside content.

In the first hour of dialysis no abnormalities of rhythm were seen in dogs receiving digoxin, except for one in which ventricular tachycardia developed at a serum sodium level of 53 mEq/liter. Sinus dysrhythmia was evident in control tracings and in those recorded during the hour of dialysis. Electrocardiographic abnormalities were observed in animals depleted of sodium whenever dialysis continued longer than 60 minutes. These will be described in detail in another communication.

Discussion

Repke (6), Skou (7), and others (8, 9) have found inhibition of Na$^+$- and K$^+$-stimulated adenosine triphosphatase activity by cardiac glycosides at high concentrations ($10^{-7}$ to $10^{-5}$M) but stimulation at low concentration ($10^{-9}$ to $10^{-12}$M). Whether the inotropic action of glycosides in the living animal is related to the stimulation or inhibition of this adenosine triphosphatase is unknown, but the active transport of sodium and potassium across cell membranes is dependent on the enzyme. It has been suggested that potassium competes with glycosides for binding sites on cellular membranes (10). However, it has been shown (1) that early myocardial content (at 1 hour after injection) of digoxin-3H in rats is depressed by potassium depletion. At 24 hours after injection of digoxin-3H, there was retention of radioactivity within the myocardium. These findings seemed to be incompatible with the competition hypothesis and suggest the possibility that myocardial influx and efflux of glycosides are inhibited by potassium deficiency.

Matsui and associates (2, 3, 11) isolated a highly ouabain-sensitive adenosine triphosphatase from calf heart muscle. They concluded that inhibition of the enzyme activity by ouabain was not due to direct or indirect displacement of potassium at the binding site but was dependent on the Na-K ratio. In the present study, the serum sodium level was decreased by 50% and the myocardial sodium level by 40%. No significant changes were detected in other cations, pH, or osmolality in dogs dialyzed against the low-sodium solution. The 1-hour myocardial content of...
digoxin-3H in the sodium-depleted dogs was 53% of control values, which is comparable to the data (56 ± 3%) derived from potassium-depleted rats (1). These data suggest that the myocardial binding of digoxin-3H depends on the myocardial or serum sodium and potassium concentrations and that alteration of either cation can affect cardiac glycoside content. Since chloride was removed from the dogs with sodium, the question arises as to the role of chloride in the inhibition of myocardial glycoside binding. The information presented by Weatherall (12) indicates that cations are more likely to influence the binding of glycosides.

The cardiac outputs and mean arterial pressures of control animals subjected to dialysis against a physiologic (standard solution) bath did not differ from those of dogs dialyzed against a low-sodium solution during the first hour. Since osmolality was maintained constant by infusion of mannitol or urea, venous return to the heart probably was not altered significantly in the experiments in which sodium was removed from the animal. Although myocardial calcium and magnesium contents were not measured, there were no significant changes in the serum concentrations of these bivalent cations. Abnormalities in rhythm were recorded after 1 hour of dialysis but were not present within the hour in 19 of the 20 dogs in which myocardial radioactivity was measured. Blood radioactivity was considerably lower than myocardial radioactivity.

The initial delivery of digoxin to the heart depends on coronary blood flow. The quantity ultimately present is a function of the trapping mechanisms in the heart and the mean transit time of the glycoside through the myocardium. The tritiated digoxin was injected at 5 minutes after onset of dialysis, a time when it is likely that there was no significant difference in myocardial blood flow between the two groups. The animals were killed 1 hour after the injection, when the maximal radioactivity was contained within the sarcoplasmic reticulum, according to Dutta and his associates (13). About 90% of a single injected dose (14) of the glycoside disappears from the blood within 15 minutes after administration. The lower myocardial radioactivity in the sodium-depleted animals at 1 hour after injection should not be due to a lower initial delivery of digoxin to the heart. We did not measure coronary blood flow. However, coronary blood flow is in part dependent on cardiac output and systemic arterial blood pressure. Since there were no significant differences in blood pressures and cardiac outputs of the control and sodium-depleted animals, the 50% reduction of the myocardial radioactivity of the sodium-depleted dogs is more likely secondary to alteration in the myocardial trapping mechanism than to decreased myocardial blood flow.

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References


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