Effects of Hypothermia and Pronethalol on Ionic Correlates of Ouabain Arrhythmias in Dogs

By S. B. I. El-Fiky, M.D., Ph.D., and B. G. Katzung, M.D., Ph.D.

ABSTRACT
The ionic basis of ventricular arrhythmias induced by a cardiac glycoside was studied in pentobarbital-anesthetized dogs. The effects of ouabain alone and in combination with hypothermia or pronethalol on myocardial and plasma Na\(^+\) and K\(^+\) were determined. Hypothermia (26°C) and pronethalol (15 mg/kg, iv) had similar effects on the standard lead II of the ECG, i.e., a decrease in heart rate and increases in P-R, QRS, and Q-T intervals. In the absence of ouabain, hypothermia and pronethalol each produced small and inconsistent changes in intracellular Na\(^+\) and K\(^+\) concentrations. Both hypothermia and pronethalol were found to significantly increase the threshold toxic dose of ouabain required to produce a ventricular arrhythmia within the test period of 90 minutes. Both pronethalol and hypothermia effectively prevented ouabain-induced changes in myocardial Na\(^+\) and K\(^+\) when arrhythmias were prevented. When sufficient ouabain was given to produce an arrhythmia, there was always a significant fall in myocardial K\(^+\). In unprotected and in hypothermic animals, these arrhythmias were also associated with a significant increase in left ventricular Na\(^+\). The effect of ouabain on Na\(^+\) was not significant in pronethalol-treated dogs.

ADDITIONAL KEY WORDS antiarhythmic action of hypothermia antiarrhythmic action of pronethalol cardiac Na and K plasma Na and K ouabain toxicity

Many of the effects of cardiac glycosides on the cardiac transmembrane potential and ECG have been convincingly ascribed to the effects of these drugs on Na\(^+\) and especially K\(^+\) movements across the cell membrane (1-3). Most workers agree that the toxic effects of cardiac glycosides on the heart are functions of changes in fluxes of K\(^+\) or perhaps both ions (2, 4-6). On the biochemical level, the toxic potency of glycosides has been correlated with inhibiting effects on a Na\(^+\)-K\(^+\)-activated ATPase located in the membrane of many cells including cardiac muscle (7). Toda and West (8), on the other hand, have suggested that the rate of onset of ouabain toxicity may be linked primarily to entry of Na\(^+\) into cardiac cells.

Certain antiarrhythmic drugs and hypothermia increase resistance to the toxic effects of the glycosides in intact animals. The protective action of hypothermia has been shown by Satoskar and Trivedi (9), Angelakos et al. (10), and by Nahum and Phillips (11). The mechanism by which it protects against digitalis toxicity is not known, but hypothermia has been shown to have effects on the transmembrane action potential (12) opposite to those of digitalis (13). Furthermore, hypothermia is known to in-
fluence cation concentrations in the heart and other tissues (14, 15).

Pronethalol, a beta-receptor blocking agent first described by Black and Stephenson (16), was reported to protect against digitalis arrhythmias by Sekiya and Vaughan Williams (17). This finding has been confirmed by a number of authors (18-22) for pronethalol and certain other beta-receptor blocking agents. Quinidine has also been shown to significantly increase acetylstrophanthidin tolerance (23). The mechanism by which pronethalol and other antiarrhythmic drugs reverse or protect against digitalis toxicity is the subject of conflicting views. Erlig and Mendez (19) and Levitt and Roberts (24) suggested that this effect might be due to its beta-receptor blocking effect; Somani and Lum (18) and Lucchesi (21), on the other hand, ascribed it to a quinidine-like or local anesthetic effect. Sekiya and Vaughan Williams (25) found that pronethalol affects the transmembrane action potential of the cardiac cell membrane in a manner similar to that of quinidine. These effects are in some ways similar to those of hypothermia and opposite to those produced by cardiac glycosides. Lucchesi et al. (22) have shown that the protection against acetylstrophanthidin toxicity afforded by propranolol, another antiarrhythmic beta-receptor blocking agent, is associated with diminished K+ loss from isolated myocardium. Rahn and Reuter (26) have shown that quinidine and pronethalol have similar (stabilizing) effects on 42K+ fluxes in electrically driven isolated guinea pig atria. However, there has been no systematic comparison of the effects of these drugs and hypothermia on myocardial ion content in control and cardiac glycoside-treated animals and as indicated, there is no general agreement regarding the relative importance of Na+ and K+ movements in glycoside toxicity.

This study was carried out to compare the effects of hypothermia and pronethalol on myocardial and plasma Na+ and K+ in the presence and absence of ouabain arrhythmias and to evaluate the role of increased myocardial Na+ in this type of arrhythmia.

**Methods**

Male adult mongrel dogs selected for a weight range from 8 to 12 kg were anesthetized with pentobarbital sodium, 30 mg/kg, iv. A femoral artery-femoral vein shunt was made through a siliconized stainless steel coil immersed in a water bath. Heparin sulfate, 200 units/kg, was then given intravenously. The shunt was used for controlling body temperature and continuous monitoring of blood pH (Beckman combination glass pH electrode). Hypothermic respiratory depression and blood pH variation were prevented with artificial respiration adjusted to keep blood pH constant at 7.4. A Sigamamotor pump was used to maintain circulation through the shunt during hypothermia. Body core temperature was monitored by means of a Telethermometer with a probe introduced into the esophagus of the animal as close to the heart as possible. The electrocardiogram was recorded on a Grass Polygraph (Model 7) using standard limb lead II. Blood pressure was measured at the femoral artery with a Statham transducer and recorded on the polygraph.

The dogs were divided into eight groups of five dogs each for treatment (Table 1). Group A was the normothermic control group; no ouabain was given. Dogs in groups B, E, and H were given toxic doses of ouabain, 0.25 mg, iv, every 5 to 10 minutes until an arrhythmia occurred as indicated by the appearance of frequent ventricular extrasystolic beats or ventricular tachycardia in the ECG recording. The arrhythmia was maintained (by additional doses if necessary) at the same severity, i.e., 60% to 100% extrasystoles for the duration of the experiment. Dogs in groups C, D, and E were cooled and maintained at 26°C for the duration of the experiment. Dogs in groups F, G, and H were given pronethalol1 in a dose of 15 mg/kg, iv, followed by supplements of 2 mg/kg every 10 minutes for the duration of the experiment. Dogs in groups D and G were treated with subtoxic doses of ouabain and were given incremental doses of the glycoside to a fixed total which was larger than the minimum threshold toxic dose in the unprotected group (B) by approximately 50% of the difference between the group B threshold toxic dose and the threshold toxic dose for the pretreated toxic groups (E and H).

Each experiment lasted 2 hours; 1 hour for induction of hypothermia or protective drug treatment and 1½ hours for ouabain treatment.

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1Supplied by Ayerst Laboratories.
Observations of esophageal temperature, pH of the blood, ECG, and blood pressure were made continuously for the 2-hour period, after which a sample of arterial blood was drawn for Na+ and K+ analysis. Next, the chest of the dog was opened rapidly, and the heart was removed and transferred for dissection to a dish containing Tyrode’s solution (NaCl, 8.25 g; KCl, 0.171 g; MgCl₂H₂O, 0.224 g; CaCl₂, 0.202 g; NaHCO₃, 0.841 g; KH₂PO₄, 0.057 g; and glucose, 0.990 g dissolved in 1 liter of distilled water). Three full-thickness fat-free pieces (0.2 to 0.5 g) were taken rapidly from the free wall of each of the four chambers of the heart, blotted thoroughly on filter paper, weighed on aluminum foil to the nearest 0.1 mg, and then dried at 130°C to constant weight.

The dried tissue samples were digested in a hot mixture of 5 ml of concentrated HNO₃ and 5 ml of concentrated HCl. The digest was evaporated to a small volume and then redissolved in 0.1N HCl and filtered into a 50-ml flask using ashless acid-resistant filter paper (Whatman no. 40). Samples were analyzed for sodium and potassium using a Perkin Elmer flame photometer model 52-C. Three blanks were carried through the same procedure with each set of samples.

To measure the extracellular space, pieces of muscle, not more than 100 mg in weight and 0.5 mm in thickness, were taken from the free wall of the four chambers of the heart. They were then incubated for varying lengths of time at 37°C or 26°C in oxygenated Tyrode’s solution containing ¹⁴C-inulin (45,000 counts/min/ml). After incubation, the tissue slices were blotted and radioactivity was extracted and measured by a liquid scintillation spectrometer (Packard Tri-Carb). Extracellular space was calculated, and the results were plotted against incubation time. Regression lines were extrapolated to zero time as described by Tuttle et al. (4) to eliminate the influence of adsorption of inulin to the surface of the cells or diffusion into the intracellular space. The inulin space was 21.0 ml/100 g for normothermic tissue and 20.6 ml/100 g at 26°C (not significantly different).

Statistical significance of group differences was examined using analysis of variance and the Scheffé technique for multiple group comparisons (27). Comparison of the number of ouabain doses required to produce toxicity was carried out with the Mann-Whitney rank order test (28).

**Results**

At 37°C, a mean of 3.3 doses of ouabain was required to produce a sustained ventricular arrhythmia. This was equivalent to 0.08 mg/kg (Table 1, group B). In sharp contrast, a mean of 8.1 doses (0.20 mg/kg) was required to produce an ectopic rhythm at 26°C (Table 1, group E). Hypothermia was also capable of reversing arrhythmias which had been produced by ouabain (unpublished data). A mean of 5.3 doses of ouabain (0.13 mg/kg) was required to produce an arrhyth-
mia in the presence of pronethalol (group H). Pronethalol was also capable of reversing pre-existing arrhythmias.

The electrocardiographic effects of a large dose of ouabain given to normothermic animals (Table 2, group B) were those usually described for cardiac glycosides, namely an increase in P-R interval, a decrease in QRS duration, and a decrease in heart rate. In addition, the Q-T interval was prolonged. However, none of these changes were statistically significant. Hypothermia had very marked effects (Table 2, group C). These changes were in the same direction as the changes produced by ouabain in normothermic dogs except for the effect on QRS duration. However, when a large nontoxic dose of ouabain was given after induction of hypothermia (group D), the effects of the drug on QRS, Q-T interval, and rate were reversed, i.e., the direction of the changes was in the opposite direction from those produced by ouabain at 37°C. Pronethalol produced prolongation of the P-R and Q-T intervals (Table 2, group F) and slowing of the heart rate. When ouabain was given after pronethalol (group G), there was

<table>
<thead>
<tr>
<th>Group</th>
<th>P-R (msec)</th>
<th>QRS (msec)</th>
<th>Q-T (msec)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>84 ± 1.4</td>
<td>37 ± 2.8</td>
<td>164 ± 42.7</td>
<td>168 ± 5.1</td>
</tr>
<tr>
<td>B</td>
<td>92 ± 8.1*</td>
<td>31 ± 4.2*</td>
<td>219 ± 4.9*</td>
<td>154 ± 9.4*</td>
</tr>
<tr>
<td>C</td>
<td>208 ± 42.2</td>
<td>50 ± 4.5</td>
<td>988 ± 394</td>
<td>34 ± 9.0</td>
</tr>
<tr>
<td>D</td>
<td>236 ± 16.0</td>
<td>74 ± 9.8</td>
<td>784 ± 138</td>
<td>36 ± 7.0</td>
</tr>
<tr>
<td>E</td>
<td>110 ± 8.9</td>
<td>38 ± 6.6</td>
<td>252 ± 13.5</td>
<td>123 ± 6.8</td>
</tr>
<tr>
<td>F</td>
<td>108 ± 7.3</td>
<td>51 ± 7.5</td>
<td>200 ± 21.9</td>
<td>144 ± 17.9</td>
</tr>
<tr>
<td>G</td>
<td>A±</td>
<td>A±</td>
<td>A±</td>
<td>A±</td>
</tr>
</tbody>
</table>

*Measurement made just before onset of the arrhythmia.

Values are means ± 1 SEM. Letters under values indicate statistically different populations and symbols indicate the probability (P) of the null hypothesis: *P < 0.001, †P < 0.005, §P < 0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>111.8 ± 4.2</td>
<td>119.0 ± 6.8</td>
<td>147.9 ± 3.3</td>
<td>148.8 ± 2.8</td>
</tr>
<tr>
<td>B</td>
<td>98.5 ± 4.4</td>
<td>114.4 ± 2.8</td>
<td>136.5 ± 3.2</td>
<td>130.3 ± 3.1</td>
</tr>
<tr>
<td>C</td>
<td>109.5 ± 5.7</td>
<td>125.8 ± 4.8</td>
<td>148.7 ± 5.1</td>
<td>145.9 ± 2.4</td>
</tr>
<tr>
<td>D</td>
<td>90.9 ± 5.1</td>
<td>114.0 ± 1.7</td>
<td>146.7 ± 4.7</td>
<td>142.7 ± 3.0</td>
</tr>
<tr>
<td>E</td>
<td>68.0 ± 4.8</td>
<td>109.0 ± 8.7</td>
<td>130.2 ± 2.7</td>
<td>128.7 ± 3.0</td>
</tr>
<tr>
<td>F</td>
<td>103.3 ± 4.9</td>
<td>114.1 ± 7.3</td>
<td>A±, C±, D±</td>
<td>A†, C†, D†</td>
</tr>
<tr>
<td>G</td>
<td>109.3 ± 6.5</td>
<td>119.4 ± 6.8</td>
<td>147.5 ± 5.3</td>
<td>148.3 ± 7.3</td>
</tr>
<tr>
<td>H</td>
<td>95.6 ± 4.6</td>
<td>113.5 ± 2.7</td>
<td>138.9 ± 1.9</td>
<td>128.0 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM. Letters under values indicate statistically different populations and symbols indicate the probability (P) of the null hypothesis: *P < 0.05, †P < 0.005, §P < 0.001. §P < 0.01.
some reversal of the pronethalol effect on P-R and Q-T intervals and rate, but duration of QRS was prolonged more.

The arrhythmia produced by ouabain during hypothermia was usually in the form of ventricular extrasystoles progressing to complete A-V block and asystole; ventricular tachycardia was rarely seen. The average heart rates during both normothermic and hypothermic arrhythmias were lower than the control normothermic rates. The most common arrhythmia produced by excess ouabain in dogs pretreated with pronethalol was ventricular tachycardia. The average rate during these arrhythmias was also less than the control rate.

Potassium and sodium analyses are summarized in Tables 3, 4, and 5. Qualitative changes were similar for all four chambers of the heart, even though concentrations of ions in the atria were significantly different from those in the ventricles, i.e., atrial potassium values were lower and sodium values higher than in the ventricles (group A). These differences parallel those reported by Mazel and Holland (29) and Tuttle et al. (4). The former authors concluded that the intrinsic rhythmicity of the various chambers may be directly correlated with sodium and inversely related to potassium concentration.

Ouabain in a toxic dose (group B) lowered myocardial K⁺ concentration significantly as previously reported by many authors (3). There was a reciprocal increase in plasma K⁺ which was statistically significant (Table 5). Hypothermia (group C), on the other hand, produced small and variable changes in myocardial potassium and a decrease in sodium which was not statistically significant. There was some decrease in plasma K⁺ suggesting uptake of this ion by some tissues at the lower temperature or increased excretion. When ouabain was given to a hypothermic group (D) in a dose of 0.14 mg/kg (almost double

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### TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64.0 ± 7.3</td>
<td>88.3 ± 6.2</td>
<td>18.9 ± 3.5</td>
<td>16.3 ± 4.0</td>
</tr>
<tr>
<td>B</td>
<td>63.0 ± 5.6</td>
<td>63.0 ± 5.6</td>
<td>95.0 ± 9.8</td>
<td>62.0 ± 6.9</td>
</tr>
<tr>
<td>C</td>
<td>65.1 ± 3.4</td>
<td>75.3 ± 9.9</td>
<td>75.8 ± 5.4</td>
<td>75.4 ± 4.7</td>
</tr>
<tr>
<td>D</td>
<td>60.1 ± 3.4</td>
<td>74.0 ± 1.4</td>
<td>19.0 ± 3.6</td>
<td>26.2 ± 5.1</td>
</tr>
<tr>
<td>E</td>
<td>61.0 ± 6.9</td>
<td>48.7 ± 4.6</td>
<td>16.8 ± 3.9</td>
<td>18.9 ± 2.9</td>
</tr>
<tr>
<td>F</td>
<td>75.4 ± 5.4</td>
<td>50.7 ± 4.7</td>
<td>26.2 ± 5.1</td>
<td>22.9 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM. Letters under values indicate statistically different populations and symbols indicate the probability (P) of the null hypothesis: *P < 0.05, †P < 0.005.

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### TABLE 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺ (mEq/liter)</th>
<th>K⁺ (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>160 ± 1.0</td>
<td>5.3 ± 1.3</td>
</tr>
<tr>
<td>B</td>
<td>160 ± 1.6</td>
<td>6.5 ± 0.24</td>
</tr>
<tr>
<td>C</td>
<td>158 ± 2.0</td>
<td>4.6 ± 0.12</td>
</tr>
<tr>
<td>D</td>
<td>156 ± 2.0</td>
<td>6.7 ± 0.72</td>
</tr>
<tr>
<td>E</td>
<td>155 ± 2.5</td>
<td>7.9 ± 0.21</td>
</tr>
<tr>
<td>F</td>
<td>154 ± 3.7</td>
<td>6.2 ± 0.72</td>
</tr>
<tr>
<td>G</td>
<td>157 ± 2.8</td>
<td>7.2 ± 0.74</td>
</tr>
<tr>
<td>H</td>
<td>154 ± 1.7</td>
<td>10.7 ± 1.43</td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM. Letters under values indicate statistically different populations and symbols indicate the probability (P) of the null hypothesis: *P < 0.05, †P < 0.005, ‡P < 0.001.

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the normothermic toxic dose), there was no arrhythmia and there were no significant changes in the intracellular K⁺ and Na⁺ concentrations as compared to the control hypothermic and normothermic groups. There was, however, a slight downward trend for both ions and a significant increase in plasma K⁺. When ouabain was given in a toxic dose to hypothermic animals (group E), there were significant decreases of ventricular and right atrial K⁺ and increases of Na⁺ when compared to the control hypothermic and control normothermic levels. Plasma K⁺ showed a further increase with the toxic dose of ouabain (Table 5). The plasma increase at the higher ouabain dose may be partially explained on the basis of myocardial K⁺ loss, but the significant change at the lower dose (group D) suggests that other tissues may be more sensitive to the K⁺ displacing effect of ouabain than the heart at this temperature.

Pronethalol alone (group F) had small effects on myocardial Na⁺ and K⁺ (Tables 3 and 4) which tended in the same direction as those of ouabain. Pronethalol plus a subtoxic dose of ouabain (group G) resulted in normal myocardial Na⁺ and K⁺. However, there was an increase in plasma K⁺ (Table 5) suggesting loss of K⁺ by some tissue other than the heart. When a toxic dose of ouabain followed pronethalol (group H), there was a significant fall in myocardial K⁺. There was no significant change in myocardial Na⁺ compared to control groups A and F.

Discussion

Our results confirm the findings of others, cited in the introduction, that hypothermia and pronethalol are capable of increasing the threshold toxic dose level for cardiac glycosides in dogs. In neither case was protection absolute since it was always possible to produce severe ventricular arrhythmias or arrest by further increases in the ouabain dose. A possible complicating factor is the slower heart rate in both protected groups. It is well known that time to onset of glycoside toxicity is a function of the frequency or total number of beats at physiological heart rates (30). Thus, the very slow rate in hypothermic animals could have significantly delayed the full manifestations of the administered ouabain dose. A similar, smaller effect might be postulated in the pronethalol-treated animals. Had this been a major factor, progressive increase in severity of the arrhythmia, once it was established, should have been observed. Furthermore, no supplemental doses of ouabain should have been required once the arrhythmia was established. Neither of these observations were made. In fact, more supplements of ouabain were required to maintain arrhythmias as well as to induce them in hypothermic animals.

It has been reported that quinidine has relatively small or variable effects on myocardial Na⁺ and K⁺ content (31, 32). This is also true of hypothermia and pronethalol (Tables 3 and 4). The latter drug, however, did consistently increase plasma K⁺, which was not true of hypothermia in this study. Pronethalol also produced a consistent although not statistically significant increase in left ventricular sodium, an effect not seen with hypothermia. In spite of these differences both hypothermia and pronethalol profoundly reduced the ability of ouabain to change myocardial K⁺ and Na⁺. This stabilizing effect on ion concentrations is consistent with the hypothesis that the observed increase in membrane resistance and decreased automaticity reported for hypothermia (33) and for antiarrhythmic drugs (25) are due to decreased ion fluxes. Of the two protective interventions, pronethalol proved to be the most effective stabilizer of Na⁺. Not even an arrhythmia-producing dose of ouabain produced a significant change in myocardial Na⁺ in dogs pretreated with pronethalol. The latter observation suggests that an increase in intracellular myocardial Na⁺ is not a necessary concomitant of an arrhythmia induced by ouabain, but a decrease in K⁺ is required. However, it must be noted that the changes we observed in ordinary myocardial muscle do not necessarily imply identical changes in the specialized conduct-
IONIC CORRELATES OF OUABAIN TOXICITY

ing tissue of the heart. Since there is evidence that ouabain arrhythmias usually originate in conducting tissue (34), it would be most desirable to determine the degree of correlation of ionic changes in specialized and nonspecialized cardiac cells. Furthermore, it is possible that the ionic mechanism of ouabain toxicity in unprotected myocardium is different from the mechanism in protected animals.

References

2. MÜLLER, P.: Kalium and digitalisintoxicität. Car


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