Studies on the Effects of Hyperkalemia on Serum and Myocardial Digoxin Concentration in Dogs

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SUMMARY

The effects of hyperkalemia on serum and myocardial digoxin (DX) concentration was studied in conjunction with hemodynamic changes in 31 normal dogs. The myocardial DX concentration in the hyperkalemic (HK) group was significantly lower than that in normokalemic (NK) group, despite a significantly higher serum DX concentration in the HK group. In the HK group, the myocardial sodium concentration was significantly lower than in the NK group. Coincident with these biochemical changes, no increase of LV max dP/dt after DX administration was observed in the HK group. These results suggest that there might be competitive antagonism between myocardial uptake of potassium and DX.

Additional Indexing Words:
Potassium Atrial pacing Tissue digoxin concentration LV max dP/dt Myocardial sodium concentration

There have been many reports on the correlation between electrolytes, especially potassium, and the effects of digitalis. With the recent development of immunological assays for digitalis, this problem has been studied from the standpoint of the concentration of digitalis in the body fluid or various tissues. However, the site of action of digitalis, has not been demonstrated unequivocally. In this study, adult mongrel dogs were divided into 2 groups: those with normokalemia (hereafter abbreviated as the NK group) and those with hyperkalemia (hereafter abbreviated as the HK group). In each group, the correlation between the digoxin concentration in the blood and that in the myocardium was observed in association with hemodynamic changes.
MATERIALS AND METHODS

Thirty-one normal adult mongrel dogs, weighing 8.8–15.0 Kg, were used in this study. In the HK group, KCl diluted with physiological saline was infused at a rate of 0.525 mEq/ml/min into the femoral vein over a period of 40 min, according to the method of Goldman et al.5) Thereafter, the infusion of KCl solution was continued at a rate of 0.3 mEq/ml/min until completion of the experiment. In the NK group, physiological saline was infused at a rate of 1 ml/min until completion of the experiment. The dogs were divided into 2 groups, with 16 in the HK group and 15 in the NK group. Both groups were pretreated with 2 mg/Kg of morphine hydrochloride followed by 25–30 mg/Kg of sodium pentobarbital. After insertion of a tube in the trachea, controlled respiration was performed with room air using a respirator and PaO₂ was kept over 80 mmHg and the pH between 7.35 and 7.50 until the end of the experiment. Catheters were inserted into the external carotid artery, the femoral artery and the femoral vein, and the left ventricular end-diastolic pressure, the left ventricular dP/dt, the aortic pressure and mean right atrial pressure were measured. The electrocardiogram (ECG) was recorded at lead II. In the HK group, right atrial pacing was performed at the rate of 150–180 per minute to eliminate the effects of arrhythmias or bradycardia induced by digitalis on the left ventricular dP/dt.

Seventy min after the administration of the KCl solution, 0.04 mg/Kg of digoxin (hereafter abbreviated as DX) was given intravenously for 30 sec, and hemodynamic parameters were measured after 5, 10, 15, 20, 30, 45, 60, 90 and 120 min. In the NK group, 1 ml/min of physiological saline was administered until the end of the experiment and the same dose of DX was administered as in the HK group. For the measurement of hemodynamic changes, each parameter was recorded at a paper speed of 50 mm/sec using a Fukuda electronic polyrecorder. At each period mentioned above, blood samples were collected from the catheter placed in the right atrium and serum electrolytes and DX concentrations were measured. To determine time-dependent changes in the concentrations of DX in organs, 7 of the 15 dogs in the NK group and 7 of the 16 dogs in the HK group were sacrificed 5 min after DX administration and the remaining dogs in each group were sacrificed 120 min after DX administration.

1) Method of the measurement of DX concentration in organs

The concentration of DX in organs was measured by a modified Brock’s method,8)–18) i.e. 200±20 mg of each tissue was taken and homogenized with 4 ml of physiological saline containing 0.4% gelatin. After centrifugation of the homogenate, 5 ml of dichloromethane was added to the supernatant and
the DX was extracted and evaporated to dryness in a hot water bath at 40–50°C. It was then dissolved in phosphate buffer and measured by radioimmunoassay. The variation coefficient of this method was 12% between assays and the recovery was 88–90%. To ascertain the influence of the serum digoxin level on tissue digoxin concentration, 6 mongrel adult dogs weighing 8.0–15.0 Kg were sacrificed 5 min after DX administration. The organs having been removed, an ATOM venous catheter (5F) was wedged in the artery of each organ and about 5 Gm of the tissue distal to the wedged artery was washed once with 10 ml of physiological saline. After washing, about 200 mg of the washed tissue was removed and the blood vessels on the incision face were rapidly ligated. Further washing was performed using 10 ml at each time for a total of 150 ml and the DX concentration was measured in 200 mg of the tissue removed after each washing.

2) Determination of the electrolytes in the myocardium and the serum

The measurement of electrolytes in the myocardium was done on samples of 100±10 mg wet weight obtained from three parts of the heart, i.e., the apex of the left ventricle, the papillary muscle and the base of the heart. After drying for 72 hours at 85°C, 1 ml of concentrated nitric acid was added and digestion was performed for at least 4 hours at 100 °C. Five ml of 1% Li solution and 3.05ml of deionized water were added to 2 ml of the diluent and measurements were made with a Hitachi 205 flame photometer. The measurements of the serum electrolytes were also made with a Hitachi 205 flame photometer. Statistical analysis of the data was performed by Student’s t-test with p<0.05 as a significant level. Results are expressed as the mean ±SEM.

**Results**

There was no significant difference between the serum potassium (K) concentration 70 min before DX administration in the NK (3.6±0.18 mEq/L) and HK (3.4±0.20 mEq/L) groups (Fig. 1). The serum K concentration in the HK group after the administration of KCl solution was 5.6–7.1 mEq/L and the values were significantly higher at all periods than those in the NK group with physiological saline infusion (p<0.001). The K concentrations of left ventricular myocardium 120 min after DX administration were 29.2±0.5 mEq/100 Gm dry weight in the NK group and 35.8±0.5 mEq/100 Gm dry weight in the HK group (right side of Fig. 1). The value in the HK group was significantly higher than that in the NK group (p<0.001). The sodium concentration in the left ventricular myocardium was 26.4±0.8 mEq/100 Gm dry weight in the NK group and 20.6±0.8 mEq/
Fig. 1. Time course of serum potassium and sodium concentration after saline or potassium chloride infusion. Right bars indicate left ventricular potassium and sodium concentration 120 min after digoxin administration in normokalemic (NK) and hyperkalemic (HK) groups. Each value represents the mean ± SEM. ***p<0.001, ±p<0.02.

100 Gm dry weight in the HK group (p<0.02). There were no significant differences in serum sodium (Na) and calcium (Ca) concentrations, between the NK and HK groups at any period before and after DX administration.

Fig. 2 shows the changes in serum DX concentrations after intravenous DX administration. In the NK group, the values were 41.3±6.7 ng/ml after 5 min and 5.2±1.6 ng/ml after 120 min. In the HK group, these values were 86.4±6.6 ng/ml (p<0.001) after 5 min and 8.0±1.0 ng/ml (p<0.005) after 120 min. The values for the HK group were significantly higher than those of the NK group at each period.

Since the serum DX concentration revealed high levels at early periods after its administration, it is important to determine how high levels of serum DX concentration influenced the tissue DX concentration. The results are shown in Fig. 3. The DX concentrations in each tissue before washing are shown as 100% on the ordinate and the washing volumes are shown on the abscissa. The DX concentrations in each tissue rapidly decreased after washing with 10 ml. With 20 ml, they formed a plateau and they began to rapidly decrease again at greater than 30 ml. When the various organs were washed by this method, the blood in the vessels was first washed out and, thereafter, the DX in the tissues appeared to be released. As shown in Fig. 3, the washing out of the blood may have been completed when the concentration in the tissue reached an approximate plateau. The values for the
Fig. 2. Time course of serum digoxin concentration in the 2 groups. Each point represents the mean ± SEM. ***p<0.001, †p<0.005, ‡p<0.05.

Fig. 3. Correlation between tissue digoxin concentration and saline volume used for washing the tissues. Each point represents the mean volume.

left ventricle, kidney, lung and liver were 90.5±2.03%, 82.7±1.84%, 67.5±2.56% and 54.7±3.84%, respectively, of the values before washing. These results indicate that DX concentrations in the organs which have a large amount of blood such as lung and liver are more apt to be influenced by the serum DX level than muscle tissues such as the myocardium.
Fig. 4. Comparison of tissue digoxin concentration in various organs 5 and 120 min after digoxin administration between the 2 groups. Each value represents the mean±SEM. †p<0.02, ‡p<0.01, **p<0.005, ***p<0.001. LV=free wall of left ventricle; RV=free wall of right ventricle; LA=left atrium; RA=right atrium; Ske.Mus.=skeletal muscle; Diaph.=diaphragm.

Fig. 5. Time course of tissue digoxin concentration after digoxin administration in the 2 groups. Each value represents the mean±SEM. *p<0.05, †p<0.02, ‡p<0.01, **p<0.005, ***p<0.001.

The DX concentrations in various tissues in the 2 groups were compared at 5 and 120 min after DX administration (Fig. 4). The values were necessarily lower in the HK group than in the NK group, and this tendency was more clearly revealed in the values after 120 min than in those after 5 min (p<0.001). Fig. 5 shows the comparison between the values 5 min and
Table I. Comparison of Hemodynamic Change after Digoxin Administration between the 2 Groups (Mean±SEM)

<table>
<thead>
<tr>
<th></th>
<th>Normokalemia (n=8)</th>
<th>P</th>
<th>Hyperkalemia (n=9)</th>
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<tr>
<td></td>
<td>Before</td>
<td>120 min</td>
<td>Before</td>
<td>120 min</td>
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<tr>
<td>Heart Rate (beats/min)</td>
<td>143.8±8.34</td>
<td>&lt;0.05</td>
<td>165.1±5.02</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>99.7±10.1</td>
<td></td>
<td>165.1±5.02</td>
<td>NS</td>
</tr>
<tr>
<td>Pacing Rate (beats/min)</td>
<td>120.0±3.83</td>
<td>&lt;0.05</td>
<td>133.6±3.94</td>
<td>NS</td>
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<tr>
<td></td>
<td>132.0±3.49</td>
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<td>125.1±4.18</td>
<td>NS</td>
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<tr>
<td>Aortic Pre. (mmHg)</td>
<td>syst.</td>
<td>87.0±2.91</td>
<td>92.1±3.90</td>
<td>&lt;0.05</td>
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<td></td>
<td>diast.</td>
<td>92.1±3.90</td>
<td>89.5±3.28</td>
<td>NS</td>
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<td></td>
<td>3.3±0.21</td>
<td>NS</td>
<td>4.2±0.36</td>
<td>NS</td>
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<tr>
<td></td>
<td>2.6±0.39</td>
<td></td>
<td>4.5±0.42</td>
<td>NS</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>3.1±0.23</td>
<td>NS</td>
<td>3.5±0.29</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>2.4±0.40</td>
<td></td>
<td>3.8±0.28</td>
<td>NS</td>
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</table>

120 min after DX administration in the NK and HK groups. In the NK group, the 120 min values were significantly higher than the 5 min values, except for the liver and lungs. In the HK group, the 120 min values were significantly lower than the 5 min values except for the right atrium, skeletal muscle, diaphragm and kidney. The ratios of the tissue DX concentration to the serum DX concentration after 5 min were 49.7±5.04/41.3±6.7=1.20 for the left ventricle in the NK group and 31.7±2.48/86.4±6.6=0.37 in the HK group. For the skeletal muscle, they were 14.4±3.0/41.3±6.7=0.35 and 5.78±0.37/86.4±6.6=0.07, respectively. After 120 min, the ratio in the left ventricle in the NK group was 130.9±5.54/5.2±1.6=25.2 and 19.66±2.1/8.0±1.0=2.46 in the HK group. The respective values for skeletal muscle were 25.1±2.82/5.2±1.6=4.83 and 5.03±0.61/8.6±0.58=0.58.

Table I shows the changes in the hemodynamic parameters induced by DX administration in NK and HK groups. Either systolic or diastolic aortic pressure significantly increased after DX administration in the NK group (p<0.05). In the HK group, there were no significant changes. The heart rate did not change in the HK group because atrial pacing was performed, but, in the NK group, there was a significant decrease after DX administration. No significant differences in left ventricular end-diastolic pressure (LVEDP) and mean right atrial pressure were found in the NK and HK groups. Fig. 6 shows the percent change of the left ventricular dP/dt after DX administration, compared with the values before the administration. In the NK group, there was a marked increase after DX administration which reached a peak value after 60 min (p<0.001), followed by a tendency to decrease slightly. However, in the HK group, there were no significant changes. After 5 min, there were significant differences between the 2 groups (p<0.02).
DISCUSSION

1) Changes in Na and K concentrations in the serum and myocardium induced by KCl administration

Data regarding the influence of hyperkalemia (HK) on the inotropic effects of digitalis are not conclusive. Either positive\(^9\) or negative\(^10\) results have been presented with regard to whether HK decreases the inotropic effect. It appears to be caused by differences in experimental condition; e.g., the differences between in vivo and in vitro studies, different potassium concentrations in the serum or perfusion solution, and different doses of digitalis. In the present study KCl solution was administered according to the method of Goldman et al\(^5\) to yield target values for HK within the physiological range which can be observed clinically. Consequently, the serum K levels in the NK group of normal dogs were 3.6±0.18 mEq/L, while those in the HK group were 5.6–7.1 mEq/L for 2 hours from the start of KCl administration to the completion of the experiment. Morgan et al\(^3\) reported the following results concerning the concentration of K in various tissues in the HK group. Dogs were given a maintenance dose of DX with KCl solution and were sacrificed when the serum K value reached an average of 12.3±3.6 mEq/L. The K concentration in the myocardium was 25.1±2.0 mEq/100 Gm dry weight, which is significantly higher than the value of...
20.7±0.5 mEq/100 Gm dry weight obtained in controls that were not given KCl solution. We measured the Na contents of the myocardium simultaneously and found that there was also a significant difference in the Na contents in the myocardium between the 2 groups. In the NK group, the K and Na concentrations in the myocardium of the left ventricle were 29.2±0.5 mEq/100 Gm dry weight and 26.4±0.8 mEq/100 Gm dry weight, respectively, while in the HK group, these values were 35.8±0.5 mEq/100 Gm dry weight and 20.6±0.8 mEq/100 Gm dry weight, respectively. The myocardial contents of K were significantly higher and those of Na were significantly lower in the HK group than in the NK group (p<0.001, p<0.01). These results suggest that there is an active transport mechanism in the electrolyte exchange in the myocardium.

2) Effect of hyperkalemia (HK) on concentrations of digoxin (DX) in serum and tissues

It has long been considered that potassium supplements are useful in the treatment of digitalis intoxication, but the detailed mechanism underlying this phenomenon are not clear at this time. Therefore, we compared the concentrations of digitalis in serum and tissues between the HK and NK groups and also investigated the effects on cardiac contractility. The serum concentration of digitalis is regulated by three factors: absorption, distribution and excretion. In this experiment, the serum DX concentrations were significantly higher at all periods of observation in the HK group than in the NK group (Fig. 2). Many authors have reported different serum DX concentrations during HK, but Morgan et al reported almost the same results as this experiment. Since the DX dose employed and the administration route were the same both in the NK and HK groups in this experiment, the differences in the pattern of serum DX concentration between the 2 groups is thought to be mainly due to distribution and excretion. The values of DX concentrations in the myocardium are shown in Figs. 4 and 5. From these figures, the cell/blood concentration ratio of DX was calculated. The ratios for the left ventricle after 5 min were 1.20 in the NK group and 0.37 in the HK group and 0.35 and 0.07 for the skeletal muscle, respectively. After 120 min, the ratio for the left ventricle was 25.2 in the NK group and 2.46 in the HK group and for the skeletal muscle, the values were 4.83 and 0.58, respectively. Thus the HK group showed significantly lower ratios of cellular intake of DX, despite the high serum DX concentration. This clearly indicates that DX uptake in the myocardium and skeletal muscle were suppressed by HK.

The concentration of DX in the skeletal muscle was always lower than the concentrations in the myocardium. Because the skeletal muscle occupies
a high percentage of the body as a whole, it is suggested that the skeletal muscle plays an important role as a digitalis pool. Goldman et al.\(^7\) noted that the DX concentration of skeletal muscle was 12.9% of that of the left ventricle and they suggested that it was mainly based on differences in blood flow. Doherty,\(^12\) Morgan\(^3\) and Moran\(^14\) et al. reported similar findings, and they suggested that there may be fewer binding sites for digitalis in the skeletal muscle than in the myocardium or that the uptake of digitalis may be promoted by both contact with digitalis and the frequency of muscle contraction.

The excretion of digitalis must also be considered. The comparison of DX concentrations in various tissues (Fig. 4) showed that the concentration in the kidneys was much higher than that in other tissues. This suggests that the kidney is the main route of DX excretion. A comparison of the DX concentrations in the kidneys from the NK and HK groups showed that the values in the HK group were significantly lower than those in the NK group after both 5 and 120 min. This indicates that the excretion of DX is delayed in the HK group. In this experiment, concentrations in the urine and bile were not measured, but Morgan et al.\(^3\) reported that DX concentrations in the urine were much higher in the NK group than in the HK group, and there were no differences in concentrations in the bile between the 2 groups. From these data, then, it is evident that the tissue uptake of administered DX was less in the HK group than in the NK group and that the excretion in the urine was decreased. Consequently, the DX concentration remained high in the blood in the HK group.

3) \(\text{DX concentration in the myocardium and the inotropic effect of digitalis}\)

As a prerequisite for digitalis showing its inotropic effect on the heart, it must bind to receptor sites in the heart. However, these sites are still obscure. In this experiment, the DX concentrations in the myocardium were measured and correlated with the changes in LV max dP/dt. As can be seen in Fig. 6, the LV max dP/dt in the NK group reached a peak 60 min after DX injection. However, the value after 5 min was only 25% of the peak and there was no statistically significant difference from the control value. However, the DX concentration in the myocardium was 132 ng/Gm after 120 min and 52 ng/Gm after 5 min (about 40% of the 120 min values). In the HK group, no clear conclusion could be drawn due to atrial pacing. Although there were no significant changes in the LV dP/dt, the DX concentration in the myocardium were significantly lower than those in the NK group. These results indicate that there is not necessarily a parallel relation between the DX concentration in the myocardium and the LV max dP/dt. Deutscher et al.\(^13\) administered 0.08 mg/Kg of DX to dogs and observed the changes in
LV dP/dt. They found an increase after 5 min, a value of 33–66% of the peak after 15 min and a peak after 60 min. The concentration of DX in the myocardium was already 50% of the maximum value after 5 min and reached the maximum value after 15 min. Goldman et al\(^7\) studied the amounts of DX which induced the same level of inotropic effects in HK and NK dogs and found that twice the amount was required in the former dogs. From the results described above, it is evident that the K concentration in the myocardium is higher and the Na concentration is lower in the HK group than in the NK group, and that the DX concentration in the myocardium of the HK group is lower than in the NK group, which coincides with a reduced inotropic effect.

In this experiment, the myocardial tissue was not analyzed by tissue fractionation methods and no definite conclusions can be drawn. However, the facts that HK caused not only an increase in the K concentration in the myocardium but also a decrease in the Na concentration, in addition to low DX concentration in the myocardium, strongly suggest that the sodium pump is closely related to the myocardial uptake of K and digitalis. From a number of previous reports,\(^1\) it is assumed that Na-K ATPase in the myocardial cells provides an effective site for manifestation of the inotropic effect. Dutta\(^14\) made the observation using \(^3\)H-DX in isolated heart of guinea pigs (modified Langendorff’s technique) and pointed out that a large amount of DX was initially found in the supernatant fraction. However, when the inotropic effect appeared after 60 min, most of the DX was found in the microsomal fraction. Goldman et al\(^6\) reported that the grade of inhibition of Na-K ATPase under DX administration and the amount of microsomal DX are correlated with the inotropic effect. However, Okita et al\(^15\) reported that the administration of strophanthidin inhibited membrane Na-K ATPase activity with a positive inotropic effect. However, the inhibition of the enzyme continued even when the inotropic effect had disappeared after the wash-out of digitalis, and he suggested that Na-K ATPase is not a real binding site for the manifestation of the inotropic effect of digitalis. Since the location of the receptor site of digitalis is a matter of controversy, it would be reasonable to consider at the present time that even if Na-K ATPase itself is not an effective site of digitalis (Goldman et al\(^6\)), they are substantially very close to Na-K ATPase.

From the discussions above described, it seems reasonable to conclude that there might be a competitive antagonism between myocardial uptake of potassium and DX.
CONCLUSION

In order to elucidate the effects of hyperkalemia on serum and myocardial digoxin concentration, observations were made in normal dogs with these hemodynamic changes. Results obtained were as follows:

1. The serum DX (digoxin) concentrations up to 2 hours after intravenous DX administration in normal dogs were significantly higher in the HK (hyperkalemia) group than in the NK (normokalemia) group.

2. The DX concentrations in various organs 120 min after DX administration were significantly lower in the HK group than in the NK group, and the myocardial DX concentration in the HK group was particularly low compared with the NK group.

3. The LV max dP/dt after DX administration increased gradually in the NK group and reached a peak after 60 min, but there were no significant changes of this parameter in the HK group.

4. In the NK group, there were significant increases in both systolic and diastolic aortic pressures after DX administration, but there were no significant changes in the HK group.

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REFERENCES