Influence of Potassium Depletion on Myocardial Concentration of Tritiated Digoxin

By Keith E. Cohn, M.D., Robert E. Kleiger, M.D., and Donald C. Harrison, M.D.

ABSTRACT

The influence of potassium deficiency on myocardial 3H-digoxin concentration in the mouse was studied. Tritiated digoxin, 400 µg/kg, was injected intraperitoneally into mice, which were killed at varying time intervals, and the 3H-digoxin was extracted. One-half of the mice were made potassium deficient by a diet free of potassium.

In control animals, myocardial 3H-digoxin was maximal by 30 min and declined through 24 hours. There was no significant difference between the myocardial 3H-digoxin of the control and potassium-depleted groups at ½, 1, 2, 6, or 16 hours, but at 24 hours a significantly higher concentration of digoxin was present in the potassium-deficient mouse hearts (23.6 ± 5.2 ng/g) than in the control group (3.1 ± 1.5 ng/g). Potassium-depleted mice with total renal failure, produced by bilateral ligation of the renal pedicle, showed increased myocardial 3H-digoxin levels at 20 hours as compared with mice with renal failure but without potassium deficiency.

It is concluded that potassium deficiency may lead to increased concentrations of cardiac digoxin. The findings in the anuric animals suggest that diminished renal function produced by potassium depletion is not the sole mechanism of this retention of cardiac 3H-digoxin.

ADDITIONAL KEY WORDS kaliopenic nephropathy electrolytes heart digitalis toxicity ATPase anuria mice

For many years it has been appreciated that complex interrelationships between potassium and digitalis glycosides exist. A number of investigators have established that potassium administration will abolish toxic arrhythmias produced by digitalis (1) and Lown and associates (2) have demonstrated that depletion of total body potassium will enhance the development of digitalis toxicity. Toxic and therapeutic doses of digitalis have been shown to induce a loss of myocardial potassium (3-6) mainly by slowing potassium influx (3), although a decrease in intracellular potassium does not regularly occur when therapeutic amounts of digitalis are employed (5, 6). Decreases in extracellular potassium have also been shown to augment the potassium loss from the heart produced by digitalis (6, 7).

The mechanism by which potassium affects the cardiac response to the glycosides remains undefined. The present study was designed to determine the effect of potassium depletion on the myocardial uptake and storage of radioactive digoxin. It seemed possible that changes in myocardial content of digoxin could account for the interesting interaction between digitalis and potassium. Injection of tritiated digoxin into mice provided a convenient model for studying this problem.

Methods

One hundred eighteen Swiss-Webster mice weighing 20 to 30 g were divided into two groups: a control group on a Hartroft diet with
added potassium and a potassium-deficient group, placed for 3 weeks on a potassium-free Hartroft diet. No tissue levels were determined in this study because previous studies in the rat have demonstrated that a potassium-deficient diet for only 14 days results in a significant decrease in myocardial potassium concentration and in histological changes within the heart (9). Serum potassium concentrations were determined by flame photometry and blood urea nitrogen values by the urease method with direct nesslerization on Folin-Wu filtrate.

The tritium-labeled digoxin was prepared by the Willzach hydrogen exchange method, and radioactive impurities were removed by column partition chromatography. The digoxin was supplied with a specific activity of 112.4 mc/mg. Each of 86 mice received a single intraperitoneal injection of tritiated digoxin, 400 μg/kg body weight (approximately 10 μg per animal). The digoxin was dilute in normal saline and administered in a volume of 0.25 ml to prevent tissue necrosis produced by the alcohol base in which the digoxin is supplied.

The animals were killed by cervical fracture at 8, 1, 2, 6, 16 or 24 hours after digitalization. At each interval, 4 to 11 mice were studied in both the control and potassium-deficient groups. The hearts were removed, frozen and weighed. They were then homogenized in chloroform and ethanol, and the tritiated digoxin was extracted by thoroughly shaking in 10 ml of 20% ethyl alcohol in chloroform, and the mixture was filtered. The supernatant fluid was evaporated to dryness, dissolved in 0.2 ml of 100% ethyl alcohol, counting solution was added, and the specimens were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Series 2000). The counting solution was prepared by dissolving 3 g 2,5-diphenyloxazole (PPO) and 0.1 g dimethyl 1,4-bis-(4 methyl-5-diphenyloxazolyl-benzene) (POPOP) in 1 liter of toluene. The samples were corrected for quenching by addition of tritiated calcium carbonate, 19.7% magnesium sulfate, 14.8% sodium phosphate dibasic, 41.0% calcium phosphate tribasic, 3.2% ferric phosphate, 15% cupric sulfate, 0.4% manganese sulfate, 0.2% cobaltous chloride, 0.1% sodium iodide and 11% zinc sulfate. KCl, 1.2 g, is added to each 100 g of control diet.

Results

The time course of myocardial 3H-digoxin concentration is shown in Table 1. The concentration was maximum by 30 min. In the control animals, the myocardial digoxin was almost entirely depleted by the twenty-fourth hour, the radioactivity being at background levels in four of the seven mice in this group.

There was no significant difference between the myocardial 3H-digoxin concentrations of the control and those of the potassium-depleted groups at either 8, 1, 2, 6, or 16 hours. At 24 hours, however, a significantly higher concentration of digoxin was present in the hearts from potassium-deficient mice (23.6 ± 5.2 μg/g). When elevated blood urea nitrogen values, presumably due to kaliopenic nephropathy, were detected in the potassium-deficient mice, it was decided to eliminate differences in renal function between the two groups of mice as a variable. Thirty-two mice, divided into control and potassium-deficient groups, were anesthetized with 10 mg pentobarbital administered intraperitoneally, laparotomies were performed, and the renal pedicles were ligated bilaterally, thus producing acute, total renal failure. The animals were killed 20 hours after intraperitoneal injection of 3H-digoxin, 400 μg/kg, and the hearts were handled exactly as in the unoperated mice. Statistical analysis was performed employing Student's t-test.

<table>
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<td><strong>Myocardial 3H-Digoxin Concentrations</strong></td>
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* Number in parenthesis is number of mice.
mug/g heart wt.) than was found in the controls (3.1 ± 1.5 mug/g heart wt.; P < .001; Table 1).

The serum potassium averaged 5.2 ± 0.26 mEq/liter in the control animals and was significantly lower (3.3 ± 0.24 mEq/liter) in the kaliopenic mice (P < .001). Slight hemolysis, often produced during the bleeding of the mice, likely caused a minor spurious increase in the serum potassium values. The blood urea nitrogen values in the control and potassium-depleted mice averaged 23 ± 0.8 and 40 ± 3.6 mg/100 ml.

Twenty hours after digoxin administration to the anuric mice, the mean cardiac 3H-digoxin concentration was significantly greater in the 15 potassium-deficient animals (90.4 ± 8.7 mug/g heart wt.) than in the 17 controls (51.7 ± 5.3 mug/g; P < .001). The serum potassium averaged 8.2 ± 0.5 and 4.5 ± 1.1 mEq/liter, and the mean blood urea nitrogen levels were 84 ± 8 and 87 ± 5 mg/100 ml in control and potassium-depleted groups respectively.

Discussion

The data presented in this study demonstrate the time course of myocardial 3H-digoxin concentration in normal mice following a single, intraperitoneal loading dose. The initial uptake and storage of tritiated digoxin in the hearts of potassium-depleted mice was essentially identical to that of the controls. At 24 hours, however, the cardiac 3H-digoxin concentration was significantly greater in the potassium-deficient animals.

The mechanism of this myocardial digoxin retention induced by potassium depletion is not known. It is generally accepted that digoxin is mainly excreted by the kidneys (9, 10), and that in renal failure an increase in serum and cardiac digoxin concentrations are found (12, 13). Potassium deficiency may lead to renal dysfunction. Although usually affecting tubular structure and function (14), potassium depletion may also decrease the glomerular filtration rate and produce azotemia (15). It therefore seemed possible that a diminished glomerular filtration rate in the potassium-deficient mice, reflected in this study by the elevated blood urea nitrogen, was responsible for the observed retention of myocardial digoxin. However, even after bilateral ligation of the renal pedicle, the chronically potassium-depleted mice again manifested increased myocardial 3H-digoxin levels compared with the nonpotassium-deficient, anuric animals. It therefore appears that the impaired renal excretion of digoxin produced by potassium deficiency does not solely account for the observed elevations of cardiac 3H-digoxin concentrations in the potassium-depleted mice.

The reason for the increased cardiac concentrations of 3H-digoxin caused by low potassium levels is still uncertain. Perhaps the movement of digoxin across the cell membrane is slowed, efflux being impaired to a greater extent than influx. Several authors have indicated that digitalis and potassium compete for a locus at the cell membrane (7, 16, 17). Myocardial binding sites left vacant by potassium ions could thus be occupied by available digitalis. Recent studies employing radioautography have localized the site of digitalis binding primarily to the A band, or myosin portion, of the myofibril (18, 19). It remains a possibility that alterations in physiochemical properties of actomyosin produced by variations in potassium concentration, as have been described by Szent-Gyorgi (20), may influence the myocardial binding of digitalis.

Hypokalemia also affects circulatory dynamics, evoking a positive inotropic effect (21), histological changes in the myocardium (8, 22), vasodepression, and a diminished response to the administration of catecholamines (23). Although it is conceivable that one of these mechanisms induced by potassium depletion may indirectly influence tissue retention of digoxin, there is no evidence in this study to support or deny this hypothesis.

The present observations correspond with those of Ebert et al., who studied the effect of acute potassium infusion on the digoxin content of the canine heart (24). These workers detected a 14% decrease in cardiac 3H-digoxin, and attributed this change to either drug egress from the myocardium or impaired
entry. The blood $^3$H-digoxin levels were not significantly changed.

Caution should be exercised in attempting to relate the present observations to clinical problems in man. It is not known whether myocardial digoxin concentrations will reach toxic levels in a typical clinical setting of digitalis administration and potassium deficiency. Furthermore, it is likely that variations in cellular transmembrane potentials caused by changes in intracellular and extracellular potassium influence, in a more direct manner, cardiac responsiveness to digitalis. Nonetheless, cardiac retention of digoxin does occur during potassium deficiency in mice and may possibly be one mechanism whereby toxic levels of intracellular digoxin could accumulate.

References

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