Effect of Therapeutic and Toxic Concentrations of Ouabain Upon Potassium Content of Myocardium.* (26427)

PULAK N. SANYAL AND PAUL R. SAUNDERS
Department of Pharmacology, School of Medicine, University of Southern California, Los Angeles

In recent years, considerable attention has been devoted to the possibility that the actions of cardiac glycosides may be related to effects upon the electrolyte content of the myocardium. Several investigations(1-6) have indicated that a loss of potassium from the myocardium occurs in presence of toxic concentrations of these drugs. Other studies have demonstrated inhibition by cardiac glycosides of potassium uptake by red blood cells(7,8) and skeletal muscles(9). It is not clear, however, if the positive inotropic action of cardiac glycosides is also associated with a change in intracellular potassium concentration. We have, therefore, investigated the effect of both "therapeutic" and toxic concentrations of ouabain upon intracellular potassium content of isolated, contracting myocardium.

Methods. Ventricle strip preparation. Strips were prepared from the right ventricular wall of hearts of male guinea pigs (150-250 g) and stimulated at a frequency of 100/min. at 37.5°C. in a bicarbonate medium (pH 7.4) gassed with 1% CO_2-99% O_2 mixture as described previously(10).

Determination of extracellular space. Ventricle strips were equilibrated in medium containing 1% (w/v) inulin (dissolved by gentle heating) for the 3 hour experimental period. Inulin content of the blotted tissue was determined by the method of Ross and Mokotoff(11), adapted to approximately 50 mg of wet tissue, and extracellular (inulin) space then calculated.

Determination of potassium. At the conclusion of the experimental period, the ventricle strips were blotted with filter paper, weighed and total water determined by drying at 110°C. Each strip was then digested for 10 minutes with 1.5 ml of 0.75 *N* HNO_3 in a boiling water bath(3). The extracts were filtered and potassium determined with a Baird Atomic KY flame photometer employing an internal lithium standard. Intracellular potassium concentration was calculated from the determined values of total water, extracellular space, total potassium and potassium concentration of the medium. Intracellular potassium concentration was determined for each strip, using the corresponding mean extracellular space values for strips in the absence of drug or which had been exposed to ouabain at various concentrations.

Experimental procedure. Control strips were set up and stimulated for 3 hours, then extracellular space and potassium content were determined. Because of their small size, separate strips were used for determination of potassium and extracellular space. Other strips were stimulated for a 2-hour period, ouabain was then added and the strips were stimulated for an additional hour after which extracellular space and potassium content were measured.

Results. Ouabain at a concentration of 0.16 μg/ml produced a marked increase in the force of contraction without any evidence of toxicity (*e.g.*, spontaneous beats or increase in resting tension). Maximum increase in force (Table I) usually occurred within about one-half hour and was sustained during the remainder of the experimental period (one hour after addition of ouabain). A similar response occurred to a somewhat higher concentration of ouabain (0.33 μg/ml), although 3 of a total of 17 strips showed evidence of toxicity (Table I) at this concentration. With a consistently toxic concentration of the drug (1 μg/ml) there was initially a large increase in force of contraction to a mean value of about 110% of the initial force just prior to the point at which spontaneous contractions independent of the frequency of stimulation first appeared (the mean time to

* This investigation was supported in part by research grants from Nat. Heart Inst., P.H.S., and Los Angeles County Heart Assn.

TABLE I. Effects of Therapeutic and Toxic Concentrations of Ouabain upon Intracellular Potassium Concentration of Guinea Pig Ventricle Strips.

Ouabain conc., μg/ml	Increase* in force of contraction, %†	Evidence of toxicity	Total water	Extracellular space	Intracellular space	Total potassium, mmoles/kg wet wt†	Intracellular potassium conc., mmoles/l†
			ml/kg wet wt†				
0	—	None	780 ± 3 (12)‡	264 ± 6 (10)‡	516 ± 3	69 ± 1 (12)‡	131 ± 2
.16	52 ± 5 (20)‡	"	788 ± 8 (12)	277 ± 12 (8)	511 ± 8	66 ± 2 (12)	126 ± 2
.33	80 ± 8 (14)	" §	783 ± 6 (6)	275 ± 8 (8)	508 ± 6	65 ± 1 (6)	125 ± 2
1.0	See text (16)	Arrhythmia & increase in resting tension	804 ± 5 (8)	253 ± 12 (8)	551 ± 5	58 ± 1 (6)	103 ± 2

* Expressed as % of initial force. Initial force defined as steady force attained at end of 2 hr equilibration period (immediately prior to addition of drug).
† Mean value ± S.E.
‡ No. of experiments given in parentheses throughout table.
§ 3 strips at this concentration of ouabain displayed evidence of toxicity (spontaneous contractions of irregular force at a rate independent of frequency of stimulation, although no increase in resting tension occurred). Values given in the table are those for strips which did not show such evidence of toxicity at this concentration of ouabain.

appearance of first spontaneous contractions was 8 minutes and spontaneous frequency was about 250/min). The resting tension of the strips began to increase greatly at approximately the time of first appearance of spontaneous contractions, and after approximately an additional 5 minutes all visible contractile activity of the muscle ceased except for a slight quivering motion at a greatly increased resting tension. A concentration of ouabain of 0.16 μg/ml, therefore, produced a positive inotropic response such as that which occurs after therapeutic doses of such drugs, while a concentration of 1 μg/ml led to obvious evidence of toxicity.

The mean intracellular potassium concentration of the strips in presence of concentrations of ouabain which increased the force of contraction without evidence of toxicity (0.16 and 0.33 μg/ml) did not differ significantly from that of control strips ($P>0.1$, t test). On the other hand, a toxic concentration of ouabain (1.0 μg/ml) caused a significant decrease (21%) in intracellular potassium concentration by comparison with control strips ($P<0.01$). Intracellular space was not affected by ouabain at concentrations of 0.16 or 0.33 μg/ml, but was increased significantly ($P<0.01$) at a concentration of 1.0 μg/ml.

Discussion. The effect of therapeutic concentrations of cardiac glycosides upon potassium content of the heart has long been disputed, and a decrease, an increase or no change(12) have been reported. In many of the studies reporting a loss of this ion, however, clear evidence of a positive inotropic action without toxicity was lacking. Vick and Kahn(4) state that detectable losses of potassium from guinea pig hearts occurred with concentrations of ouabain producing a positive inotropic action. Their data showed a small and almost identical potassium loss over a wide range (10-75%) of positive inotropic action, but no statistical evaluation was presented. Later work(5) indicated that low concentrations of dihydro-ouabain, which produced a substantial positive inotropic action, resulted in at most a small loss of potassium for a brief period after which the ventricles were in potassium balance for several hours. Vick concluded that the positive inotropic action was not due to a loss in potassium, as several non-steroid lactones caused potassium loss without producing an increase in the force of contraction. Brown *et al.*(13) have shown recently that potassium loss occurred for a brief period only after injection of a cardiac glycoside into the circulation of heart-lung preparations in which cardiac failure had been induced with

pentobarbital. The significance of this fleeting effect with respect to the positive inotropic action is unknown. There is also no clear evidence in the work of Hajdu(3) that concentrations of cardiac glycosides producing increases in the force of contraction of frog heart without toxicity caused potassium loss, although concentrations producing contracture in 20-25 minutes did result in such loss. Loss of potassium occurring after exposure of guinea pig hearts to K-strophanthoside was followed within a few minutes by contracture(14).

Our studies indicate clearly that there is no significant change in intracellular potassium concentration or total potassium content in the presence of concentrations of ouabain producing a marked increase in the force of contraction but no evidence of toxicity. The view that an alteration in the potassium balance of the heart is related to the positive inotropic action is, therefore, untenable in our opinion unless loss of potassium from only certain regions of the heart occurred, in which case such loss might not have been detectable when total potassium was determined.

On the other hand, a significant decrease in intracellular potassium concentration and total potassium occurred in our experiments in presence of a toxic concentration of ouabain. Loss of potassium from cardiac tissue after toxic doses of cardiac glycosides has been reported by a number of other workers (1-6).

Summary. Intracellular concentration and total amount of potassium in isolated guinea pig ventricle strips in the presence of both therapeutic and toxic concentrations of ouabain have been investigated. A concentration of the drug which produced an increase in the force of contraction without evidence of toxicity did not alter either intracellular concentration or total amount of this ion in the heart. A toxic concentration of ouabain which produced automaticity and a marked increase in the resting tension, followed by decline in force of contraction to zero, caused a mean decrease of 21% in intracellular potassium concentration.

1. Hagen, P. S., *J. Pharmacol. Exp. Therap.,* 1939, v67, 50.

2. Wood, E. H., Moe, G. K., *Am. J. Physiol.,* 1942, v136, 515.

3. Hajdu, S., *ibid.,* 1953, v174, 371.

4. Vick, R. L., Kahn, J. B., *J. Pharmacol. Exp. Therap.,* 1957, v121, 389.

5. Vick, R. L., *ibid.,* 1959, v125, 40.

6. Holland, W. C., Greig, M. C., Dunn, C. E., *Am. J. Physiol.,* 1954, v176, 227.

7. Glynn, I. M., *J. Physiol.,* 1957, v136, 148.

8. Solomon, A. K., Gill, T. J., 3rd, Gold, G. L., *J. Gen. Physiol.,* 1956, v40, 327.

9. Johnson, J. A., *Am. J. Physiol.,* 1956, v187, 328.

10. Sanyal, P. N., Saunders, P. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 156.

11. Ross, G., Mokotoff, R., *J. Biol. Chem.,* 1951, v190, 659.

12. Hajdu, S., Leonard, E., *Pharm. Rev.,* 1959, v11, 173.

13. Brown, T., Grupp, G., Acheson, G. H., *J. Pharmacol. Exp. Therap.,* 1960, v129, 42.

14. Sulser, F., *Experientia,* 1959, v15, 97.

Received January 19, 1961. P.S.E.B.M., 1961, v106.