The Effect of Myocardial Contraction on the Uptake and Retention of a Tritium-labelled Cardiac Glycoside

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Cardiac glycosides are a family of drugs derived from several plant and animal sources such as the leaves of *Digitalis purpuria* (purple foxglove), *Strophanthus gratus,* and toad venom. This entire family of drugs is commonly referred to simply as digitalis.

The most important property of digitalis is its unique ability to increase the force of contraction of heart muscle. By this action, digitalis exerts its therapeutic effects in congestive heart failure by improving the mechanical efficiency of the heart as a pump.

This cardio-stimulatory action of digitalis has been demonstrated repeatedly in a variety of preparations of heart muscle (Goth, 1966). However, considerable controversy exists as to whether digitalis' action

to stimulate the heart is dependent upon the rate of contraction or upon the time of contact between the drug and the myocardium. For over 50 years it has been reported that the action of digitalis to increase contractile force depends directly upon the frequency of contraction. This concept stems from early work done with frog hearts, but, more recently, similar findings have been reported for mammalian heart muscle (Weizsaecker, 1913; Wilbrandt et al., 1963). On the other hand, several investigators have reported recently that digitalis action is time-dependent rather than contraction-dependent (Sanyal and Saunders, 1958; Moran, 1963, 1967).

Since considerable controversy still exists, the present experiments were designed to determine whether contractility itself affects the ability of heart muscle to take up and retain a tritium-labelled digitalis compound (ouabain).

METHODS

Experiments were conducted with ventricle strips from guinea pigs of either sex weighing between 200 and 400 g. After cervical dislocation, the hearts were removed rapidly and immersed in a dish containing a modified Chenoweth-Koelle solution aerated with 95% O_2 - 5% CO_2 (pH 7.35-7.4). This solution was used as bath fluid in all experiments and contained in 1 liter: 7.0 g of NaCl, 0.42 g of KCl, 0.32 g of $CaCl_2 \cdot 2H_2O$, 0.43 g of $MgCl_2$, 1.8 g of dextrose, 2.1 g of $NaHCO_3$, and 10.8 mg of disodium ethylenediaminetetraacetic acid (Na,EDTA). Pairs of muscle strips, weighing between 25 and 60 mg, were cut from the free wall of the right ventricle. The strips were connected to muscle holders by impaling one end on platinum electrodes contained in the holders. The tissues and muscle holders were placed in water-jacketed muscle baths containing 10 ml of aerated Chenoweth-Koelle solution, and the free ends of the muscle strips were attached to Grass FT-03 force-displacement transducers. Two g of initial tension were placed on the muscles which were driven electrically at a frequency of 2/sec using Grass S5 stimulators to deliver square wave pulses of 5 msec duration at superthreshold voltages. Isometric contraction was recorded on a Grass Polygraph via the force transducers. The tissues were allowed to equilibrate in the aerated Chenoweth-Koelle solution for 30 min to 1 hr before exposing them to drugs. In all experiments, the temperature of the bath was kept constant at 29 ± 1 C. ^{3}H-ouabain, obtained from New England Nuclear Corporation, had a specific activity of 720 mc/ml and was measured and delivered into the bath medium with microliter pipettes. The muscles were exposed to the drug for a period of 20 min after which the baths were drained and flushed twice with fresh Chenoweth-Koelle solution which was constantly aerated and maintained at the appropriate temperature in a water-jacketed reservoir. The ventricle strips were then removed and homogenized in 2 ml of distilled water. The homogenate was centrifuged at 0 C for 10 min at 10,000 rpm (12,000 × g) in a Sorvall RC-2 refrigerated centrifuge. A 500λ aliquot of the supernatant fluid was pipetted into a vial containing 15 ml of TDE counting solution and counted for 10 min in a Packard Tri-Carb Liquid Scintillation Spectrometer for ^{3}H content. Radioactivity, expressed as counts/min/mg of tissue (cpm/mg), was determined for each sample. The efficiency of counting was approximately 18% with an internal standard ^3H_2O. Samples were uncorrected for quenching.

In other experiments, ventricle strips were prepared in the same manner with the exception that these strips were not stimulated and the tension was at zero. Other experimental procedures were identical to those described above.

All data are expressed as mean values ± one standard error. Student's *t* test was used to determine significance between groups of data. A P value of < 0.05 was considered to be statistically significant (Snedecor, 1956).

RESULTS

Uptake of ^{3}H-ouabain in the myocardium—In order to determine the importance of contraction on the tissue uptake of ^{3}H-ouabain, experiments were conducted in both contracting and quiescent isolated guinea pig ventricle strips. Groups of both contracting and noncontracting ventricle strips were prepared and allowed to equilibrate in the bath medium for the same period of time. The handling of the groups of strips differed only in that contracting strips were stimulated electrically under 2 g of tension while quiescent strips were not stimulated and no tension was applied.

The myocardial uptake of ^{3}H-ouabain by ventricle strips was determined after adding one of four different concentrations to the bath medium. The concentrations of ^{3}H-ouabain used in these studies were 4×10^{-8}, 1.2×10^{-7}, 2×10^{-7}, and 4×10^{-7} g of ouabain/ml containing 0.05, 0.15, 0.25, and 0.5μc radioactivity per ml, respectively.

The radioactivity measured in the tissue, expressed as counts/min/mg of heart muscle, after exposure to tritium-labelled ouabain for 20 min was compared statistically for each group of the ouabain concentrations studied. There was no significant difference between contracting and quiescent ventricle strips in the tissue uptake of ^{3}H-ouabain at concentrations of 4×10^{-8}, 1.2×10^{-7}, and 2×10^{-7} g ouabain/ml. Only at the highest concentration of ouabain used, 4×10^{-7} g/ml, was the uptake of ouabain significantly different between contracting and noncontracting ventricle strips. The uptake of ^{3}H-ouabain appears to be roughly proportional to the dose administered in both contracting and noncontracting ventricle strips.

Effect of ouabain on myocardial contractile force — Records were made of myocardial contraction in electrically stimulated ventricle strips contracting at the rate of 120/min, before and during the action of ^{3}H-ouabain. The lowest dose of ^{3}H-ouabain, 4×10^{-8} g/ml, failed to produce an increase in the force of myocardial contraction which was significantly different from the control contractile force in that group. With larger doses of ^{3}H-ouabain, myocardial contractile force was increased significantly above control force in the remaining three groups of contraction ventricle strips. The maximum effects on contractile force were reached by the two higher doses of ^{3}H-ouabain, 2×10^{-7} and 4×10^{-7}. The maximum increase in contractile force developed within 16 min in the group of strips given 2×10^{-7} g/ml and arrhythmias developed in 5 of the 12 strips in this group. In the group of strips given 4×10^{-7} g/ml, the maximum increase in contractile force developed after only 7.5 min exposure to the drug and all of the ventricle strips developed arrhythmias. Arrhythmias in the isolated ventricular muscle preparation consists of either the occurrence of spontaneous, automatic contractions or the failure of the ventricle strip to respond uniformly to every electrical stimulus.

DISCUSSION

Since radioactive labelled ouabain has become available in recent months, it is now possible to determine with sufficient accuracy small amounts of the labelled glycoside in isolated tissue and to determine the influence of other drugs or various procedures upon its uptake by tissue.

Of the four concentrations of ^{3}H-ouabain used in these experiments, the three lower concentrations (4×10^{-8}, 1.2×10^{-7} and 2×10^{-7} g ouabain/ml) represent "therapeutic" concentrations, somewhat similar to concentrations achieved in man. However, even with a supposedly "therapeutic" concentration (2×10^{-7} g/ml) evidence of toxicity was observed and 5 of 12 contracting ventricle strips developed arrhythmias. All contracting ventricle strips exposed to the highest concentration of ouabain

(4×10^{-7} g/ml) developed arrhythmias.

These results show that ^{3}H-ouabain is taken up by isolated guinea pig ventricle strips in a somewhat linear relationship to the concentration of ouabain administered. The total amount of radioactivity accumulated in ventricle strips did not appear to be dependent upon the presence of myocardial activity since no significant difference was found between quiescent or contracting muscle at the three lowest concentrations of ^{3}H-ouabain used. The only significant difference between the amount of ^{3}H-ouabain retained in contracting or noncontracting muscle was seen when a "toxic" concentration (4×10^{-7} g/ml) of ^{3}H-ouabain was given. In this case, all contracting ventricle strips developed arrhythmias and the amount of glycoside retained in these arrhythmic strips was significantly greater than that retained in noncontracting strips. A slightly higher, but not statistically significant, amount of ^{3}H-ouabain was also retained by contracting strips in the presence of 2×10^{-7} g/ml of ^{3}H-ouabain in which 5 of 12 strips developed arrhythmias. Weizsaecker (1913) suggested that myocardial activity can accelerate the "toxic" effect of glycosides. These results suggest that myocardial activity has no effect on the ability of heart muscle to retain "therapeutic" concentrations of cardiac glycosides, but that when "toxic" concentrations are administered, either myocardial activity itself or the arrhythmic state may produce a change in the myocardium to permit larger amounts of the glycoside to be taken up by the muscle.

The results of this study are in agreement with observations made by Okita et al. (1967) who reported that guinea pig atria exposed to ^{3}H-digoxin for 30 min while contracting took up no more radioactive digoxin than did noncontracting atria similarly treated. On the other hand, Kushinsky et al. (1967) found that contracting guinea pig atria took up ^{3}H-digoxin more rapidly than noncontracting atria, but that after 3 hr the maximum amount of radioactivity taken up was the same in both groups. These results are not necessarily contradictory to those found by Kushinsky et al. (1967), but suggest that perhaps more experiments should be conducted to determine the rate of uptake of ouabain by varying the time of exposure.

CONCLUSIONS

Present results permit the conclusion that myocardial activity has no effect on the ability of heart muscle to retain cardiac glycosides in "therapeutic" concentrations, but when "toxic" concentrations are administered, either myocardial activity itself or the arrhythmic state may produce a change in the myocardium to permit larger amounts of the glycoside to be retained by the muscle.

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