The influence of cardiac glycosides on membrane permeability in guinea-pig atrial tissue, determined by means of ^{86}Rb

SIR,—It seems that *toxic* doses of cardiac glycosides provoke a reduction of intracellular potassium, accompanied by an increase of the sodium concentration in cardiac tissue, thus explaining cardiac arrhythmia during digitalis intoxication (Holland, 1964; Klaus, 1964). But, with lower non-toxic doses, there is disagreement about the effects on membrane permeability. Tracer experiments by Klaus, Kuschinsky & Lüllman (1962) have shown that therapeutic concentrations of digitoxigenin neither affect the intracellular K-concentration nor the ^{42}K-efflux in guinea-pig isolated atria. However, Greeff, Meng & Moog (1962) observed a small increase in the K-efflux from guinea-pig isolated perfused hearts upon treatment with non-toxic ouabain doses. According to Repke (1963) and to Piechowski, Grobecker & Greeff (1963) ouabain in low concentrations may influence the K-Na sensitive membrane ATPase in myocardial tissue, although it should be emphasized that experimental evidence for this assumption is mainly based on investigations with isolated red cells.

We have found that ^{86}Rb may be used conveniently to measure changes in permeability towards ions, ^{86}Rb being suitable as a tracer for ion movements in atrial tissue (Zwieten, 1968) and easier to handle than ^{42}K.

Isolated atria were loaded with ^{86}Rb upon incubation in a K-free Tyrode solution that contained an equivalent amount of RbCl (2·7 mmole/litre), part of the Rb^+ ions being radioactive (^{86}Rb). The atria thus loaded (2 hr) were attached in a small organ bath (volume 1·8 ml) that was perfused continuously with K-free, Rb-Tyrode solution (see above). By means of an infusion pump the perfusate was drawn with a constant rate of 1·5 ml/min through a plastic tube, wound within the counting vial of an ECKO N664C liquid scintillation spectrometer. The β-radiation of the ^{86}Rb in the perfusate penetrated the wall of the plastic tube with a loss of 25%, and provoked light impulses in the counting vial that was filled with a liquid scintillation phosphor. The impulses were counted, integrated and recorded continuously by means of a Rikadenki device. The curve thus obtained reflected the ^{86}Rb content of the perfusate, i.e., the amount of ^{86}Rb released by the isolated atrium. The bath fluid was gassed with 5% carbon dioxide in oxygen and kept at 30°. The atria were stimulated electrically with a frequency of 180/min (Grass S4H). Mechanical activity was recorded continuously by a transducer and Helco-scriptor recording device (Zwieten, 1968). Isolated atria incubated in K-free, Rb-containing Tyrode solution show unimpaired mechanical activity, whereas the response to electrical stimulation and to cardioactive drugs of various categories is the same as that found during incubation of the organs in normal, K-containing Muralt-Tyrode-solution (Zwieten, 1968).

The loss of ^{86}Rb from atria, beating with a frequency of 180/min occurs by means of two different rate constants, i.e., $22{\cdot}2 \times 10^{-4}$ and $2{\cdot}0 \times 10^{-4}$ sec^{-1}, the slower process becoming predominant after a perfusion of about 20 min. The straight line obtained upon plotting the ^{86}Rb level of the perfusate towards the perfusion time is a direct measure for the rate of ^{86}Rb release by the atrial tissue. The straight line, fitted by the method of the least squares had the general equation $\log Y = bX + a$, where $Y = {}^{86}Rb$-content of the perfusate, expressed in % of initial, maximal value and X the perfusion time in min (see Table 1). To determine the effect of cardiac glycosides in therapeutic dosage, the drugs were added to the perfusion Tyrode solution.

The following cardiac glycosides were studied: ouabain, digoxin, peruvoside (the α-thevetoside of cannogenin) and digitoxin. In the dosage used the

drugs caused a pronounced therapeutic effect without provoking any cardiac arrhythmia or contracture. Again, the rate of ^{86}Rb release was characterized by means of the regression line, representing the slow process of wash-out. The results are summarized in Table 1. The influence of each drug has been studied on 5–10 different atria.

TABLE 1. INFLUENCE OF CARDIAC GLYCOSIDES IN THERAPEUTIC CONCENTRATIONS ON THE RATE OF ^{86}Rb-RELEASE. General equation of the regression lines, representing the rate of ^{86}Rb-release: $\log Y = bX - a$. (a and b are given)

Cardiac glycoside	Concentration in perfusion Tyrode solution (M)	Increase in contractile force in % (mean ± s.e.)	b	a (± S log Y)
Controls	—	—	$5{\cdot}35 \times 10^{-4}$	1·6787 ± 0·1802
Ouabain	$3{\cdot}4 \times 10^{-7}$	56 ± 8·3	$6{\cdot}33 \times 10^{-4}$	1·8120 ± 0·2054
Digoxin	$3{\cdot}2 \times 10^{-7}$	50 ± 6·9	$5{\cdot}69 \times 10^{-4}$	1·7136 ± 0·1765
Peruvoside	$3{\cdot}6 \times 10^{-7}$	60 ± 6·9	$7{\cdot}01 \times 10^{-4}$	1·7195 ± 0·2632
Digitoxin	$3{\cdot}3 \times 10^{-7}$	46 ± 2·2	$5{\cdot}34 \times 10^{-4}$	1·6715 ± 0·2123

None of the equations shown in Table 1 are significantly different from that representing the ^{86}Rb-release under control circumstances. Accordingly, the rate of ^{86}Rb release is not changed by cardiac glycosides in therapeutic concentrations. Therefore, the Rb-efflux also remains unchanged, since this parameter is directly proportional to the rate constant of the release process.

Toxic concentrations of the cardiac glycosides caused arrhythmia and finally contracture after an initial positive inotropic action. The following doses were used: ouabain $1{\cdot}7 \times 10^{-6}$M; digoxin $6{\cdot}4 \times 10^{-6}$M; peruvoside $4{\cdot}3 \times 10^{-6}$M; digitoxin $6{\cdot}5 \times 10^{-6}$M. During the initial positive inotropic effect no increase in ^{86}Rb release could be observed. However, an irregular though large acceleration of the release process accompanied the toxic symptoms of the glycoside effect. A quantitative evaluation of the accelerated ^{86}Rb-release could not be given since the irregular contractile activity of the atria in this stage also enhanced the release of $^{86}Rb^{+}$-ions, the arrhythmia being accompanied by an increased frequency of beating. Evidence has been given that the release of ^{86}Rb is directly related to the number of contractions (Zwieten, 1968). Consequently, the membrane permeability in atrial tissue is increased by toxic concentrations of cardiac glycosides, whereas therapeutic concentrations of the same drugs do not affect this parameter. This finding is in satisfactory agreement with the studies using ^{42}K as a tracer (Klaus, Kuschinsky & Lüllman, 1962).

The ouabain-induced increase in ^{86}Rb-release, and also the mechanical arrhythmia, could be antagonized by the simultaneous infusion of diphenylhydantoin-sodium into the organ bath. This drug has proved of some use in the therapy of digitalis-induced arrhythmia in patients (see, e.g., Delius, 1966). Electrophysiological studies by Lüllman & Weber (1968) suggest that a reduction of passive ion fluxes on the membrane may explain the therapeutic effect of diphenylhydantoin-sodium. The investigations with ^{86}Rb also demonstrate that the ouabain-induced increase in membrane permeability may be antagonized by the hydantoin.

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Effects of some spasmolytic agents on the lipid-facilitated transport of calcium ions

SIR,—Depolarization of smooth muscle cells is accompanied by an influx of calcium ions. Woolley (1963) proposed that lipids were involved in the transport of calcium ions and phospholipids extracted from skeletal and cardiac muscle and nervous tissue were found to facilitate the transport of calcium ions from an aqueous to the chloroform phase (Feinstein, 1964; Nayler, 1966a). Several substances were found to elicit effects on this simple system consistent with their pharmacological properties (Feinstein, 1964; Blaustein & Goldman, 1966; Nayler, 1966a,b; Sandow & Isaacson, 1966; Blaustein, 1967; Piccinini & Pomarelli, 1967). We (Santi, Ferrari & Contessa, 1964; Tóth, Ferrari & others, 1966) implicated two effects in the mechanism of action of spasmolytics: (i) the inhibition of oxidative phosphorylation shared by papaverine and its main oxy-alkyl-benzylisoquinoline derivatives, and (ii) interference with calcium ions presumably assuming a prominent importance in the myolytic activity of other compounds devoid of inhibitory effects on energy production (Ferrari & Gaspa, 1965; Tóth & others, 1966). We now describe the effects of some myolytic agents on lipid-facilitated calcium transport.

Phospholipids were extracted (1 hr) from a homogenate of calf stomach muscle with chloroform–methanol (2:1) solution (1·5 ml)/g wet weight (Feinstein, 1964; Blaustein, 1967). The extract was washed and diluted with chloroform–methanol (2:1) to obtain a phospholipid concentration of 1·5 mg/ml (Folch, Lees & Stanley, 1957). The drugs (papaverine hydrochloride, eupaverin hydrochloride, isoxsuprine hydrochloride and aminopromazine hydrochloride) were dissolved at concentrations ranging from 0·1 to 2 mM in a medium containing 116 mM NaCl, 2·5 mM KCl, 0·5 mM $CaCl_2$, 0·2 μc/ml $^{45}CaCl_2$. Samples (0·5 ml) of this solution were added to 1 ml of chloroform–methanol phospholipid extract; the mixture was shaken for 1 min in a cyclomixer and then centrifuged for 10 min at 2500 *g*. Aliquots of 0·2 ml of the chloroform phase were tested for radioactivity in an end window Geiger counter.

Under these experimental conditions it was observed that aminopromazine, papaverine, eupaverin and isoxsuprine inhibit the lipid-facilitated calcium transport from the aqueous to the chloroform phase. Aminopromazine is the