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A COMPARISON OF THE ACCUMULATION AND RELEASE OF ^{3}H-OUABAIN AND ^{3}H-DIGITOXIN BY GUINEA-PIG HEART MUSCLE

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The tissue uptake and excretion of radioactively labelled cardiac glycosides have been studied in whole animals and also in man (for review, see van Zwieten, 1967). There are, however, only a few reports in the literature of the accumulation of the labelled drugs by isolated organs or tissue slices. Nevertheless such studies with isolated organs would be of great interest, because the pharmacological action of cardiac glycosides can only be studied properly if various extra-cardiac factors can be ruled out. Sjoerdsma & Fischer (1951) described the accumulation of ^{14}C-digitoxin by isolated perfused hearts of various animal species. The uptake of ^{14}C-digitoxin by slices of rabbit auricles and ventricles was estimated by Godfraind & Lesne (1967). Systematic investigations of the accumulation and release of ^{3}H-digoxin by beating, isolated guinea-pig atria have recently been carried out (Kuschinsky, Lahrtz, Lüllmann & van Zwieten, 1967). In these investigations, the influence of various parameters—for example, the concentration of digoxin in the bath and the frequency of beating of the organs—has been established. The exchange process of tissue-bound digoxin against ^{3}H-digoxin in the bath has been described in a separate paper (Kuschinsky, Lüllmann, Schmitz & van Zwieten, 1967). Similar experiments on uptake, release and exchange have been carried out with ^{3}H-peruvoside, the α-thevetoside of cannogenin (Kuschinsky, Lüllmann & van Zwieten, to be published). The pharmacological and clinical properties of digoxin and peruvoside, whose kinetics of uptake and release by guinea-pig atria we have already studied, lie between those of the two "extremes," digitoxin and ouabain. Digitoxin and peruvoside belong to the intermediate category of cardiac glycosides. In animals and in man, digitoxin shows a prolonged action, whereas the duration of the effect of ouabain is comparatively short. It therefore seemed of interest to investigate the uptake and release of ^{3}H-digitoxin and ^{3}H-ouabain by guinea-pig atria. The results of these studies have also been compared with those previously obtained for digoxin and peruvoside.

METHODS

Guinea-pig isolated atria were prepared and suspended in an organ bath as previously described (Kuschinsky, Lahrtz, Lüllmann & van Zwieten, 1967). All experiments were carried out at 30° C. The calcium content of the Tyrode solution was 1.2 m-equiv/l. Throughout the experiments the

atria were stimulated electrically with a frequency of 180/min. The total radioactivity of the organs which had been treated with the tritium-labelled cardiac glycosides was determined according to the same procedure as that used in our preceding experiments with ^{3}H-digoxin.

The contraction amplitude and the influences of the various glycoside concentrations on this parameter were established in separate experiments by means of a transducer and a Helcoscriptor recording device, type HE 86-t.

^{3}H-digitoxin and ^{3}H-ouabain had been prepared according to the Wilzbach exchange procedure at the Institute for Nuclear Research at Karlsruhe. The purification of the crude radioactive materials was carried out at the Isotope Department of Merck A.G., Darmstadt.

We regularly checked the purity of the radioactive drugs, which were stored at −20° C. Thin-layer chromatography of ethanolic solutions of the radioactive compounds was carried out on glass plates, covered with an 0.2 mm layer of silica gel and $CaSO_4$. The following solvents were used for the development of the chromatograms: ^{3}H-digitoxin ethylacetate/*n*-butanol (9:1, v/v); ^{3}H-ouabain chloroform/methanol/H_2O (65:30:5, v/v). The dried chromatograms were scanned by means of a Packard Radiochromatogram scanning system. Both drugs gave rise to a single peak on the chromatogram. The following R_F values were thus obtained: ^{3}H-digitoxin 0.55; ^{3}H-ouabain 0.70. The values were identical with those of the non-radioactive glycosides subjected to thin-layer chromatography in the same conditions.

In order to determine whether the radioactive tissues contained any radioactive metabolites besides the cardiac glycosides, the atria were homogenized in 50% aqueous ethanol (approximately 1 ml./atrium). The homogenate was extracted with approximately the same volume of chloroform. ^{3}H-ouabain could not be extracted with chloroform: almost all the radioactive material remained in the alcoholic solution. ^{3}H-digitoxin, however, was transferred almost entirely to the chloroform extract. Samples of the solutions which contained the radioactive material were subjected to thin-layer chromatography and scanned as described above. In none of our experiments, even after exposure for 2–3 hr, could the formation of radioactive metabolites in the tissues be demonstrated. Consequently, the total tissue-radioactivity determined may be related directly to the ^{3}H-digitoxin or ^{3}H-ouabain content of the organs.

RESULTS

Accumulation of ^{3}H-ouabain and ^{3}H-digitoxin

The accumulation of both radioactively labelled cardiac glycosides was investigated using two different concentrations of each drug in the medium, 1×10^{-7} and 5×10^{-7} g/ml. For ouabain these concentrations are 1.7×10^{-7} and 8.5×10^{-7} M respectively, and for digitoxin to 1.3×10^{-7} and 6.5×10^{-7} M. The lower concentration of both glycosides caused a clear-cut increase of contractile force, which was not followed by contracture. The maximum positive inotropic action was reached 15–20 min after administration of either ouabain or digitoxin. The higher concentration of both drugs (5×10^{-7} g/ml.) gave rise to the development of contracture after an initial increase of the contraction amplitude. Again, the positive inotropic effect was maximal 15–20 min after drug administration.

The tissue/medium radioactivity ratio (T/M ratio) determined for both radioactive glycosides was plotted against the incubation time (Figs. 1 and 2). The uptake of ^{3}H-ouabain reached equilibrium after 20 min, whereas about 30–60 min were necessary before the accumulation of ^{3}H-digitoxin had attained this stage. For ^{3}H-ouabain, the maximal value of the T/M ratio proved to be about 0.5–0.7. The atria could accumulate a far greater relative amount of ^{3}H-digitoxin: for this glycoside, the T/M ratio at equilibrium was approximately 10. Neither the time required until equilibrium was

reached nor the maximal T/M ratio were measurably influenced by the concentration of both glycosides in the medium. Using the equation $Y=A\ (1-e^{-kt})$, where Y is the uptake at time t and A the uptake at equilibrium, the rate constant of the uptake process (k) was obtained by plotting t versus $\log \frac{A}{A-Y}$. This plot yielded straight lines for both ^{3}H-ouabain and ^{3}H-digitoxin. This implies that the uptake processes may be described by means of an exponential function. The following values were calculated for the rate constants k: ouabain 31×10^{-4} sec^{-1}; digitoxin 14×10^{-4} sec^{-1}. The half-lives ($t\frac{1}{2}$) of the uptake processes were calculated from k, using the equation $k=\frac{0.693}{t\frac{1}{2}}$. For H-ouabain $t\frac{1}{2}$ of the uptake proved about 3.7 min, and for ^{3}H-digitoxin approximately 8.25 min.

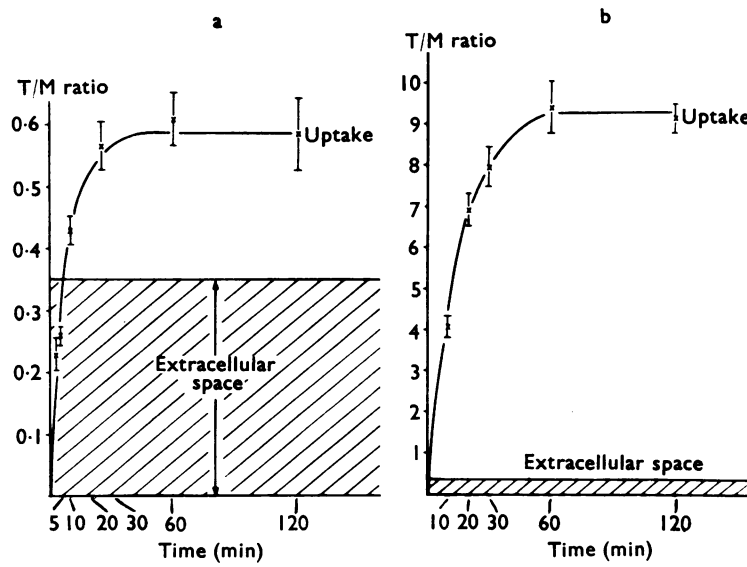

Fig. 1. Uptake of ^{3}H-ouabain (a) and ^{3}H-digitoxin (b) by isolated atria. Medium concentration of both drugs: 1×10^{-7} g/ml. The tissue/medium radioactivity ratio (T/M ratio) has been plotted against the incubation time in min. Each point in this figure represents the mean of eight to ten atria (mean ± S.E.M.). The hatched part of the figure represents the relative amount of radioactive material present in the extracellular space.

In order to emphasize the difference in tissue uptake between ^{3}H-ouabain and ^{3}H-digitoxin, the relative amount of radioactive material in the extracellular space has also been shown in Figs. 1 and 2 (hatched area). To calculate this amount we used the value for extracellular space of guinea-pig atrial tissue (0.35 ml./g), established in previous investigations (Lüllmann & van Zwieten, 1967). The tissue clearance achieved at equilibrium was calculated for both drugs at the concentrations studied (Paton & Rang, 1965). The following values were established for this parameter: ouabain 0.36 and 0.32 ml./g (bath concentrations 1×10^{-7} and 5×10^{-7} g/ml.), respectively; digitoxin 14 and 13 ml./g (bath concentrations 1×10^{-7} and 5×10^{-7} g/ml.).

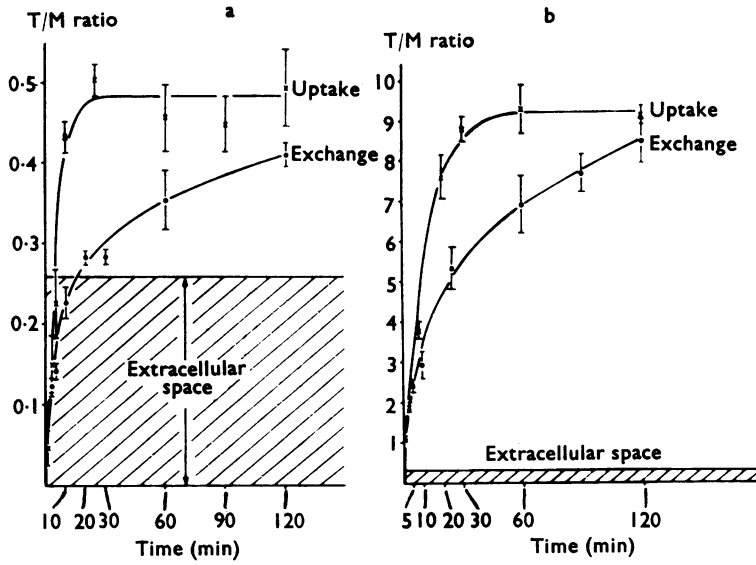

Fig. 2. Uptake of ^{3}H-ouabain (a) and ^{3}H-digitoxin (b) by isolated atria. Medium concentration of both drugs: 5×10^{-7} g/ml. The tissue/medium radioactivity ratio (T/M ratio) has been plotted against the incubation time in min. Each point on the curves represents the mean value (±S.E.M.) of at least eight atria. The hatched part of the figure represents the relative amount of radioactive material in the extracellular space. The lower curves show the uptake of the two tritium-labelled cardiac glycosides after pre-saturation of the atria with the corresponding non-radioactive drugs ("exchange").

Exchange of tissue-bound glycosides against radioactive glycosides in the medium

In order to obtain a better picture of the tissue-binding of the two cardiac glycosides, the exchange process between previously bound, non-radioactive glycosides and the corresponding, radioactively labelled drugs in the incubation medium was investigated.

Electrically driven, isolated atria were incubated as usual in Tyrode solution containing either ouabain or digitoxin (non-radioactive; bath concentration 5×10^{-7} g/ml. for both drugs). The organs were incubated for at least 20 min in the solution containing ouabain, whereas the minimal time of exposure to digitoxin was 30 or 60 min, to ensure that the uptake process had reached the equilibrium phase. The atria thus saturated with the non-labelled glycosides were subsequently transferred to Tyrode solution containing the appropriate, radioactively labelled drugs in the same concentration (5×10^{-7} g/ml.) as that used for the non-radioactive glycosides. Consequently, the exchange process has been studied at equilibrium. After determination of the tissue radioactivity as usual, the T/M ratio was plotted against the incubation time (lower curves in Figs. 2a and 2b). These figures clearly demonstrate that the uptake of ^{3}H-ouabain and ^{3}H-digitoxin occurs more slowly after preincubation of the atria with the corresponding, non-labelled glycosides. After 20 min (ouabain) or 30 min (digitoxin) of incubation the difference between the "uptake" and the "exchange" curves proved highly significant ($P<0.01$) for both drugs. If the total tissue radioactivity is known, the cellular concentration of the drugs can be calculated. This calculation was carried out for both the "uptake"

and the " exchange " processes after 2 hr of incubation in the radioactive medium, using the appropriate values for extracellular space (Lüllmann & van Zwieten, 1967). The relative amount of tissue-bound drug which had exchanged against the tritium-labelled compounds in the medium could thus be estimated. After 2 hr approximately 50–60% of the ouabain bound by the cells had exchanged against ^{3}H-ouabain in the medium. For digitoxin this relative amount was at least 80%.

For the reasons explained in DISCUSSION, it seemed of interest to study the binding of ^{3}H-ouabain by atria which had previously been saturated with dihydro-ouabain. Accordingly, the electrically stimulated atria were pre-incubated for at least 20 min in Tyrode solution which contained dihydro-ouabain 5×10^{-7} g/ml. Subsequently, ^{3}H-ouabain was added in order to study the uptake of the tritium-labelled glycoside by the atria pretreated with dihydro-ouabain. Again, the total radioactivity of the organs was determined as a function of the incubation period. In a series of simultaneous experiments, atria saturated with non-radioactive ouabain (5×10^{-7} g/ml., 20 min of exposure) were incubated for various periods in Tyrode solution which contained ^{3}H-ouabain. The uptake of ^{3}H-ouabain by these atria was much slower than that by atria which had been pre-saturated with dihydro-ouabain (Fig. 3). The uptake of ^{3}H-ouabain after pre-incubation with dihydro-ouabain approximately corresponded to

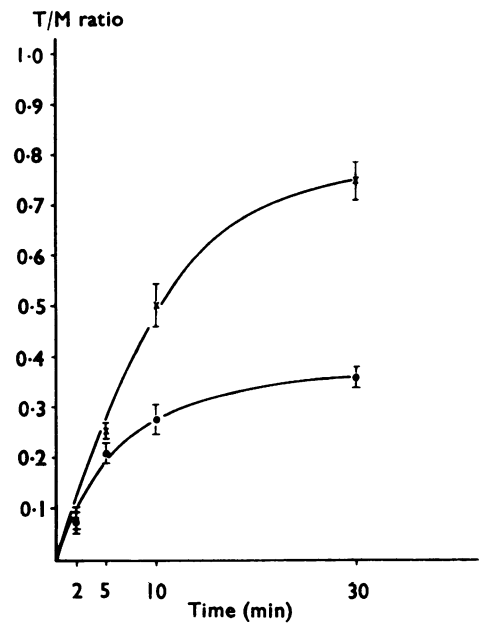

Fig. 3. Uptake of ^{3}H-ouabain by isolated atria after pre-treatment with and in the presence of dihydro-ouabain (upper curve) or non-radioactive ouabain (lower curve). After pre-saturation with either dihydro-ouabain (5×10^{-7} g/ml.) or ouabain (5×10^{-7} g/ml.) the accumulation of ^{3}H-ouabain was determined as usual. The tissue/medium radioactivity ratio (T/M ratio) has been plotted as a function of the incubation time (min) in the ^{3}H-ouabain solution. Each point on the curves represents the mean value (±S.E.M.) of eight to ten atria.

the uptake of the labelled drug by glycoside-free tissue (Fig. 2a, upper curve). The uptake of ^{3}H-ouabain after pre-saturation with ouabain corresponded to the "exchange" process (lower curve in Fig. 2a).

Release of ^{3}H-ouabain and ^{3}H-digitoxin

In order to study this process, isolated atria were initially saturated with ^{3}H-ouabain or ^{3}H-digitoxin and subsequently transferred to glycoside-free Tyrode solution. The residual radioactivity of the organs was determined after various periods of incubation in the Tyrode solution. The wash-out curves thus obtained are shown in Figs. 4 and 5. As for the uptake curves (Figs. 1 and 2), the relative size of the extracellular space has also been represented in Figs. 4 and 5. After approximately 20 min of wash-out, the initially accumulated ^{3}H-ouabain had disappeared from the extracellular space. The amount left in the tissue after this period was approximately half the original concentration. The release of cellular ^{3}H-ouabain proceeded very slowly.

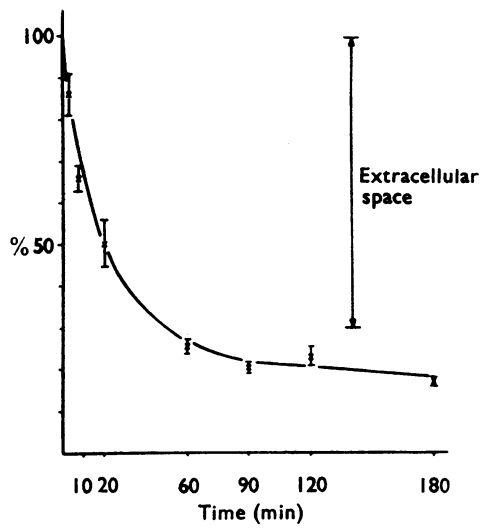

Fig. 4. Release of previously bound ^{3}H-ouabain on incubation of the atria in glycoside-free Tyrode. After accumulation of ^{3}H-ouabain until the equilibrium had been reached (medium concentration 1×10^{-7} g/ml.) the atria were transferred to glycoside-free Tyrode solution and incubated for various periods. The radioactivity of the organs was expressed as a percentage of the initial tissue radioactivity and subsequently plotted against the incubation time in the glycoside-free Tyrode solution. Each point on the curve represents the mean value (±S.E.M.) of at least ten atria. The relative amount of radioactive material which occupies the extracellular space has been represented by arrow.

^{3}H-digitoxin will probably be washed out from the extracellular space at approximately the same rate as ^{3}H-ouabain. After 20 min of incubation in glycoside-free Tyrode solution, however, as much as fifteen to twenty times the amount of ^{3}H-digitoxin present in the extracellular space had been released by the tissues. Obviously, cellular ^{3}H-digitoxin, bound by the cells in excess of ouabain, is released much more rapidly than ^{3}H-ouabain bound by cellular structures.

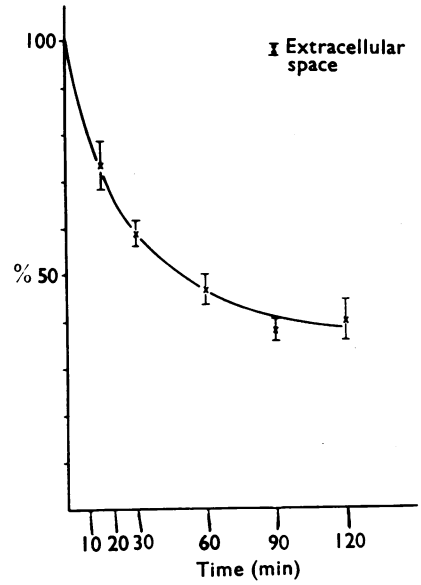

Fig. 5. Release of previously bound ^{3}H-digitoxin on incubation of the atria in glycoside-free Tyrode. After accumulation of ^{3}H-digitoxin until the equilibrium had been reached (medium concentration 1×10^{-7} g/ml.) the atria were transferred to glycoside-free Tyrode solution and incubated for various periods. The radioactivity of the organs was expressed as a percentage of the initial tissue radioactivity and subsequently plotted against the incubation time in the glycoside-free Tyrode solution. Each point on the curve represents the mean value (±S.E.M.) of at least ten atria. As in Fig. 4, the relative amount of radioactive material which occupies the extracellular space has been represented by an arrow.

DISCUSSION

The results described in the present paper have revealed striking differences between the uptake process of ouabain and digitoxin. The relative accumulation of digitoxin, achieved in the equilibrium phase of the uptake process proved as high as ten times the glycoside content of the medium. For ^{3}H-ouabain on the other hand, only 0.5–0.7 times the amount of glycoside in the medium could be recovered in the tissues. Moreover, the uptake of ouabain occurred more rapidly than that of digitoxin. The rate of the uptake process for ouabain approximately corresponds to the uptake rate of molecules of comparable size into the extracellular space and to the rate by which the positive inotropic effect of the drug develops. The accumulation of digitoxin on the other hand continues for about 60 min until it reaches equilibrium, whereas the maximal positive inotropic action of this glycoside is usually observed some 20 min after its administration. Digoxin is also taken up more slowly than the development of its pharmacological effect takes place (Kuschinsky, Lahrtz, Lüllmann & van Zwieten, 1967). Notwithstanding the very modest uptake of ouabain by the atria, the small amount of the drug present in the tissues is obviously sufficient for the development of a positive inotropic effect of the same order of magnitude as that of digoxin or digitoxin.

The development of the maximal positive inotropic action takes about 20 min for all cardiac glycosides investigated in our kinetic experiments (ouabain, digoxin, peruvoside

and digitoxin). A correlation between the uptake into the extracellular space and the time required for the development of the pharmacological effect only exists, however, for ouabain. Obviously, the small amount of ouabain, which is only a little more than the amount which occupies the extracellular space, may react rather rapidly with the "receptors" for the positive inotropic action. The small amount of ouabain which is taken up and also the rate of uptake would suggest that such receptors are located on the cell-membrane or possibly in the T-tube system as well. The penetration of cardiac glycosides through the cell-membrane may be expected to occur more slowly than the occupation of the extracellular space. Accordingly, the rate of uptake determined for digitoxin and also for digoxin would suggest that these glycosides do penetrate into the cells to a considerable extent. Moreover, this assumption is supported by the observation that digitoxin and digoxin show a much more pronounced cellular accumulation than ouabain. If indeed the ouabain bound by the cells is chiefly attached to the membrane, it would be possible to estimate the number of molecules which occupy the membrane. The cellular surface of muscular tissue may be calculated using the equation: $O_F = \frac{G-O}{S-V}(I-E)$ [cm^2], where O_F is the surface to be calculated, G = weight (in g), S = specific weight, E = extracellular space (ml./g tissue) and V/O = the ratio volume/surface of the muscle fibres. For guinea-pig atrial tissue this ratio was estimated to be about 2 μ (Klaus, Kuschinsky & Lüllmann, 1962). According to this equation a surface of about 3×10^3 cm^2 was calculated for 1 g of guinea-pig atrial tissue (wet weight). The amount of ouabain bound by the membrane corresponds to about 3×10^{-11} moles/g tissue for the lower bath concentration studied (1×10^{-7} g ouabain/ml.).

Consequently, about 10^{-14} moles of ouabain have become attached to 1 cm^2 of membrane surface. This figure may be a measure for the maximal glycoside receptor capacity of heart muscle cells. Similar calculations have been carried out for the binding of atropine to smooth muscle cells (Paton & Rang, 1965) and for that of propranolol to guinea-pig atrial cells (Potter, 1967). In accordance with the estimations made by these authors, the receptor capacity for glycosides seems to be only an extremely small part of the membrane surface. Since the surface of the biologically active part of ouabain (that is, the genin) was assumed to be approximately 10^{-14} cm^2, the amount of ouabain which is bound by the cells (10^{-14} moles/cm^2 tissue) could cover as a monolayer only 0.006% of the membrane surface. Electron microscopic studies have shown that in guinea-pig atrial tissue the T-tube system is not of particular importance (E. Lindner, personal communication). Although it cannot be denied that ^{3}H-ouabain may also be bound by these cellular structures we have not considered this possibility in our estimation of the receptor capacity. If this factor had been considered, the figure of 0.006% (membrane surface covered by ouabain) would be lower still. Both digitoxin and ouabain give rise to approximately the same increase of contractile force. If it is assumed that 1 molecule of ouabain influences the receptors to the same extent as 1 molecule of digitoxin, this necessarily implies that the greater part of the large amount of digitoxin accumulated by the cells is probably not of any importance for the pharmacological action of the drug.

Our experiments have shown that previously bound digitoxin exchanges rather easily against ^{3}H-digitoxin in the bath. For ouabain, however, this exchange occurs more slowly.

Previous investigations with ^{3}H-digoxin (Kuschinsky, Lüllmann, Schmitz & van Zwieten, 1967) suggest that the dissociation of digoxin from so far unknown cellular structures is the rate-limiting step in the exchange process. Consequently, the exchange of tissue-bound digitoxin and also that of digoxin does not allow any conclusions concerning the binding of the cardiac glycosides to the specific receptors on the membrane. The relatively small amount of digitoxin bound to the receptors cannot be expected to influence the exchange phenomenon to a measurable extent. In case of ouabain on the other hand, the greater part of which is probably attached to the receptors, the exchange process might reflect the kinetic behaviour of the drug at these receptors. The much slower exchange of ouabain, provided the extracellular space is excluded, would support this hypothesis.

If indeed the binding of ^{3}H-ouabain to the specific receptors were correlated to the pharmacological effect, pretreatment of the organs with a compound, structurally related to ouabain but without pharmacological action at the particular concentration should not impair the binding of ^{3}H-ouabain. Chemically, dihydro-ouabain is very similar to ouabain, although it does not possess any positive inotropic or other cardiac action in the concentration used. Moreover, the pharmacological effect of ouabain is not impaired by dihydro-ouabain. It thus seems fairly certain that dihydro-ouabain does not occupy the specific glycoside-receptors after treatment of isolated atria with this compound. Pre-saturation of the atria with dihydro-ouabain did not cause any impairment of the ^{3}H-ouabain-uptake (Fig. 3); the uptake curve obtained after pretreatment with dihydro-ouabain corresponded to the uptake curve, determined for atria which were devoid of dihydro-ouabain. Pretreatment with non-radioactive ouabain, however, provoked a significant impairment of the ^{3}H-ouabain uptake, because in this case the receptors had been occupied already by the cardiac glycoside. The experiments with dihydro-ouabain confirm the hypothesis that most (or all) of the ouabain which exchanges slowly against medium-^{3}H-ouabain is indeed responsible for the pharmacological effect. It was estimated that only some 3% of the accumulated digitoxin would occupy the specific receptors. The remaining 97% would then be redundant for the pharmacological action. For digoxin the relative amount which occupies the receptors was estimated to be about 10% (Kuschinsky, Lahrtz, Lüllmann & van Zwieten, 1967) and for peruvoside about 8% (Kuschinsky, Lüllmann & van Zwieten, to be published). On the other hand, all or almost all the bound ouabain may be necessary for the positive inotropic action.

Until now, few morphological studies on the cellular localization of digoxin or digitoxin have been reported in the literature (Conrad & Baxter, 1964; Fozzard & Smith, 1965; Harvey & Pieper, 1955). As stated above, the greater part of the digoxin or digitoxin accumulated is of no importance for the pharmacological action of these drugs. Consequently, morphological studies on the localization of digitoxin or digoxin within the cell do not allow any relevant conclusions concerning the binding of these drugs to specific receptors.

The greater part of the cellular ^{3}H-ouabain was released from the extracellular space in about 20 min. The release of ^{3}H-ouabain bound to cellular structures, however, proved much slower. This observation is in agreement with the slow exchange of tissue-bound ouabain against ^{3}H-ouabain in the medium. The wash-out of ^{3}H-digitoxin from the extracellular space is negligible in comparison with the release of the drug bound to

cellular structures, because such a small relative amount of drug fills the extracellular space. The release from cellular structures occurs much more rapidly than the detachment of ^{3}H-ouabain, which is probably bound to specific receptors to an important degree.

Although our investigations have shown that the release of ^{3}H-ouabain from the heart occurs rather slowly, this process is still more rapid than the total elimination of ^{3}H-ouabain by humans. According to Marks, Dutta, Gauthier & Elliott (1964), the slower component of elimination of ^{3}H-ouabain from human plasma has a half-life of approximately 5 hr, whereas the release from isolated guinea-pig atria certainly occurs considerably faster. Moreover, ^{3}H-ouabain is scarcely bound to plasma proteins (Kuschinsky, 1968), so the dissociation of ^{3}H-ouabain from proteins cannot be the rate-limiting factor in the elimination process either.

Because in addition, the biliary excretion of ^{3}H-ouabain is probably of little importance (Marks, Dutta, Gauthier & Elliott, 1964) the excretion of ^{3}H-ouabain by the kidney seems to be the rate-limiting step in the elimination process. We also believe that for the other glycosides (digoxin, digitoxin, peruvoside) the kidney excretion is the rate-limiting step. During renal insufficiency, both ^{3}H-digoxin (Doherty, Perkins & Wilson, 1964) and ^{3}H-peruvoside (Lahrtz & van Zwieten, 1968) disappear from the plasma more slowly than in patients with healthy kidneys. These clinical findings also suggest that urinary excretion determines the rate of plasma elimination for cardiac glycosides.

The present studies have enabled us to compare the binding to heart muscle tissue of a few clinically important cardiac glycosides with different kinetic properties. We have thus been able to demonstrate that the amount of drug taken up by the cells differs greatly for the various glycosides, whereas the amount of drug necessary for the pharmacological effect is probably about the same. In spite of the considerably different kinetic properties, the pharmacological effects of these drugs are similar when studied on isolated heart muscle preparations. A relatively modest amount of ouabain is bound by the cells, but most or perhaps all of this " specifically " bound ouabain seems to be essential for the pharmacological effect. Digitoxin, on the other hand, is accumulated to a considerable extent by cardiac muscle, but only a small part of the accumulated drug is responsible for the pharmacological action.

SUMMARY

1. The accumulation and release of ^{3}H-ouabain and ^{3}H-digitoxin by electrically stimulated guinea-pig atria have been compared. The exchange of tissue-bound glycoside (non-radioactive) against tritium-labelled glycoside in the medium has also been studied for both drugs.

2. The uptake of both tritium-labelled drugs may be described by means of an exponential function. ^{3}H-ouabain is taken up more rapidly than ^{3}H-digitoxin. The atria could accumulate a far greater relative amount of ^{3}H-digitoxin. For this glycoside the tissue/medium radioactivity ratio at equilibrium was about 10, whereas for ^{3}H-ouabain this value proved as low as 0.5–0.7.

3. After preincubation with non-radioactive glycosides, the corresponding ^{3}H-glycosides are taken up more slowly than by glycoside-free atria. At equilibrium, approximately 50–60% of the ouabain bound by the cells has exchanged against ^{3}H-ouabain in the medium after 2 hr. For digitoxin this figure was at least 80%.

4. After pre-saturation of the atria with dihydro-ouabain, no change of the ^{3}H-ouabain uptake could be detected.

5. The release of cellular ^{3}H-ouabain proceeds more slowly than that of cellular ^{3}H-digitoxin.

6. It is suggested that ouabain becomes bound chiefly to specific receptors on the membrane surface, whereas the greater part of the relatively larger amount of accumulated digitoxin is bound within the cells. Accordingly, most of the digitoxin taken up is not of any importance for the positive inotropic action of this drug. On the other hand all or almost all the bound ouabain may be necessary for the pharmacological action.

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