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REVIEW

The kinetic behaviour of cardiac glycosides *in vivo*, measured by isotope techniques

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The usual clinical doses of cardiac glycosides are low (0·002–0·02 mg/kg) because of their potent pharmacological activity and relatively narrow therapeutic range. Accordingly, the classical analytical procedures are not sensitive enough for the quantitative determination of the low concentrations of these drugs in biological material. The introduction of radioactively labelled cardiac glycosides, however, has greatly facilitated investigations of the fate of therapeutic amounts of the drugs in the organism and also enabled kinetic studies in isolated organ systems to be made. The radiochemical analysis has proved so sensitive that the kinetic behaviour of cardiac glycosides even in *subthreshold* dosage or in concentrations, devoid of any pharmacological or toxic action, can be examined. Such investigations have led to a better understanding of the kinetic and clinical properties of these valuable drugs.

In earlier reviews (Rothlin & Bircher, 1954; Wright, 1960; Zwieten, 1967) comprehensive compilations of references on the subject have been given. The present paper aims rather to present a critical appreciation of current development in this field, partly based on our own experience. At first, the kinetic behaviour of the drugs in isolated organ systems will be discussed. Investigations in organs, incubated in Tyrode solution will be emphasized, but also studies made in isolated organs incubated in oxygenated whole blood will be considered. *In vivo* studies on the fate of radioactively labelled cardiac glycosides have been made in animals and in man. Distribution, absorption, elimination and metabolism may thus be examined under normal and also pathological circumstances. The sequence: isolated organs (Tyrode solution) $<$ isolated organs (whole blood) $<$ animals $<$ man shows an increasing complexity of the systems studied, although it should be recognized that conclusions drawn from these studies are, at the same time valuable in throwing light on the behaviour of cardiac glycosides under clinical circumstances.

Kinetic studies on isolated atria, incubated in oxygenated Tyrode solution

Electrically driven isolated atria, suspended in oxygenated Tyrode solution provide a simple system for the determination of kinetic properties of drugs under reproducible circumstances, the number of variables in the system being limited and well-defined. Moreover, the influences of, for example, changes in drug concentration, frequency of beat, and ion concentration on the kinetic behaviour may be conveniently examined in such an experimental system. The results probably allow relevant conclusions about the kinetic behaviour of the drugs in human heart muscle tissue if the experiments are made with atria of the guinea-pig, a species that shows pronounced sensitivity to cardiac glycosides. Although Sjoerdsma & Fischer (1951) studied the uptake of [^{14}C]digitoxin by isolated perfused hearts of the rabbit, systematic investigations on

uptake and release of the various cardiac glycosides by isolated heart muscle preparations have only been done recently. In isolated atria kept in Tyrode solution, the kinetic properties of 4 different glycosides were established: [^{3}H]digoxin (Kuschinsky, Lahrtz, & others, 1967; Kuschinsky, Lüllmann & others, 1967); [^{3}H]ouabain and [^{3}H]digitoxin (Kuschinsky, Lüllmann & Zwieten, 1968a); peruvoside (= α-thevetoside of cannogenin) (Kuschinsky, Lüllmann & Zwieten, 1968b). These experiments were extended to [^{3}H]digitoxigenin to establish whether the sugar moieties in the glycoside molecules are pertinent to their kinetic and pharmacological properties (Kuschinsky, Lüllmann & Zwieten, 1968c).

The uptake of the four glycosides and the aglycone showed some similarity. For all five substances the uptake process reached an equilibrium phase after a given time of incubation. The time course of the uptake could be described algebraically by means of e-functions; that is to say, the well-known equation $Y=A\,(1-e^{-kt})$ could be applied, Y being the uptake at time t, A the uptake at equilibrium and k the rate constant. Apart from k, the tissue/medium (T/M) radioactivity ratio at equilibrium is also a parameter of particular importance for the character of the uptake process since it allows a direct comparison of the relative accumulation of the various compounds examined. The rate constants (k), the half lives ($t_{\frac{1}{2}}$) and also the T/M ratios obtained for "therapeutic" medium concentrations of the glycosides and the aglycone are listed in Table 1. [^{3}H]Ouabain is seen to be taken up much more rapidly than the other drugs. The rate of the ouabain uptake process suggests that the drug is restricted mainly to the extracellular space, since its uptake rate is similar to that of the uptake rate of molecules of similar size in this compartment (Lüllman & Zwieten, 1967). The other glycosides and the genin also penetrate into the cell and become attached to intracellular structures (see literature quoted above).

Table 1. *Rate constants* (k), *the half lives* ($t_{\frac{1}{2}}$) *and also the* T/M *ratios obtained for therapeutic medium concentrations of the glycosides and the algycone*

Drug	Medium concn (g/ml)	Positive inotropic action (%)	Rate constant k s^{-1} ($\times 10^{-4}$)	$t_{\frac{1}{2}}$ min	T/M
[^{3}H]Ouabain	5×10^{-7}	132	31·0	3·7	0·6
[^{3}H]Digoxin	$2{\cdot}5 \times 10^{-7}$	100	5·0	23·1	2·8
[^{3}H]Peruvoside	$2{\cdot}5 \times 10^{-7}$	120	5·2	22·2	3·2
[^{3}H]Digitoxin	1×10^{-7}	90	14·0	8·3	9·3
[^{3}H]Digitoxigenin	1×10^{-7}	150	5·7	20·0	8·0

Although the pharmacological effects of the various drugs in the given concentrations occur within the same range (compare the relative increases of the contractile force in Table 1), there are quite large differences between the T/M ratios achieved at equilibrium. Whereas the ouabain content of the atrial tissue is but 60% of that in the medium, a more than nine-fold accumulation relative to the concentration of the bath fluid was seen with digitoxin. The decreasing polarity in the sequence: ouabain $>$ digoxin $>$ digitoxin $\approx$ digitoxigenin (Waldi, 1962) is obviously accompanied by an increased cellular accumulation. This finding would suggest that an important part of the cardiac glycosides (and the aglycone) that accumulate is probably bound to lipid-, and protein-containing, structures of the cells. The binding of the labelled drugs to serum proteins also increases in the same sequence: no measurable protein binding could be demonstrated for ouabain, whereas about 80% of the [^{3}H]digitoxin

or its genin are firmly attached to serum proteins (Scholtan, Schlossmann & Rosenkranz, 1966; Kuschinsky, 1968). Moreover, previous removal of lipid material from atrial tissue upon extraction with aqueous glycerol reduced the T/M ratio for [^{3}H]-digitoxin to approximately 4·5 without changing that obtained for [^{3}H]ouabain (0·5) (Kuschinsky, Lüllman & Zwieten, 1968d). Obviously the removal of lipid material reduces the binding capacity of the tissues for digitoxin without affecting the size of the compartment that contains ouabain.

If we assume that a certain degree of receptor occupation is related to a given positive inotropic action and that this degree will be about the same for all the glycosides used in our experiments, the large differences in T/M ratio achieved suggest that not all of the accumulated drug is involved in the therapeutic effect. In other words, the higher the relative accumulation, the larger the amount of drug that is bound to those cellular structures or dissolved in those cellular lipids that are not involved in the development of the pharmacological effect. For [^{3}H]digitoxin and its aglycone, especially, the major part of the amount taken up is probably bound to or dissolved in cellular compartments that are not involved in drug action. For [^{3}H]ouabain the picture seems to be different: the relatively small amount of drug bound by the tissue is probably of vital importance for the pharmacological effect. Concomitantly, [^{3}H]ouabain probably combines to a major degree with specific "receptors", necessary for the development of positive inotropic action. Such "receptors" will probably be accessible from the extracellular space quite easily, since [^{3}H]ouabain is mainly contained in the extracellular space (see below). The "receptors" might for instance be located on the outward membrane or otherwise in the T-tube system, the latter being accessible from the extracellular space. If the membrane location of the "receptors" is assumed, these structures would occupy but 0·006% of the membrane surface (Kuschinsky, Lüllmann & Zwieten, 1968a).

The comparison between the uptake process of the cardiac glycosides and their pharmacological effect has provided some information about the existence of glycoside "receptors" and cellular structures to which an unspecific binding of the drugs takes place. It also seemed of interest to compare the release of initially bound [^{3}H]glycosides (or aglycones) with the disappearance of the pharmacological effect during wash-out with glycoside-free Tyrode solution (Lüllmann, Weber & Zwieten, 1968). The release processes could not be described by means of e-functions, since the rate constant gradually decreased upon prolonged incubation. It drew the attention, however, to the fact that the loss of [^{3}H]labelled glycosides from the tissue occurred at roughly the same rate for each of the five drugs studied: after a wash-out period of 2 h about 40–50% of the initially accumulated drugs had been released by the tissues. The disappearance of the positive inotropic effect, however, took place far more rapidly, no measurable effect being left after 15–20 min of wash-out for ouabain, digoxin or digitoxin. The positive inotropic effect of digitoxigenin disappeared after only 5 min, although the total tissue concentration determined by isotope studies was 50% of its original value 2 h after wash-out. There seemed to be no correlation between the disappearance of the pharmacological effect and the reduction in total tissue issue concentration of all 4 drugs studied. The disappearance of the positive inotropic action must be a reflection of the dissociation of the glycoside or aglycone molecules from the "receptors". Since these "receptors" are easily accessible from the extracellular space, it might be assumed that the diffusion of the glycoside molecules from this space would be the rate-limiting step in the wash-out process of the

pharmacological effect. Indeed, experimental evidence is available to show that the diffusion of the glycoside molecules from the extracellular space largely determines the rate by which the positive inotropic effect is washed out, although the major part of the glycosides bound to cellular structures or dissolved in cellular lipids disappears more slowly (Lüllmann & others, 1968). These findings once more confirm that, particularly with digitoxin and its genin but also with digoxin and peruvoside, by far the greater part of the accumulated drug plays no part in the pharmacologic effect. Obviously, the differences in "fixation" to heart muscle tissue, postulated by clinicians to explain the differences in loss of activity per day for the various glycosides does not really exist. The different clinical decay rates of ouabain, digoxin and digitoxin must be caused by differences in the elimination rate, a parameter that is governed by a much longer half life than the "wash-out" process from myocardial tissues.

Kinetic studies on isolated atria, incubated in oxygenated whole blood

The use of oxygenated blood for the incubation of isolated organs has given rise to insurmountable experimental difficulties like excessive foam formation and haemolysis. However, with the aid of a specially designed oxygenator, Lüllmann, Peters & Zwieten have developed an experimental procedure allowing them to incubate beating guinea-pig atria and other organs in circulating, oxygenated whole blood of the same species. No significant haemolysis occurred over several hours and the usual serum electrolyte concentrations were also maintained. Foam formation could be avoided. This newly developed technique proved convenient for kinetic studies with radioactively labelled cardiac glycosides. Of course, this method more closely approaches *in vivo* conditions than does the incubation of isolated atria in Tyrode solution. In whole blood both the binding of the glycosides to serum proteins and also the presence of erythrocytes give rise to a more complicated, although a more realistic, over-all picture. On the other hand, elimination by either the liver or kidneys cannot occur, thus metabolic degradation hardly takes place. Therefore, these processes cannot interfere with the partition of the radioactively-labelled drug over the various biological compartments within the given system.

It can be demonstrated that both [^{3}H]digitoxin and its aglycone are taken up by erythrocytes, much less, however, than by atrial tissue. Neither [^{3}H]ouabain nor [^{3}H]digoxin are taken up by the erythrocytes (Lüllmann, Peters & Zwieten, to be published).

At equilibrium, the following apparent T/M radioactivity ratios were reached for the partition between the blood and the isolated atria: [^{3}H]ouabain 0·52; [^{3}H]digoxin 1·25; [^{3}H]digitoxin 1·12; [^{3}H]digitoxigenin 0·61 (serum = 1). These values are clearly different from those obtained in the experiments with oxygenated Tyrode solution as a medium. However, the binding of the drugs to serum proteins (mainly albumin) should be taken into consideration. By means of the Sephadex gel filtration method, Kuschinsky (1968) obtained the following values for the amount of *free* drug in guinea-pig serum: ouabain $\approx$ 100, digoxin 70, digitoxin 12, digitoxigenin 8%. These determinations were made with [^{3}H]labelled drugs in "therapeutic" concentrations. If the ratio of glycoside concentration in the tissues to *free* glycoside concentration in the serum is calculated, the following "true" T/M values are obtained: ouabain 0·52, digoxin 1·79, digitoxin 9·32, digitoxigenin 7·63. These ratios are in satisfactory agreement with the T/M ratios obtained in Tyrode solution as the medium of incubation (see Table 1). It is evident, then that the cardiac glycosides are taken up by heart

muscle tissue only when they are available in the free form, that is to say, not bound to serum proteins. The same conditions may be expected to hold true for the partition of cardiac glycosides administered to man. The pharmacologically *active* concentration of the different glycosides probably lies within the same range.

Fate of [^{3}H]*labelled glycosides in animals and man*

The distribution of radioactively labelled cardiac glycosides between various organs has been investigated in a number of animal species (Repke, 1958; Gonzalez & Layne, 1960; Bretschneider, Doering & others, 1962; Dutta, Marks & Smith, 1963; Marks, Dutta & others, 1964; Fauconnet & Widmer, 1965; Lüllmann & Schaum, 1968) In most of these studies no particular affinity of the cardiac glycosides for heart muscle tissue could be demonstrated. No particular accumulation in heart muscle tissue was observed, since the highest concentration was found in the excretion organs like liver, colon and kidneys. However, myocardial tissue accumulated somewhat more of the glycosides than did striated or smooth muscle.

Virtually the same observations were made by Okita, Talso & others (1955) for the distribution of [^{14}C]digitoxin, administered to moribund patients shortly before death. A five-fold accumulation in the serum level, however, was found for [^{3}H]-ouabain in human auricle tissue obtained by biopsy (Marks & others, 1964). The usually modest accumulation of [^{3}H]labelled cardiac glycosides *in vivo*, both in animals and man is in agreement with the unpublished observations of Lüllmann, Peters & Zwieten on the accumulation of the drugs by isolated atria, incubated in circulating, oxygenated whole blood (p. 4).

From clinical experience it is well known that the enteral absorption of cardiac glycosides shows large differences for the various drugs: thus, whereas digitoxin is absorbed completely, the absorption of ouabain is particularly uncertain and in most cases negligible. These clinical observations confirmed early animal studies with the Hatcher procedure (for reviews see Rothlin & Bircher, 1954; Wright, 1960). In recent experiments (Lahrtz, Sattler & Zwieten, 1968) it was shown that in the cat, *intraduodenally* applied [^{3}H]labelled digitoxin reached a much higher serum level than did [^{3}H]digoxin by the same route. [^{3}H]Ouabain on the other hand gave widely varying though usually low serum levels when administered in this manner. No measurable radioactivity could be demonstrated in the serum of human subjects who had been given an oral, therapeutically subthreshold dose of [^{3}H]ouabain. The low urine radioactivity in these subjects indicated that about 0·5–2% of the given dose had been absorbed (Lahrtz, Sattler & Zwieten, 1968). These isotope studies in both animals and man have confirmed the empirically obtained clinical experience about the absorption of cardiac glycosides—the uselessness of oral administration of ouabain is once more emphasized.

The time course of the serum radioactivity after intravenous or oral administration of radioactively labelled cardiac glycosides has been studied in animals (Harrison, Brandenburg & others, 1964; Katzung & Meyers, 1965; Abel, Luchi & others, 1965; Lüllmann & Schaum, 1968) and also in man (Okita & others, 1955; Doherty, Perkins & Mitchell, 1961; Doherty & Perkins, 1962; Marcus, Pavlovitch & others, 1967). Usually, serum concentration of the cardiac glycosides was found to decline by at least two e-functions, the first process being much faster than the second one. The exact significance of these two phases is not yet known. Possibly the first phase may represent the rapidly occurring uptake of the [^{3}H]cardiac glycosides in the blood by

the various organs, whereas the second phase might reflect the elimination process. The rate of the elimination is *not* determined by the partition of the drug between blood and tissues, but by the kidney or the liver, or both. The presumed tissue "fixation" to the heart does *not* determine this rate. During renal failure the excretion of cardiac glycosides is retarded (Doherty, Perkins & Wilson, 1964; Doherty & Flanigan, 1967; Lahrtz & Zwieten, 1968a, 1968b). However, the exact mechanism of the retarded excretion is not fully understood (Lahrtz & Zwieten, 1968b). Although in man ouabain is almost entirely excreted in the urine (Lahrtz & Zwieten, 1968b), [^{3}H]-digitoxin and its metabolites are eliminated via kidneys and liver (Lahrtz & Zwieten, unpublished). The excretion via the kidney by glomerular filtration may be expected to become preponderant if the binding to serum proteins of the glycoside in question is low. Most of the protein-bound glycoside becomes attached to serum albumin, a molecule that normally cannot be filtered by the glomerular system.

Impairment of the glycoside excretion via the liver as a result of a pathologically reduced biliary flow does not seem to give rise to an increased tissue concentration in patients (Marcus & Kapadia, 1964; Lahrtz & Zwieten, 1968a, 1968b). Accordingly, no accumulation of cardiac glycosides can be expected during liver disease, in contrast to the observations made in patients suffering from renal failure. Again, the isotope studies have confirmed previous clinical observations.

CONCLUSIONS

The kinetic experiments with tritium-labelled cardiac glycosides in isolated atria incubated either in oxygenated Tyrode solution or in oxygenated whole blood have shown that the distribution of the drugs between the various compartments in the *in vitro* system occurs rapidly. Since this rate of distribution is much higher than that of the drug elimination *in vivo*, the latter process exclusively determines the decay rate *in vivo*. The elimination depends rather upon the activity of the excretion organs like the kidney and the liver. The kinetic behaviour of cardiac glycosides in heart muscle is not primarily responsible for the duration of the cardiac effect elicited by these drugs.

The different T/M ratios obtained for the four cardiac glycosides and for the genin in isolated atrial tissue, incubated in an aqueous, protein-free medium suggest that their distribution between the various compartments within the cell differs widely from drug to drug. With those glycosides achieving a high T/M ratio at equilibrium, most of the accumulated glycoside seems to be bound to cellular structures or is present in compartments that have nothing to do with the positive inotropic effect. A small part of the molecule (e.g. digitoxin or its aglycone), however, combines with more specific "receptors" that are obviously involved in the development of the positive inotropic glycoside effect. With ouabain, the *major* part of the tissue bound drug combines with these "receptors", that are probably located on the outward membrane surface or may also be in the T-tube system. The kinetic studies with [^{3}H]ouabain suggest that the "receptors" must be easily accessible from the extracellular space. The combination of the drugs with the "receptors" probably reaches the equilibrium phase quite rapidly.

In vivo, and also in isolated atria incubated in oxygenated whole blood, the relative accumulation of the glycosides by the tissues *apparently* shows little difference for the drugs studied. However, if the protein-binding of the drugs is considered, a different picture emerges: if the amount of *free* glycoside in the blood is taken as a base for the calculation of the T/M ratios at equilibrium, approximately the same values are

obtained as for isolated atria, incubated in Tyrode solution where protein-binding cannot occur. Concomitantly, cardiac glycosides are taken up by heart muscle in accordance with the concentration of free and not protein bound, molecules.

In vivo, the protein-binding of the cardiac glycosides may be expected to influence their elimination through the kidneys. The protein-bound drugs cannot pass through the glomerular system; on the other hand, if free glycosides have passed the glomerular system, part of the molecules may be subject to reabsorption in the tubular system, provided the compound in question is lipid soluble, as for example is digitoxin.

The main conclusion that may be drawn from the kinetic investigations is probably the fact that no particular "fixation" of the drugs to heart muscle takes places *in vivo*. Although this parameter is frequently discussed by clinicians, there seems to be no experimental evidence for its real existence. The different properties of the various cardiac glycosides *in vivo* are most probably caused by differences in *elimination* or metabolic degradation or both, and not by a different kinetic behaviour towards myocardial tissues.

SUMMARY

In this review a critical evaluation of current developments in kinetic research on the behaviour of cardiac glycosides both *in vitro* and *in vivo* is given. The results recently obtained in this field suggest that in heart muscle tissue digitoxin and to a lesser extent digoxin predominantly combine with cellular structures that are not immediately involved in the development of the pharmacological effect. Only a part of the drugs combine with specific "receptors", and thus give rise to positive inotropic action. Probably, these "receptors" are easily accessible from the extracellular space which may be the membrane surface or T-tube system. Ouabain, however, chiefly combines directly with these "receptors". Thus, the different properties of the various cardiac glycosides *in vivo* are probably caused by differences in elimination or metabolism and not by a different kinetic behaviour towards heart muscle tissues. The kinetic studies suggest that *in vivo* no particular "fixation" of these drugs to the heart really takes place.

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