Br. J. Pharmac. (1976), **56**, 437–441

THE UPTAKE AND SUBCELLULAR DISTRIBUTION OF RADIO-LABELLED METABOLITES OF DIGITOXIN IN THE GUINEA-PIG ISOLATED PERFUSED HEART

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1 Comparisons were made of the uptake and inotropic effects of concentrations of 0.1 μM of digitoxin and its cleavage products digitoxigenin-bis-digitoxoside, digitoxigenin-mono-digitoxoside and digitoxigenin in the isolated perfused hearts of guinea-pigs.

2 Digitoxin produced the greatest inotropic responses in this series, while the sequence of cleavage products produced progressively smaller responses.

3 The uptake of digitoxin was significantly higher than that of the three metabolites, and the uptake of metabolites became progressively less with cleavage. The highest binding in each case was found in the microsomal fraction.

4 The uptake of all four digitaloids was reduced when the potassium in the perfusion medium was increased.

Introduction

The importance of the sugar component for the pharmacodynamic properties of the cardiac glycosides has been emphasized by Repke (1963). The sugars linked directly to digitoxigenin are desoxy sugars. Digitoxin undergoes stepwise cleavage yielding the corresponding genin, although *in vivo* the genin cannot usually be detected, possibly because the biotransformation as well as excretion of the released genin is far more rapid than the cleavage of the glycosides (Repke, 1959a,b; Lauterbach & Repke, 1960). In *in vivo* studies, Repke (1963) demonstrated that when digitoxin was injected in rats, in 10 min almost 5% of the material recovered from the liver was digitoxigenin-bis-digitoxoside, after a loss of one sugar molecule. The sugars alone are without any cardiotonic action (Straub, 1916) while the genins appear to be biologically active with a shorter latent period than their glycosides (Walther, 1943; Modell, Kwitt, Dayrit, Shane & Gold, 1948).

Considering the possibility of digitoxin cleavage, therefore, a systematic study was undertaken to compare the properties of digitoxin with those of digitoxigenin bis-digitoxoside, digitoxigenin mono-digitoxoside and digitoxigenin by evaluating their uptakes by perfused guinea-pig heart, their distribution in the subcellular ventricular fractions and their cardiotonic effects. Normal and high-potassium media were used.

Methods

Studies on the isolated perfused hearts of guinea-pigs were carried out with various digitoxosides of digitoxigenin, obtained from Boehringer. These were randomly tritiated by New England Nuclear Corp. using the catalytic exchange process. [^{3}H]-digitoxin was purchased from New England Nuclear Corp. All these tritiated derivatives were subjected to liquid/liquid chromatography (Repke, 1959a) which revealed one primary radioactive spot for each compound giving a R_F value characteristic for the unlabelled standards. The uptake of these glycosides by the heart tissue and their distribution in the various subcellular components was studied using the perfusion technique described by Dutta, Goswami, Lindower & Marks (1968). Langendorff perfusion of guinea-pig hearts was carried out using a peristaltic pump to deliver 4.5 ml/min of Krebs-Henseleit buffer (K-H) at 28°C (pH 7.4) through the cannulated aorta. The atria were removed to establish an idioventricular rhythm of 60-80 beats per min after which the heart was paced by bi-polar electrodes on the ventricle using threshold voltage to drive at 120 beats/minute. After

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an adequate period of equilibration, the preparation was observed for a control period of 30 min following which the medium was changed to an identical one containing a concentration of 0.1 μM of the tritiated cardiac glycoside under study.

In order to make an evaluation of the electrical and mechanical performances of the perfused heart on a four-channel polygraph, continuous records were maintained of these parameters: (1) isometric developed tension (dT) from a constant resting tension of 5.0 g recorded from the apex of the heart using a Grass force displacement transducer FTO3C; (2) the first derivative of the dT (df/dt); (3) the perfusion pressure using a Statham transducer; (4) the surface electrocardiogram. (1) was used for the comparison of drug effects, (2) paralleled (1) and is not reported separately, and (3) and (4) provided control information.

Drug perfusion was continued for 64 min at the end of which normal K-H was perfused for 8 min to wash out the drug from the vascular and interstitial spaces so that the cardiac drug content measured would primarily represent the cellular accumulation of the drug. The effect of increased levels of potassium on the uptake of these glycosides was determined by perfusing other groups of hearts for 64 min with a concentration of 0.1 μM of the tritiated drug in K-H modified to contain 1.5 times the normal concentration of potassium (8.7 mM). These perfusions also were preceded by a control period of 30–40 min and followed by an 8 min wash-out period in normal K-H.

At the end of the wash-out, the ventricular tissue was homogenized in sucrose-disodium edetate (EDTA) to obtain a 10% suspension. The various subcellular fractions—nuclear, mitochondrial, microsomal and supernatant fractions were obtained by differential centrifugation as described by Dutta & Marks (1969). The tritiated drug content in the several fractions and in the total heart homogenate was counted in a liquid scintillation counter. The protein content of aliquots of each fraction and of the homogenate was estimated by a biuret method (Gornall, Bardawill & David, 1949), so that the concentration of the drug in the various fractions could be expressed as picomoles/mg protein. Four hearts were used for each drug and control perfusion experiment. The criterion for significance in all experiments was taken as a probability of chance occurrence of less than 5% ($P < 0.05$).

Results

Experiments with digitoxigenin and its digitoxosides in normal K-H buffer

Figure 1 presents the sequence of events during the time course of the perfusion with digitoxigenin and its various digitoxosides. Digitoxin produced a mean

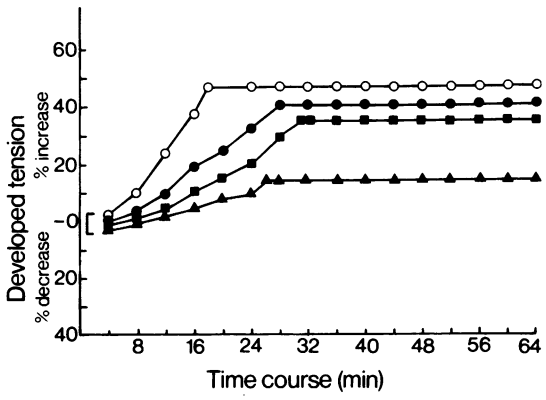

Figure 1 The developed tension (dT) during the 64 min perfusion of isolated hearts of guinea-pigs with digitoxin or its metabolites (all 0.1 μM) in normal Krebs-Henseleit buffer at a flow rate of 4.5 ml/min and temperature of 28°C. (○) Digitoxin; (●) digitoxigenin bis-digitoxoside; (■) digitoxigenin mono-digitoxoside; (▲) digitoxigenin. Each plot denotes mean value from 4 hearts.

positive inotropic response of 48.7 ± 4.6% over the control, reached in 18–20 min and maintained until the end of the drug-perfusion period. The digitoxigenin bis-digitoxoside produced a 41 ± 3.7% response in 28 min, the mono-digitoxoside a 36 ± 4.3% response in 31 min and digitoxigenin produced the least effect of 14 ± 1.6% in 26 min; with all the drugs, plateaus of increased developed tension were maintained throughout the remainder of the drug perfusion period.

The total uptake by the heart of digitoxin was 70% greater than that of the three metabolites, there being no significant difference among the quantitative uptakes of the metabolites (Table 1). The overall distribution of the drugs between the particulate and supernate fractions calculated as the supernate/pellet ratio showed a range between 0.5 to 0.77 for the four drugs, with digitoxin having the highest ratio and mono-digitoxoside the lowest ratio. Subcellular distribution showed the highest concentrations of binding in the microsomal fraction. The digitoxin accumulation in the microsomal fraction was again significantly higher than that of the metabolites, with no distinct difference among the three metabolites.

Experiments with digitoxosides in high potassium K-H buffer

Control experiments were done without any drug, but with increased potassium (1.5 × normal = 8.7 mM) in the perfusing medium for the full perfusion period of 64 minutes. On changing the perfusion medium from normal K-H to that of K-H with increased potassium at 0 time, there was a fall of 46% in the isometric

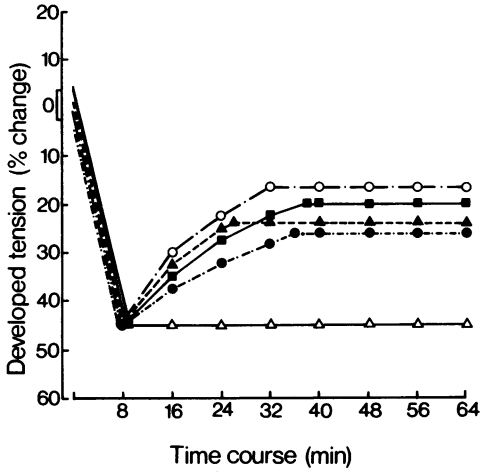

Figure 2 The developed tension (dT) of the isolated perfused hearts of guinea-pigs on changing the perfusion medium from normal Krebs-Henseleit to one with increased potassium (× 1.5) and the effect of adding digitoxin or its metabolites (all 0.1 μM) 8 min later. The flow rate and temperature were as in Figure 1. (△) Control curve for Krebs-Henseleit buffer with increased potassium; (○) digitoxin; (●) digitoxigenin bis-digitoxoside; (■) digitoxigenin mono-digitoxoside; (▲) digitoxigenin. Each plot denotes mean value from 4 hearts.

developed tension. This occurred within 8 min, after which the new plateau was maintained (Figure 2). At the end of 64 min, the 8 min wash-out with normal K-H restored the dT to control levels promptly in all experiments (not illustrated). The plateau obtained during the perfusion of high potassium K-H served as a measure to evaluate changes in dT induced by the glycoside in its presence. Inotropic responses were observed with all four glycosides, digitoxin producing the greatest increase and the bis compound the least (Figure 2).

In the presence of increased potassium in the perfusion medium, the uptake by the whole heart was decreased by 20–30% for each glycoside and the uptake by the microsomal fraction was decreased by 40–50% (Table 2). The highest concentrations were again found in the microsomal fractions and again the maximal uptake was seen with digitoxin and there was no distinct difference among the metabolites.

Discussion

These experiments show that the cleavage products of digitoxin, that have lost one, two or three of the digitoxose residues, are active positive inotropic agents. Quantitatively, digitoxin itself had the greatest effect at the one concentration determined. Although some metabolism of digitoxin has been claimed to take place in heart tissue (Sjoerdsma & Fischer, 1951) most studies emphasize that glycosides are not rapidly

Table 1 The uptake and subcellular distribution of digitoxigenin glycosides perfused in normal Krebs-Henseleit medium through isolated hearts of guinea-pigs

	Homogenate		*Subcellular fractions (pmol/mg protein)*			
Drug (All 0.1 μM)	*pmol/g heart*	*pmol/mg protein*	*Nuclear*	*Mitochondrial*	*Microsomal*	*Supernate/ pellet ratio*
[^{3}H]-Digitoxin	576.48 ± 8.83	3.751 ± 0.673	1.640 ± 0.262	2.305 ± 0.283	2.626 ± 0.110	0.775 ± 0.064
[^{3}H]-Digitoxigenin bis-digitoxoside	335.78 ± 11.72	2.264 ± 0.466	0.415 ± 0.073	1.190 ± 0.061	1.385 ± 0.190	0.631 ± 0.043
[^{3}H]-Digitoxigenin mono-digitoxoside	323.47 ± 7.06	2.293 ± 0.532	0.436 ± 0.071	1.274 ± 0.082	1.527 ± 0.160	0.504 ± 0.061
[^{3}H]-Digitoxigenin	305.42 ± 3.76	2.235 ± 0.379	0.832 ± 0.053	1.077 ± 0.081	1.377 ± 0.059	0.686 ± 0.021

Each value: mean ± s.e. of 4 hearts.

or extensively transformed in the heart (Marcus, Pavlovich, Burkhalter & Cuccia, 1967; Kuschinsky, Lahrtz, Lüllman & van Zweiten, 1967). We have administered each metabolite in its pure form directly to the heart and therefore the effects recorded represent those produced by the unchanged drug. The quantitative difference in their effects on inotropic response appears to follow a pattern. With decreasing number of digitoxose residues, the inotropic responses are progressively reduced. While the quantitative difference between the bis and mono compounds was not significant ($P > 0.05$), the differences between digitoxin and its cleavage products were significant ($P < 0.01$), as also were the differences between digitoxigenin and the other members of this group ($P < 0.01$).

Progressively diminishing effects with step-wise removal of the digitoxose residues of digitoxin were not found in previous studies either of inhibition of brain Na-K ATPase (Wilson, Sivitz & Hanna, 1970) or of pharmacological action. Thus Lage & Spratt (1966) showed a decrease in lethality following intravenous injection in mice (a digitalis resistant species) with each decrease in the number of sugar residues as far as the mono-digitoxoside but digitoxigenin was the most toxic of four agents. Furthermore in tests using guinea-pig isolated atria, Lüllman & Peters (1971) observed the greatest inotropic activity with the mono-digitoxoside and the least with digitoxigenin. Hence relative potencies appear to vary with the species and test situation.

The concentration of digitoxin attained in the ventricle was almost twice that of its metabolites, indicating that the third sugar residue promotes both the accumulation of the glycoside by the heart and the positive inotropic response. The relationship between uptake and inotropic response was anomalous for digitoxigenin, since its uptake was comparable to that of the bis and mono compounds, while its inotropic response was less. Neither the uptake nor the inotropic responses can be explained by the lipid solubility characteristics of this series of drugs. The results suggest an important contribution by the digitoxose moieties to interaction of digitalis with receptor or binding sites in heart muscle.

The decrease in digitoxigenin glycoside uptake in the presence of increased potassium is in keeping with the observations with other digitalis glycosides (Dutta *et al.*, 1968; Dutta & Marks, 1972). Potassium ions appeared to inhibit the uptake of each of the digitoxigenin glycosides to a membranous binding site. Nevertheless all four glycosides have shown a marked positive inotropic effect in the presence of elevated potassium (Figure 2) and this indicates that the increased $[K]_0$ does not interfere with the interaction of digitaloids with the inotropic receptor.

Thus, from the various standpoints of potassium effect on uptake, subcellular distribution as well as positive inotropic effectiveness, it is clear that the cleavage products of digitoxin behave as typical digitaloids, resembling their parent compound but with less biological activity. On a molar basis, the cardiac

Table 2 The uptake and subcellular distribution of digitoxigenin glycosides perfused in Krebs-Henseleit medium with elevated potassium concentration (K x 1.5) through isolated hearts of guinea-pigs

	Homogenate		*Subcellular fractions (pmol/mg protein)*			
Drug (All 0.1 μM)	*pmol/g heart*	*pmol/mg protein*	*Nuclear*	*Mitochondrial*	*Microsomal*	*Supernate/ pellet ratio*
[^{3}H]-Digitoxin	371.36 ± 7.62	2.413 ± 0.514	0.993 ± 0.043	1.194 ± 0.061	1.282 ± 0.041	0.601 ± 0.034
[^{3}H]-Digitoxigenin bis-digitoxoside	177.97 ± 4.41	1.303 ± 0.403	0.704 ± 0.041	0.738 ± 0.063	0.850 ± 0.071	0.811 ± 0.052
[^{3}H]-Digitoxigenin mono-digitoxoside	201.04 ± 4.76	1.424 ± 0.451	0.781 ± 0.027	0.793 ± 0.041	0.936 ± 0.044	0.709 ± 0.061
[^{3}H]-Digitoxigenin	207.83 ± 3.97	1.401 ± 0.394	0.509 ± 0.044	0.746 ± 0.061	0.915 ± 0.032	0.684 ± 0.063

Each value: mean ± s.e. of 4 hearts.

uptakes of digitoxin and its cleavage products were approximately three times greater than and the supernatant pellet ratios were about twice those of ouabain or digoxin studied under the same conditions (Dutta *et al.*, 1968).

This work was supported by grant HE–07051 from the National Heart Institute, U.S.A. Some of these results were presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics, Fall 1971, in Burlington, Vermont, U.S.A. Please send reprint requests to S.D.

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