Biochemical Pharmacology, Vol. 26, pp. 2427–2431. Pergamon Press, 1977. Printed in Great Britain.

SUBCELLULAR DISTRIBUTION OF DESLANATOSIDE C, OUABAIN AND DIGITOXIN IN THE HEART AND LIVER OF CONSCIOUS GUINEA PIGS

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(*Received* 25 *January* 1977; *accepted* 16 *May* 1977)

Abstract—Myocardial and liver uptake and subcellular distribution in the two organs of [^{3}H]Deslanatoside C, [^{3}H]Ouabain and [^{3}H]Digitoxin (100 μg/kg, i.v.) were investigated in conscious guinea-pigs. Myocardial uptake, the specific concentrations in its pellet fractions and the ventricle/plasma concentration ratio observed with Deslanatoside C and Ouabain were higher than those observed with Digitoxin, this being the inverse behaviour of that observed *in vitro*. This suggests that myocardial uptake depends on plasma protein in unbound glycoside, which is 100 per cent with Ouabain, about 70–80 per cent with Deslanatoside C and only about 10 per cent with Digitoxin. The liver uptake of Digitoxin was much higher than that of Ouabain and Deslanatoside C. Plasma protein binding thus seems to not interfere with the liver mechanism uptake. Subcellular distribution of the three glycosides in the liver did not differ.

In conclusion, for a given glycoside a substantial difference can be observed between uptake in perfused and in *in situ* hearts as well as between heart and liver uptake mechanisms, heart uptake provoking contractility and liver uptake biliary excretion.

A standardized method for quantitative measurement of the subcellular distribution of labelled cardiac glycosides in isolated perfused hearts by ultracentrifugation has been worked out by Dutta *et al.* [1]. Using this method Dutta *et al.* [2] studied the cardiac distribution of Ouabain, Digoxin, Digitoxin, Convallotoxol, Dihydroouabain and Proscillaridin, and Marzo *et al.* [3, 4] studied the cardiac distribution of K-strophanthoside and Deslanatoside C in isolated guinea pig hearts.

This paper reports quantitative results on the subcellular distribution of Deslanatoside C, Ouabain and Digitoxin in *in situ* hearts and livers of conscious guinea pigs.

MATERIAL AND METHODS

Ouabain (515 μC/mg), Deslanatoside C (457 μC/mg) and Digitoxin (650 μC/mg) general labelled* were used. Their chemical and radioactive purity were ascertained by the TLC technique as described previously [4]. Ouabain, Deslanatoside C and Digitoxin were dissolved in an ethylene glycol–saline solution (NaCl 9 g/l), 25:75 (v/v), at a concentration of 100 μg/ml. Male guinea pigs weighing 250–300 g were given i.v. 100 μg/kg† of each glycoside solution through the posterior paw vein. For each glycoside the animals were divided into 5 groups each consisting of 8 animals. The animals were rendered unconscious by cervical distorsion, the first group 30 min, the second 90 min, the third 3 hr, the fourth 5 hr and the last 24 hr after administration. A sample of blood and the whole heart were quickly removed from each animal. The liver was also removed, but only from the animals of the first group (30 min after injection). The heart were perfused through the aorta and the livers through the porta vein for 8 min in order to wash the glycosides from the extracellular spaces using preoxygenated K-H Medium at 28° [5]. The perfusion rate was 3 ml/min in the case of the hearts and 5 ml/min in the case of the livers. In previous experiments 8 min had been enough to remove all the glycoside from the extracellular spaces in both hearts [1–4] and livers. A sample of the right and left atria, another of the right and left ventricles and one of the liver (50–100 mg for sample) were taken from each heart and liver, carefully weighed, dissolved in Packard Soluene TM 100 and counted as total radioactivity in the liquid scintillation spectrometer (LSS). The remainder of the heart and liver were pooled in groups of two. The whole of two hearts were used whereas only about 2 g from the two livers were taken. The pooled hearts and livers were carefully weighed and homogenized in 9 vol./g of sucrose 0.32 M, EDTA (Ethylenediamino tetraacetic acid) 10^{-6} M, $MgSO_4$ 10^{-6} M and Tris (trihydroxymethyl-

*The randomly labelled glycosides were supplied by New England Nuclear Corporation, 575 Albany Street, Boston, MA 02118, U.S.A.

† Ouabain, 171 nmoles/kg; Deslanatoside C, 160 nmoles/kg; Digitoxin, 131 nmoles/kg.

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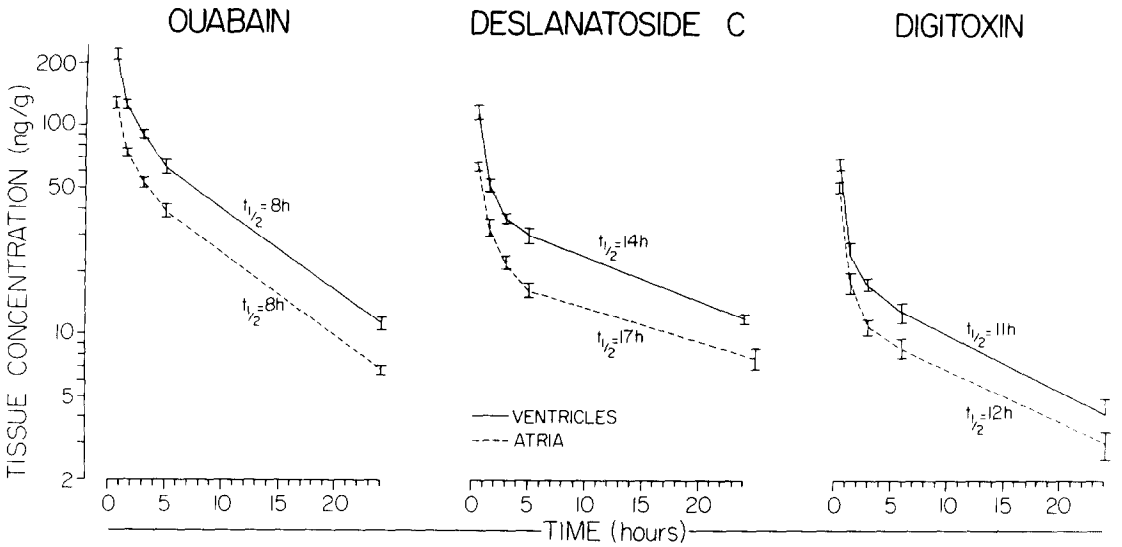

Fig. 1. Ventricle and atria concentrations of Ouabain, Deslanatoside C and Digitoxin in conscious guinea pigs treated i.v. with 100 μg/kg of each glycoside. Mean values of 8 findings ± SE.

aminomethane) $5-10^{-5}$M buffered at pH 7.2 (Medium I) according to De Robertis *et al.* [6]. The homogenate was filtered through a sheet of surgical gauze. The filtered homogenate was centrifuged for 10 min at 900 *g*. The nuclear pellet obtained was resuspended in Medium I and recentrifuged for 10 min at 900 *g*. The latter washing operation was then repeated. The pooled supernates were centrifuged for 20 min at 12,000 *g*. The mitochondrial pellet was again resuspended in Medium I and recentrifuged for 20 min at 12,000 *g*. The latter washing operation was then repeated. The pooled supernates were centrifuged for 1 hr at 166,000 *g*. The microsome pellet was resuspended in Medium I and recentrifuged for 1 hr at 166,000 *g*. Aliquots of the filtered homogenate, the final supernate of 166,000 *g* (supernate) and each of the three resuspended pellets (nuclear, mitochondrial, and microsomal) were taken to evaluate radioactivity content, as described previously, and protein content according to Lowry *et al.* [7].

A few samples of each particulate fraction were fixed in buffered 2% glutaraldehyde, postfixed in osmium tetroxide, dehydrated in a series of alcohols and then embedded in epoxy resin. These sections were poststained with uranyl acetate and lead citrate for electron microscope observation. Fragments of the endoplasmatic reticulum and ribosomes were seen in the microsome fraction together with marginally lesser amounts of mitochondrial fragments. On the other hand, both the mitochondrial and nuclear fractions were contaminated by each other and cell fragments. Similar findings have been obtained previously by Dutta *et al.* [1] and Marzo *et al.* [3, 4].

The mean recovery of radioactivity calculated in all the experiments was 103 ± 2 per cent (SE); the mean recovery of the protein in the same 44 cases was 92 ± 1 per cent (SE). For this evaluation, radioactivity and the protein content in the filtered homogenate were assumed to be 100 per cent.

In order to rule out the possibility of any intracellular redistribution of the glycosides during the analysis procedure, in glycoside-free filtered homogenates of guinea pig heart and liver a trace of each labelled glycoside was added. The homogenates were then treated as described above. According to previous investigations [1–4], 95 per cent or more of the added

Table 1. Plasma concentration of Ouabain, Deslanatoside C and Digitoxin injected i.v. to conscious guinea pigs*

	Plasma levels†					Linear correlation coefficients between plasma and ventricles concentrations
	30 min	90 min	3 hr	5 hr	24 hr	
Ouabain	65.00	42.66	21.42	13.64	2.51	0.945
100 μg/kg =	±11.00	±2.29	±1.49	±1.42	±0.24	($P < 0.05$)
171 nmoles/kg	(111.1)	(72.9)	(36.6)	(23.3)	(4.3)	
Deslanatoside C	83.30	47.55	25.20	14.24	6.52	0.986
100 μg/kg =	±7.48	±2.86	±1.67	±1.97	±0.16	($P < 0.01$)
106 nmoles/kg	(88.3)	(50.4)	(26.7)	(15.1)	(6.9)	
Digitoxin	39.14	33.99	19.32	8.17	8.24	0.841
100 μg/kg =	±2.83	±2.65	±0.55	±0.30	±0.45	($P < 0.05$)
131 nmoles/kg	(51.2)	(44.4)	(25.2)	(10.7)	(10.8)	

* Mean values of 8 findings ± SE. Coefficients of linear regression between plasma and ventricles levels are also reported.

† Values in ng/ml. In parentheses values in pmoles/ml.

radioactivity was found in the supernatant and only traces in the pellet fractions.

Metabolite studies were carried out on bile and urine of each glycoside investigated. The TLC technique was used to evaluate the unchanged glycoside and the percentage of more and less polar metabolites present. This technique is described in our previous paper [8]. Ouabain was recovered as such in both bile and urine. Bile and urine of guinea pigs treated with Deslanatoside C contained about 55% of unchanged glycoside, 11% of less polar metabolites and about 34% of more polar metabolites. In the case of Digitoxin about 10% was found as such, another 10% was found in the form of less polar metabolites while 70–80% were more polar metabolites.

Plasma protein bindings of the three glycosides were investigated as described previously [1, 3, 4] by using a Sephadex G-25 glass column. No plasma protein bindings were found in the case of the Ouabain, about 20–30% of Deslanatoside C and about 90% or more of Digitoxin were bound with the plasma proteins. The same technique applied to the supernatant (166,600 *g*) of guinea-pig hearts and livers revealed no protein bindings with any of the three glycosides.

RESULTS

Figure 1 shows ventricle and atria concentrations of the three glycosides. Both ventricles and atria levels decrease in time in all cases. Ouabain and Deslanatoside C show the highest levels in both atria and ventricles and in all times, those of Ouabain being marginally higher than Delanatoside C. Digitoxin shows levels in atria and ventricles 3 or more times lower than the previous two glycosides. The $t_{\frac{1}{2}}$ of those levels (evaluate by extrapolating the slope from 5 to 24 hr to 0 time) were as follows: 8 hr for atria and ventricles in the case of Ouabain, 14 hr for the ventricles and 17 hr for the atria with Deslanatoside C and 11–12 hr for atria and ventricles with Digitoxin.

Table 1 shows plasma levels measured with the three glycosides in time. These levels decreased in time from 30 min to 24 hr. Plasma levels in the case of Ouabain and Deslanatoside C were 2–3 times lower than ventricle levels, whereas in the case of Digitoxin they were nearly the same and in some cases higher than the ventricle levels. The $t_{\frac{1}{2}}$ evaluated from 5 to 24 hr as described above were 8 hr with Ouabain and 17 hr with Deslanatoside C. In the case of Digitoxin this value is infinity because the values measured at the 5th hour are about the same as those observed at the 24th hour. The correlation between plasma and ventricle levels, measured by the linear correlation method, was good with all three glycosides, including Digitoxin, to a statistical significant degree ($P < 0.05 - < 0.01$).

Table 2 shows the concentrations of the glycosides measured in the supernatant and in whole pellet of the guinea pig myocardium. Ouabain in the supernatant shows a peak at 90 min, after which it decreases. In the pellets, Ouabain decreased from the highest value observed at 30 min to the lowest at 24 hr. The supernatant/pellet (S/P) ratio was 0.53 at 30 min, and increased to around 1 subsequently. In the case of Deslanatoside C and Digitoxin both supernatant and pellet levels decreased from 30 min to 24 hr. The S/P ratio in the case of Deslanatoside C was 0.33 at 30 min and more than 1 at the subsequent times. In the case of Digitoxin this ratio was 1.12 at the 30th min, increased to 1.48 at the 5th hr and 1.17 at the 24th hr. Among the pellet fractions the highest specific concentrations were observed in the microsomes, those in the nuclei and mitochondria being substantially lower with all three glycosides (Fig. 2). Among the three glycosides, Ouabain and Deslanatoside C

Table 2. Concentration of three cardiac glycosides in supernatant and in pellets of conscious guinea pig hearts treated i.v. with 100 μg/kg of each glycoside*

	30 min	90 min	3 hr	5 hr	24 hr
Ouabain					
Supernatant (ng/g)	25.56 ±1.83	41.76 ±3.91	23.86 ±1.64	26.17 ±2.15	8.78 ±1.06
Pellet (ng/g)	50.57 ±5.14	35.26 ±2.45	27.24 ±2.91	28.47 ±1.00	9.19 ±1.06
S/P	0.53 ±0.08	1.21 ±0.15	0.91 ±0.11	0.92 ±0.08	0.96 ±0.04
Deslanatoside C					
Supernatant (ng/g)	27.96 ±6.15	27.87 ±1.77	14.16 ±1.22	19.03 ±1.57	17.23 ±1.31
Pellet (ng/g)	67.19 ±4.70	22.82 ±2.41	15.83 ±1.47	14.14 ±0.96	13.35 ±0.75
S/P	0.33 ±0.06	1.12 ±0.12	1.02 ±0.12	1.18 ±0.05	1.31 ±0.15
Digitoxin					
Supernatant (ng/g)	21.90 ±0.92	11.03 ±1.07	8.11 ±0.94	6.77 ±0.60	2.89 ±0.29
Pellet (ng/g)	20.33 ±2.03	6.48 ±0.59	4.32 ±0.28	4.81 ±0.72	2.45 ±0.12
S/P	1.12 ±0.15	1.70 ±0.04	1.86 ±0.12	1.48 ±0.23	1.17 ±0.08

* Supernatant/pellet ratio (S/P) of the amount of glycoside are also reported. Mean values of 4 findings ± SE.

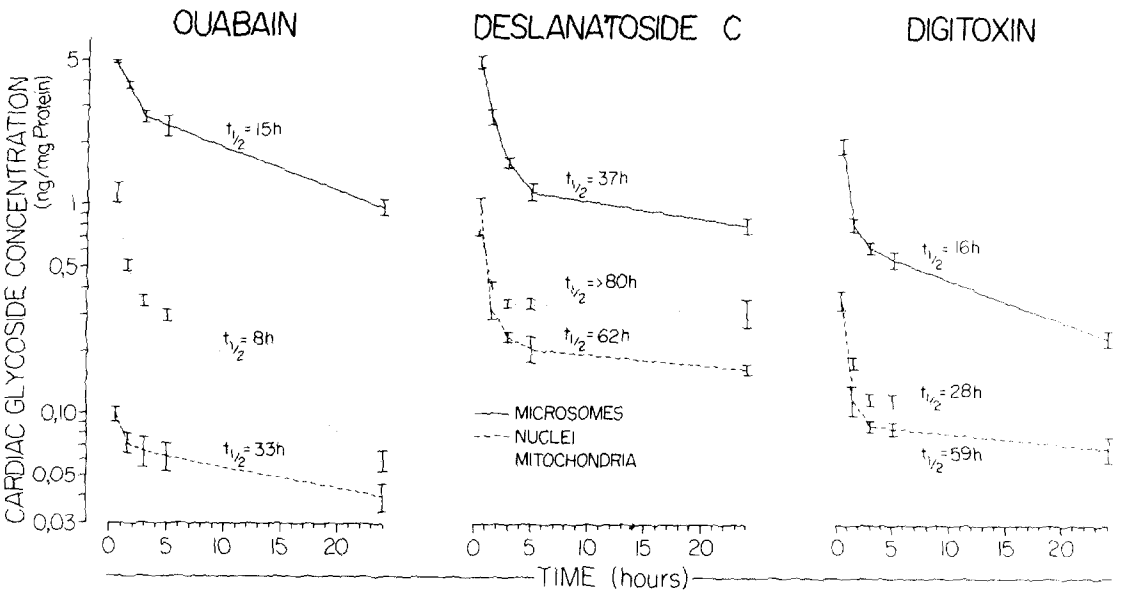

Fig. 2. Specific concentrations (ng/mg of protein) of Ouabain, Deslanatoside C and Digitoxin in microsomes, mitochondria and nuclei of conscious guinea pig hearts treated i.v. with 100 μg/kg of each glycoside. Mean values of 4 findings ± SE.

showed the highest microsomal specific concentrations, whereas in the case of Digitoxin these specific concentrations were 2–3 times lower or more (Fig. 2). The $t_{\frac{1}{2}}$ of the microsome specific concentration was 15 hr with Ouabain, 37 hr with Deslanatoside C and 16 hr with Digitoxin.

Concentration of Deslanatoside C and Ouabain in the whole liver was around 54–60 μg/g, whereas in the case of Digitoxin this value was 108 μg/g. The S/P ratio was around 1 with Ouabain and Deslanatoside C, but 4.61 with Digitoxin. Specific concentrations in microsome, nuclei and mitochondria did not greatly differ (Table 3).

DISCUSSION

In studies on isolated guinea pig hearts perfused 64 min with K-H Ringer, Dutta *et al.* [2] observed very high concentrations in both atria and ventricles with Digitoxin (700–740 ng/g) among six cardiac glycosides, whereas Ouabain showed values markedly lower (100–150 ng/g). Deslanatoside C, investigated by Marzo *et al.* [4] using the same method as Dutta *et al.* [1] showed concentrations of 77 ng/g in the ventricles, which is not very different from those observed by Dutta with Ouabain, and, consequently markedly lower than the values observed with Digitoxin. Among the particulate fractions, microsomes showed the highest specific concentrations in any case, being in 2.27 ng/mg of protein in the case of Digitoxin, 1.28 in the case of Ouabain and 2.67 in the case of Deslanatoside C. After 64 min of perfusion the S/P ratio were as follows: 0.73 with Digitoxin, 0.22 with Ouabain and 0.43 with Deslanatoside C.

In conscious guinea pigs, the heart uptake of Digitoxin and its concentration in particulate pellet fractions were invariably lower than with Ouabain and Deslanatoside C. The behaviour observed in perfused guinea pig hearts and in hearts of conscious guinea pigs are thus in evident contradiction particularly in the case of myocardial uptake of Digitoxin. A possible reason for this contradiction may arise from the plasma protein linkages of cardiac glycosides. In effect, Ouabain is not bound to plasma proteins, and

Table 3. Concentrations in the whole liver, ratio between the amounts present in supernatant and pellet, and specific concentrations in nuclei, mitochondria and microsomes of Deslanatoside C, Ouabain and Digitoxin in the livers of conscious guinea pigs treated i.v. with 100 μg/kg of each drug*

	Concentrations in whole liver (ng/g)	Supernatant/ pellet ratio	Specific concentrations (ng/mg protein) Nuclei	Mitochondria	Microsomes
Ouabain	59.79 ± 5.33	0.94 ± 0.06	0.35 ±0.02	0.29 ± 0.03	0.42 ± 0.05
Deslanatoside C	53.66 ± 2.10	1.02 ± 0.15	0.35 ±0.06	0.26 ± 0.02	0.40 ± 0.03
Digitoxin	108.01 ± 8.83†	4.61 ± 0.42‡	0.22 ±0.01	0.24 ± 0.03	0.39 ± 0.05

* Mean values of 4 findings ± SE.
† $P < 0.01$.
‡ $P < 0.001$, related to the comparison of Digitoxin with both Deslanatoside C and Ouabain.

Deslanatoside C is bound only in small amounts (20–30%), whereas Digitoxin is almost entirely linked to plasma proteins. These data on plasma protein linkages are consistent with the findings of Kolenda *et al.* [9, 10]. Myocardial uptake of cardiac glycosides does in fact seem to be related only, or principally, to free glycoside which is whole with Ouabain, 70–80% with Deslanatoside C and only about 10% with Digitoxin. This hypothesis is in agreement with the findings of Rieger and Kuschinsky [11] working in isolated perfused guinea pig hearts, who observed that the addition of albumin to the perfusion medium was able to shift the dose–response curve of the positive inotropic effect induced by Digitoxin to the right by a factor of about ten, while the Ouabain-induced effects were not significantly altered. The same Authors also observed a marked decrease in myocardial uptake of Digitoxin, but not of Ouabain in the presence of albumin. Another possible reason may account for the different behaviour of myocardial uptake observed as between conscious guinea pigs and their perfused hearts. In effect the metabolization of glycosides which is nil with Ouabain, significant but not very high with Deslanatoside C and marked with Digitoxin in conscious animals, in isolated hearts it does not occur [8]. However the main reason for the different myocardial uptake of Digitoxin *in vivo* and *in vitro* is, in our opinion, plasma protein binding.

The behaviour of liver uptake of the three glycosides observed in conscious guinea pigs was different from myocardial uptake. Liver uptake of Digitoxin is two times higher than both Ouabain and Deslanatoside C ($P < 0.01$). All the particulate pellet fractions of the liver showed specific concentrations of the three glycosides not greatly different from each other, ranging from 0.22 and 0.42 ng/mg of protein. The S/P ratio is near 1 with Ouabain and Deslanatoside C and more than 4 times higher with Digitoxin, i.e., 4.61 ng/mg protein. High liver/plasma or bile/plasma ratios have been encountered with Digitoxin by Marzo and Ghirardi [8], Russell and Klaassen [12] and Kolenda *et al.* [10]. This fact allows considerable biliary excretion of this glycoside, i.e. 68.50% 5 hr after i.v. injection in anaesthetized guinea pigs, whereas this value was 14.92 with Deslanatoside C and 3.87% with Ouabain [8]. Digitoxin specific concentrations in the particulate fraction do not greatly differ from Ouabain or Deslanatoside C; Digitoxin concentration in the whole liver is about two times higher than the other glycosides, and the S/P ratio of Digitoxin is more than 4 times higher than that of Ouabain and Deslanatoside C. The high biliary excretion of Digitoxin seems not to be correlated with subcellular distribution of this glycoside in the liver, but seems to depend only on the degree of liver uptake.

In conclusion this investigation shows that the uptake of the perfused heart may be different from that of *in situ* heart of living animals in which the drug can be bound to plasma proteins; conversely the plasma protein bindings of cardiac glycosides do not seem to reduce their liver uptake; biliary excretion of cardiac glycosides does not depend on subcellular distribution of glycoside in the liver, but only on the degree of its liver uptake; in the same glycoside it is possible to observe a higher heart uptake together with poor liver uptake, as well as the opposite behaviour.

Acknowledgements—We are most grateful to Miss T. Minoja and Messrs. M. Meroni and M. Mascheroni of the Istituto Simes di Cardiologia Sperimentale for the help they provided in the laboratory during these experiments.

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