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SODIUM-DEPENDENT CARDIAC GLYCOSIDE BINDING: EXPERIMENTAL EVIDENCE AND HYPOTHESIS

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1 The influence of increasing Na^+ concentrations on the binding of digitoxin, digoxin and ouabain was examined in a Na^+-K^+-ATPase preparation of guinea-pig hearts

2 Two distinct processes seem to be involved in this interaction: one binding process was activated at low Na^+ concentrations. The maximum binding capacities were different and the $K_{0.5}$ values were nearly identical for the cardiac glycosides studied.

3 In contrast, the second binding process was activated at appreciably higher Na^+ concentrations, the maximum binding capacities were almost identical and the $K_{0.5}$ values were different for the cardiac glycosides studied.

4 On the basis of these results attempts are made to explain the well known differences in the myocardial accumulation of cardiac glycosides.

Introduction

It is well substantiated that sodium ions may modulate the action of cardiac glycosides at different levels: (1) myocardial function, (2) cardiac uptake and subcellular distribution and (3) binding to the Na^+-K^+-adenosine triphosphatase (ATPase) (Lee & Klaus, 1971). Recently, in a more detailed study of the action of Na^+ on the binding of ouabain to a myocardial Na^+-K^+-ATPase, two different binding sites (predominant at low and high Na^+-concentrations, respectively) were demonstrated (Klaus & Fricke, 1976). The present study deals with further observations on the inter-relationship of Na^+ and the cardiac glycosides, digitoxin, digoxin and ouabain at the level of the membrane ATPase.

Methods

The binding studies were performed on a Na^+-K^+-ATPase preparation of guinea-pig hearts as previously described (Fricke & Klaus, 1977). The incubation medium contained the enzyme preparation (about 0.3 mg of protein per 4.0 ml), 5 mM $MgCl_2$, NaCl varying from 0 mM to 140 mM (osmolarity was kept constant by choline chloride), 100 mM imidazole hydrochloride (pH 7.4) and 2 mM adenosine triphosphate (ATP). The reaction was started by the addition of the respective tritiated cardiac glycoside (final concentration: 5×10^{-7} M; New Engl. Nucl. Corp.) and stopped after 2, 5, 10, 30, or 60 min by immersion in an ice bath, followed by centrifugation (40,000 *g*, 30 min; Beckman Spinco L2 65B). An identical assay was performed in the absence of ATP to determine the ATP-independent binding.

Results and Discussion

The time course of the cardiac glycoside binding to the Na^+-K^+-ATPase from guinea-pig hearts was found to be independent of the respective Na^+ concentration and of the cardiac glycoside studied. The mean half times obtained were between 24 s (digoxin) and 67 s (ouabain). Although the binding of the cardiac glycosides was found to be independent of the Na^+ concentration in the absence of ATP, in its presence an increase in cardiac glycoside binding was observed that was quantitatively related to the Na^+ concentration. To obtain some idea of the influence of Na^+ on the binding of cardiac glycosides to the Na^+-K^+-ATPase a detailed analysis of these data (ATP-dependent binding) according to Hofstee (1952) was performed. This procedure was originally developed to describe substrate-enzyme-interactions, but contrary to the more commonly used reciprocal plots (e.g. Lineweaver & Burk plot) this plot favours the

Table 1 Na^+-dependence of the binding of ouabain, digoxin and digitoxin to a myocardial Na^+-K^+-ATPase from guinea-pig hearts

	High affinity sodium site		*Low affinity sodium site*	
	Maximum binding capacity (pmol/mg prot)	*$K_{0.5}$ for sodium (mM)*	*Maximum binding capacity (pmol/mg prot)*	*$K_{0.5}$ for sodium (mM)*
Ouabain	3.6 ± 0.4	4.9 ± 0.4	4.6 ± 0.3	63.6 ± 8.3
Digoxin	19.3 ± 0.1	5.2 ± 0.3	5.8 ± 1.4	15.2 ± 3.6
Digitoxin	37.6 ± 1.2	3.0 ± 0.5	4.2 ± 0.9	5.6 ± 1.1

Drug concentration: 5×10^{-7} M; incubation time: 30 min; further details see Methods section. Original experimental data were analyzed according to Hofstee (1952). The calculated maximum binding capacities and respective $K_{0.5}$ values are given (mean ± s.e. mean, $n = 4$).

evaluation of side reactions (Lumper, 1964). Applied to the present studies two different binding sites involved in the interaction of Na^+ and the cardiac glycosides were revealed (Table 1). One binding process reflecting the 'high affinity sodium site' is activated at low Na^+ concentrations. The maximum binding capacity was found to be different for the cardiac glycosides studied, ouabain showing the lowest and digitoxin the highest binding capacity (clearly, the lipophilic character of the drugs plays a role). However, the Na^+ concentrations for half maximum activation of the cardiac glycoside binding ($K_{0.5}$) were nearly identical and in good agreement with the Na^+ concentration needed for phosphorylation of the Na^+-K^+-ATPase enzyme system ($K_{0.5} = 1$ mM; Klaus & Fricke, 1976). It was proposed earlier that this binding process represents an outward transport of cardiac glycosides in heart muscle cells.

The second binding process is activated at appreciably higher Na^+ concentrations and may represent an inward transport of cardiac glycosides (Fricke & Klaus, 1977). In contrast to the above results the $K_{0.5}$ values for Na^+ activation of the latter process were quite different for each of the three cardiac glycosides tested; half maximum binding of ouabain was obtained at a Na^+ concentration of 64 mM, whereas respective binding of the other two cardiac glycosides (digoxin and digitoxin) took place at much lower Na^+ concentrations ($K_{0.5}$ about 15 mM and 6 mM respectively). The maximum binding capacities of this second binding process reflecting the 'low affinity sodium site' were nearly identical for all the cardiac glycosides tested.

On the basis of the proposed interaction between cardiac glycosides and Na^+-K^+-ATPase (Fricke & Klaus, 1977) a proposal that includes a Na^+-dependent translocation of cardiac glycosides from an outer to an inner compartment of the cell membrane, it is tempting to suggest an explanation for the well known differences in the myocardial accumulation (Lee & Klaus, 1971) of cardiac glycosides. This would be modulated by two (major) uptake processes: (a) a non-specific one, strongly related to the lipophilic nature of the respective cardiac glycoside and (b) a specific uptake process, which is supposed to be carrier-mediated. Regarding the latter (specific) uptake process, ouabain may be taken up at a slower rate than digoxin and digitoxin because of the rather high Na^+ concentration necessary for activation of this process. On the other hand, there is (due to the hydrophilic nature of ouabain) a negligible non-specific sub-cellular binding of this cardiac glycoside. For this reason the concentration of *free* ouabain available in the inner comparment for outward transport is rather high compared to the bound drug. Because only the *free* drug-concentration is involved in the outward transport this might be high for ouabain (compared to the outward transport of the other two cardiac glycosides) resulting in a rather poor cellular accumulation. In contrast, digitoxin (and digoxin which is intermediate) can be taken up at a higher rate by the carrier-mediated transport mechanism, because a much lower Na^+ concentration is needed for activation of this process. In addition, the simultaneous non-specific uptake and (non-specific) subcellular distribution/binding may play a significant role in the accumulation of this drug.

These two processes, i.e. carrier-mediated transport and non-specific drug uptake, together with the simultaneous outward transport, are the determinant factors for the concentration of *free* digitoxin in the inner compartment and thus for improved muscular contraction. During 'steady state' the concentration of *free* digitoxin may then be well within the same concentration range as *free* ouabain which would explain the almost identical inotropic response of isolated heart muscle preparations to all cardiac glycosides studied.

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