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Effect of cardioactive steroids on the active transport of non-electrolytes

Cardiac steroids (digitalis) are powerful inhibitors of the electrolyte pump* in a variety of organ systems[1-7]. At the same time these compounds inhibit the sodium-dependent ATPase of the membrane ("pump" ATPase)[8,9] and the sodium-stimulated turnover of phosphatidic acid which is believed to be involved in the active transport of sodium[10]. All these inhibitory actions, both *in vitro* and *in vivo*, require a rather low concentration of digitalis (10^{-5}–10^{-6} M). The structural requirements of a steroid molecule necessary for the inhibition of the electrolyte pump in the red cell are identical with those essential for the well-known "digitalis-like" cardiac action. Among these requirements is the presence of an unsaturated lactone ring in position 17. Thus, Hexahydroscillaren A, with a saturated lactone ring, is void of both cardiotonic[11] and electrolyte-pump inhibitory action[3].

Recently it was shown in our laboratory that cardiac steroids inhibit not only the active transport of electrolytes, but also that of non-electrolytes, such as sugars, amino acids and pyrimidines. The active intestinal sugar transport in the frog is inhibited by a 10^{-6} M concentration of Ouabain or by a 10^{-7} M concentration of Thevetin[12]. Ouabain in identical concentration also inhibits markedly the active intestinal transport of L-tyrosine, DL-phenylalanine and uracil[13].

The present communication deals with experiments in which a number of cardioactive steroids were examined for their inhibitory action on the intestinal transport of non-electrolytes. It was also examined whether the inhibition of the non-electrolyte pumps has the same molecular configurational requirements as is needed for the cardiotonic action. For this reason the action of Hexahydroscillaren A upon the active intestinal transport of non-electrolytes was also studied.

The experiments were conducted in the surviving isolated small intestine of the frog (*Rana pipiens*). The method, described in detail earlier[16], was briefly as follows: Either a sugar ([*Me*-^{14}C]3-methylglucoside) or an amino acid (DL-[2-^{14}C]phenylalanine) was used as substrate. These substrates are known to be transporte dagainst a higher concentration in the intestine. The substrate was dissolved in a modified Ringer's solution and was placed in identical initial concentration both in the lumen and on the outside of the intestinal loop.

After an incubation at 30° in a metabolic shaker-incubator for 6 h, the loop was removed and emptied. The concentration of the sugar or amino acid was measured both in the fluid of the lumen and in the outside bathing medium. The analytical procedure, using a liquid scintillation spectrometer, was described earlier[14]. The ratio of the concentration in the outside over that in the lumen was used as a measure of the active transport. By definition, the initial value equals 1.0, any value of more than 1.0 indicates active transport. When the steroids were employed**, they were

* The expression "pump" means an active transport system *viz.* a biological membrane (or membranes), which is capable of transporting solutes by an energy-requiring process from a lower into a higher concentration, without the solute being bound on either side of the membrane or produced during the transport process or passively carried by the movement of the solvent.

** Ouabain, Convallatoxin and Convallatoxol were commercial preparations. Cymarin, Cymarol, Strophanthidin and Hexahydroscillaren A were gifts of Dr. B. BERDE of Sandoz Ltd., Basel, Switzerland.

placed in identical concentrations both inside and outside of the gut at the beginning of the experiment.

The data in Table I represent each the mean value of at least three identical experiments. In each case experiments were conducted first without the digitalis in order to establish control values. The results obtained thereafter with different concentrations of the drug were then compared with the control values. In the table the per cent inhibition exerted by the respective steroid is reproduced.

TABLE I

INHIBITION (%) OF ACTIVE INTESTINAL TRANSPORT BY VARYING CONCENTRATIONS OF CARDIAC STEROIDS

No drug = 0% inhibition.

	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M
		Ouabain			
[*Me*-^{14}C]3-methylglucoside			85	78	40
DL-[2-^{14}C]Phenylalanine		85	80	64	20
		Convallatoxin			
[*Me*-^{14}C]3-methylglucoside		89	82	68	
DL-[2-^{14}C]Phenylalanine		98	98	76	44
		Convallatoxol			
[*Me*-^{14}C]3-methylglucoside			78	43	
DL-[2-^{14}C]Phenylalanine			90	20	
		Cymarin			
[*Me*-^{14}C]3-methylglucoside		88	60		
DL-[2-^{14}C]Phenylalanine		95	44		
		Cymarol			
[*Me*-^{14}C]3-methylglucoside		82	30		
DL-[2-^{14}C]Phenylalanine		92	30		
		Strophanthidin			
[*Me*-^{14}C]3-methylglucoside		57	50		
DL-[2-^{14}C]Phenylalanine		85	42		
		Hexahydroscillaren A			
[*Me*-^{14}C]3-methylglucoside	75	60	0		
DL-[2-^{14}C]Phenylalanine	95	30	14		

The results obtained with a number of cardioactive steroids confirmed our previous findings, namely that both the sugar and the amino acid pumps in the intestine are inhibited by cardioactive steroids in a concentration of approx. 10^{-6} M or less. In contrast, the non-cardioactive compounds, Hexahydroscillaren A, has no inhibitory action at low concentration. However, if the concentration of the latter compound is increased, it will exert an inhibitory action upon the transport of non-electrolytes, its potency being about 1000 times less than that of Ouabain. In judging the inhibitory action of Hexahydroscillaren A in high concentrations one has to consider that digitalis steroids in high concentrations (10^{-3} M or higher) are capable of uncoupling oxidative phosphorylation, acting in this respect identically with

2,4-dinitrophenol[15]. This latter compound, a metabolic inhibitor, is known to inhibit all active transport processes. Clearly, this action is qualitatively different from a typical digitalis action.

It appears to be rather significant, therefore, to pay particular attention to the concentrations of the steroids whenever a specific digitalis-like action is examined. If digitalis is employed in high concentrations (10^{-3} M or higher), the effect obtained should be characterized as a non-specific cytotoxic action. There are data in the literature reporting specific inhibitory action of cardiac steroids upon different transport mechanisms in 10^{-3} M concentrations. Such reports should be disregarded as irrelevant.

The above described experimental data give additional support to the thesis that digitalis is a general pump poison and that there is a parallelism between this pump-inhibitory action and the cardiotonic action. In addition digitalis appears to be a rather specific poison of the sodium-dependent ATPase in the membrane[8,9]. This ATPase could be regarded as an essential part of all biological pumps serving to convert the chemical energy of ATP into pumping or osmotic energy. As digitalis apparently inhibits all active transport processes, ionic and non-ionic alike, it would be reasonable to assume that the pump ATPase is involved in all active transport processes. If this assumption is accepted then this enzyme could be considered as a link between electrolyte and non-electrolyte pumps. Another observation supporting such a consideration is that all non-electrolyte pumps appear to be dependent on sodium[14] and so is the pump ATPase.

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