

DIRECT RENAL ACTION OF SOME DIGITALIS STEROIDS *

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Some digitalis steroids alter the activity of cardiac muscle. Applying the bioassay technique in cats and frogs, Chen and co-workers (1-4) screened many structural variants of the clinically useful digitalis compounds for cardiac action. They were able to distinguish the active from the inactive steroids by their chemical structure. The major features common to those compounds exhibiting cardiac activity include a cyclopentanophenanthrene nucleus with a hydroxyl substituent at the C-14 position and an unsaturated, 5 or 6 membered lactone ring in β orientation at C-17. Those compounds in which the lactone ring was either saturated or in α orientation lacked cardiac activity. Synthesis of active digitalis steroids without the C-14 hydroxyl has not been accomplished to date and, therefore, the role of this constituent is unknown. In addition to their familiar actions on cardiac muscle, these steroids affect cation movement in several tissues (5-15). Inhibition of the active ion transport mechanism is the common effect produced. Glynn (9), Schatzmann (5), and others (6-8), for example, have shown that the cardiac active steroids, in a concentration of about 1 μ g/ml, depress potassium influx and sodium efflux in the human red cell. Depending upon the cell type, passive diffusion fluxes may or may not be inhibited. Glynn also studied certain molecular requirements for this inhibitory effect and found them identical to those necessary for cardiac activity. He concluded that the configuration at C-17 of the steroid nucleus is important and that saturation of the lactone ring greatly reduces activity.

The first data suggesting that digitalis steroids might directly affect renal electrolyte transport

were reported by Farber, Alexander, Pellegrino and Earle (16). In their experiments, digoxin was administered intravenously to human subjects in the course of cardiac and renal hemodynamic studies. In several instances an increase in water and electrolyte excretion preceded any alteration in glomerular filtration rate or renal plasma flow.

With this suggestive observation, Hyman, Jaques and Hyman (17) injected digoxin directly into one renal artery of a dog and observed an increase in water and sodium ion excretion from the injected kidney. More recently, Orloff and Burg (18) observed a unilateral diuresis subsequent to the injection of strophanthidin into the leg vein of the chicken. Administered by this route, the aglycone bypasses the glomeruli and is delivered to the renal parenchyma directly via the peritubular capillaries.

Schatzmann, Windhager and Solomon (19) concluded that ouabain inhibits sodium ion reabsorption in the single perfused proximal tubule of *Necturus* extending the evidence for its direct renal action.

Our work was undertaken to answer the following questions: 1) Are the compounds that are active in the heart and in red cell suspensions also active in the kidney? In other words, do the same structural requirements for activity in these other tissues also apply to the kidney? 2) What are the alterations in renal hemodynamics and urinary electrolyte excretion produced by these substances?

To these ends, we injected nine structural variants of the cardiac steroids into one renal artery of dogs. By analyzing urine samples collected separately from each kidney our experiments had the virtue of being simultaneously controlled, since, with the exception of the renal arterial infusate, the two organs had identical environments. When administered intra-arterially, only those compounds known to have activity either on the heart and/or on red cell ion movement depress sodium reabsorption in the

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kidney. This inhibition might occur throughout the nephron or at some more localized site. Partial inhibition of the proximal transport mechanism is suggested by sodium excretions which at times exceeded 35 per cent of the filtered load.

METHODS

Dogs were anesthetized with pentobarbital. A load of 800 to 900 ml of saline was infused intravenously in 1 hour. Exogenous creatinine and para-aminohippuric acid (PAH) clearances were measured to approximate glomerular filtration rate and renal plasma flow, respectively. Appropriate amounts of these materials were dissolved in a saline infusion that was administered at a rate of 5 ml per minute for the duration of the experiment.

Through a supra-pubic incision, the ureters were catheterized at the point of their junction with the bladder. The renal artery was approached through a retroperitoneal flank incision. A curved no. 25 needle fixed to a plastic catheter was introduced into the lumen of the vessel permitting infusion of the renal artery at a rate of 12 to 24 ml per hour. Normal saline was infused into the artery during the control periods. The steroids were dissolved in 3 to 5 ml of ethanol with 10 to 20 ml of saline.

The experiments described in Table I serve as controls for the effect of this infusion on renal functions. Studies were begun when urine flow had stabilized and was approximately equal from the two kidneys. Experiments were discarded when creatinine clearances of the two kidneys differed more than 10 per cent from each other.

Conventional clearance techniques were employed with separate collections of urine from the two kidneys. Creatinine was analyzed by the Phillips modification of the Jaffé reaction (20), and PAH, by the method of Smith and associates (21). Sodium and potassium were determined by flame photometry utilizing lithium for internal standardization.

RESULTS

A total of 29 experiments is included in this report. A minimum of two experiments was performed with each of the nine compounds under study.¹ Compounds classified as "diuretic" are those that increased urine flow and sodium excretion on the injected side within 3 hours of administration. Using this restrictive definition there was no difficulty in dividing the compounds into diuretic or nondiuretic groups. No attempt was made to establish a dose-response relationship

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although the effects of readily available drugs (ouabain, strophanthidin and digitoxin) were studied in several experiments at doses of from 0.5 to 3.0 mg. Diuretic response increased in rough proportion to dose.

Figure 1 illustrates an experiment in which 0.85 mg of the aglycone strophanthidin was infused during 92 minutes into one renal artery. The dotted vertical lines denote the duration of this infusion. A unilateral diuresis from the infused kidney was evident within 60 minutes. Sodium excretion increased from control period values of 360 μ Eq per minute to a maximum of 1,360 μ Eq per minute. At the peak of the diuresis only 74 per cent of the filtered sodium was reabsorbed. Sodium excretion diminished within 10 minutes of cessation of strophanthidin infusion. Changes in urine flow reflected those of sodium excretion increasing from the preinfusion rate of 2.9 to 9.7 ml per minute at the diuretic peak.

Potassium excretion was first reduced, but then increased toward the end of the period of strophanthidin infusion. Unlike sodium, potassium

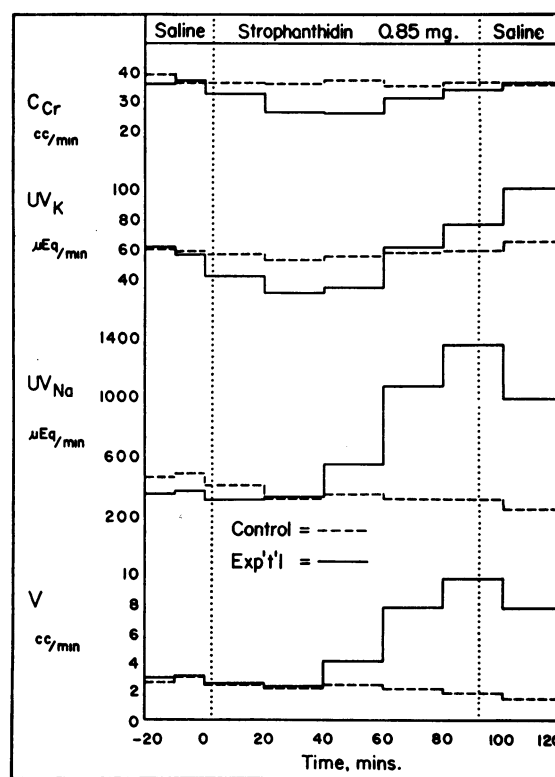


FIG. 1. THE EFFECTS OF UNILATERAL, INTRARENAL ARTERIAL INFUSION OF 0.85 MG OF STROPHANTHIDIN.

excretion continued to increase during the post-infusion period. Creatinine clearance was depressed by strophanthidin but gradually increased as the infusion of the aglycone was continued. PAH clearances, not shown in this figure, paralleled changes in creatinine clearances. The filtration fraction remained constant.

Dihydrostrophanthidin acid differs from strophanthidin chiefly in the saturation of its lactone ring (see below). As illustrated in Figure 2, this steroid, at a dose 20 times that of strophanthidin, failed to alter any of the renal functions studied.

Table I summarizes the data obtained with the steroids that produced a diuresis. The data from the control and experimental kidneys are presented as average values of two initial clearance periods and of two periods at the time of maximal diuretic action. Included in this table are the experiments at the dosage of the steroid that produced maximal diuresis with minimal depression of PAH and creatinine clearances. The total dose is listed to the left of the table under the names of the compounds. An increase in urine flow and sodium excretion occurred in each instance within 20 to 40 minutes of administration of the steroids. Maximal diuretic activity was achieved in 70 to 80 minutes with ouabain, strophanthidin and emicymarin. The peak of diuresis

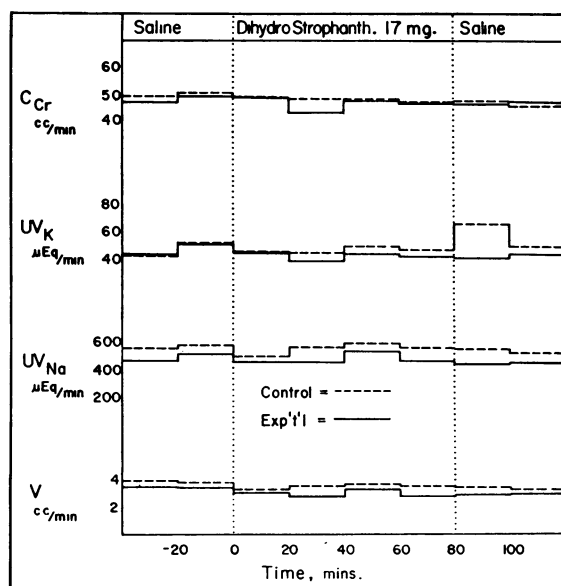


FIG. 2. THE EFFECT OF UNILATERAL, INTRARENAL ARTERIAL INFUSION OF 17 MG OF DIHYDROSTROPHANTHIDINIC ACID. See text.

TABLE I

Summary of results obtained with five steroids that increased urine flow and sodium excretion *

		V		C _{Cr}		C _{PAH}		UV _{Na}		UV _K	
		cc/min		cc/min		cc/min		μEq/min		μEq/min	
		Cont	Exptl	Cont	Exptl	Cont	Exptl	Cont	Exptl	Cont	Exptl
Digitoxin 2.1 mgs	Initial	4.4	4.5	43	43	130	130	450	400	66	64
	Max	1.4	6.0	33	35	75	84	360	720	62	76
Ouabain 0.9 mgs	Initial	2.3	1.9	36	34	100	100	410	310	49	46
	Max	1.4	9.1	37	26	75	58	210	1300	40	77
Strophanthidin 0.9 mgs	Initial	2.8	2.9	38	37	91	87	470	320	60	59
	Max	2.0	8.7	36	33	87	77	320	1200	59	69
Emicymarin 1.0 mgs	Initial	3.2	3.3	47	47	150	150	490	510	38	51
	Max	1.0	9.7	36	37	90	110	110	1380	14	55
Scillaren-A 0.5 mgs	Initial	1.6	1.3	46	44	120	120	290	260	22	16
	Max	0.7	9.3	40	44	120	110	96	1380	8	60

* See text for mode of presentation of data.

with digitoxin occurred in approximately 100 minutes and was delayed to about 200 minutes with scillaren-A. This latter compound was the most potent diuretic at the low dosage of 0.5 mg.

Typically, both creatinine and PAH clearance rates were proportionally diminished soon after the infusions were begun. This reduction of clearances often occurred on the control side as well as on the experimental side. Maximal depression of these clearance rates often preceded maximal diuresis, and recovery occurred on cessation of steroid infusion.

Potassium excretion was variable, although most often it increased concomitantly with sodium excretion. No attempt was made in these studies to obtain further information on alterations in potassium excretion through alterations in experimental protocol.

Table II summarizes the data obtained with steroids which had no effect on the renal excretion of sodium. These compounds were studied in at least two experiments, once at a dose comparable to their active analogs and once at a dose at least eight times greater. Only those experiments in which the larger dose was infused are tabulated. As in Table I, the data are presented for both the control and experimental kidneys. The figures are averages of two initial clearance periods and of two

TABLE II

Summary of results obtained with four steroids that had no effect on urine flow or sodium secretion

		V cc/min		C _{Cr} cc/min		C _{PAH} cc/min		UV _{Na} μEq/min		UV _K μEq/min	
		Cont	Expt'l	Cont	Expt'l	Cont	Expt'l	Cont	Expt'l	Cont	Expt'l
Isostrophanthidin 17 mgs	Initial	2.0	1.7	3.4	3.4	89	87	380	320	56	51
	Max	1.9	1.5	3.8	3.6	100	93	340	260	49	41
Dihydrostrophanthidin 17 mgs	Initial	3.9	3.5	51	48	160	160	570	490	48	48
	Max	3.6	3.8	48	47	140	140	560	460	55	42
17α-Emicymarin 8.0 mgs	Initial	1.9	1.7	55	54	180	180	370	360	62	70
	Max	1.8	2.1	58	58	180	210	250	280	15	19
Hexahydro- scillaren 7.5 mgs	Initial	1.8	2.0	42	45	120	130	330	350	51	54
	Max	0.8	1.0	44	45	110	110	90	130	24	29

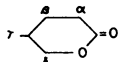
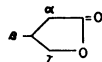
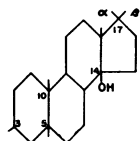
periods corresponding to the time of maximal activity of their structurally related analogs. In these and other experiments with these compounds, no significant alteration was noted in any of the studied functions.

Table III summarizes the major structural features of the nine steroids under study. At the bottom of the table is a cyclopentanophenanthrene nucleus numbered at the sites of substitution. To the right are the two forms of the C-17 lactone rings. This table is divided on the basis of diuretic

TABLE III

Major structural features of the steroid compounds used in this study*

Diuretic Compounds					Non-diuretic Compounds				
Name	C ₃	C ₅	C ₁₀	Lactone # Δ	Name	C ₃	C ₅	C ₁₀	Lactone # Δ
Digitoxin	sugar	-H	-CH ₃	5 α-β					
Oudaban	sugar	-OH	-CH ₂ OH	5 α-β					
Strophanthidin	-OH	-OH	-CHO	5 α-β	Isostrophanthidin	-OH	-OH	-CHO	5 O-bridge C ₁₄
					DihydroStroph. Acid	-OH	-OH	-COOH	5 None
Emicymarin	sugar	-OH	-CH ₃	5 α-β	17α-Emicymarin	sugar	-OH	-CH ₃	5 α-β
Scillaren-A	sugar	Δ,4-5	-H	6 α-β γ-β	Hexahydro- Scillaren	sugar	Δ,4-5	-H	6 None



* The presence of a double bond between C-4 and C-5 in hexahydro-scillaren-A is in some doubt.

activity. The inactive compounds are listed to the right of the corresponding active steroids.

A sugar at C-3 provides favorable solubility and distribution properties, but its presence is not essential for cardiac activity (2-4). The aglycone strophanthidin possesses cardiac (2) and direct diuretic activity.

DISCUSSION

A wide variety of substitutions at the C-5 and C-10 positions is possible within that group of steroids having activity on the heart (4) and on ion flux in red cells (5-9). As is evident from Table III, C-5 and C-10 substitutions of the diuretic compounds do not distinguish them from the nondiuretic compounds. The structure-activity relationship is most evident at the C-17 substitution. The lactone ring must be β oriented at this site. The single example of α orientation in this series is represented by the nondiuretic compound 17α-emicymarin. Whether 5 or 6 membered, the lactone ring of the diuretic steroids are unsaturated in the α, β position. Activity is lost with saturation of the ring as typified by dihydrostrophanthidin and hexahydro-scillaren-A. The unsaturation of the lactone ring is unstable when shifted to the β, γ position. In this state, an oxygen bridge forms from the γ carbon of the lactone ring to the C-14 hydroxyl group (23). Strophanthidin is transformed by this route into the inactive "iso" compound.

We conclude then, that the most specific constituent associated with diuretic activity is the lactone ring. Diuresis was observed solely with those steroids having a C-17, β oriented lactone ring unsaturated in the α, β position.

The molecular similarity of the digitalis steroids to the mineralocorticoids suggests that they might produce diuresis by an "anti-aldosterone effect." From the present study, evidence against anti-aldosterone activity of this group of steroids lies in the magnitude of their action on sodium reabsorption. Aldosterone and other mineralocorticoids are believed to stimulate the reabsorption of no more than about 2 to 3 per cent of the filtered sodium load (22, 24). In these studies as well as in those of Orloff and Burg (18), 15 to 40 per cent of the filtered sodium appeared in the

urine subsequent to the administration of the digitalis steroids.

The evidence to date points to an inhibition of ion carrier mechanisms as the mode of action of these compounds, although the possibility of interference of energy supply to ion transport cannot be completely excluded (9). In red cell suspensions digitalis does not alter glycolysis or oxygen uptake (5). Likewise, organic phosphate levels and adenosine triphosphate resynthesis are not affected (25). These observations favor the hypothesis of carrier mechanism interference. Added to these are the observations of both Glynn (9) and Schatzmann (5) who report kinetic alterations studied in red cell suspensions highly suggestive of competitive inhibition for carrier states. Glynn noted that the cardiac steroids inhibit certain passive diffusion fluxes, thus suggesting a direct effect on the carrier mechanism rather than interference with energy supply. Translating this hypothesis to the kidney has its dangers, but in view of the remarkable structural specificity of the compounds common to activity in all three tissues, we suggest that ion carrier inhibition is also the mode of their renal action.

SUMMARY

Nine digitalis steroids have been studied with respect to diuretic properties. These compounds were administered to dogs in acute experiments by infusion into one renal artery. Ureteral urines were collected separately.

Five of these steroids, known to affect cardiac activity and cation movement in *in vitro* preparations, increased sodium and water excretion from the infused kidney. This effect was independent of changes in renal hemodynamics. The dose necessary to produce diuresis ranged from 0.5 to 2.1 mg. In optimal quantity, these compounds blocked the reabsorption of 35 per cent of the filtered sodium.

Four of the steroids failed to increase sodium or water excretion in doses up to 17 mg. These latter compounds are those which lack significant cardiac activity or inhibitory action on cation movement *in vitro*.

The biologically active compounds are further distinguished by their chemical structure. Activ-

ity on the heart, kidney and on *in vitro* ion fluxes is most closely associated with the following structural criteria: a 5 or 6 membered lactone ring attached in β orientation to C-17 of the steroid nucleus, unsaturated between the α and β carbons. The probable mode of action is inhibition of a carrier mechanism.

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