Structure-Activity Relationships of Several Cardiotonic Steroids with Respect to Inhibition of Ion Transport in Frog Muscle

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ABSTRACT Cesium uptake by sodium-loaded frog sartorius muscles was inhibited 100% by 10^{-6} M ouabain and 10^{-6} M cymarin. The doses for 50% inhibition of cesium uptake by five cardiotonic aglycones were 1.5×10^{-7} M for strophanthidin, 2×10^{-7} M for telocinobufagin, 1.6×10^{-6} M for digitoxigenin, 2.4×10^{-6} M for periplogenin, and 6.3×10^{-6} M for uzarigenin. Because of the limited solubility of sarmentogenin the maximum concentration studied was 2×10^{-6} M which inhibited cesium uptake about 36%. Inhibition of cesium uptake by cymarin was not reversed during a 3.5 hr incubation in fresh solution while the muscles treated with ouabain and strophanthidin recovered partly during this time. Cymarin was a more potent inhibitor of sodium efflux than strophanthidin and periplogenin was less potent. Increased cesium ion concentration in the external solution decreased the strophanthidin inhibition of cesium uptake but 25 mM cesium did not overcome the inhibition by 10^{-8}–10^{-6} M strophanthidin. Increased potassium ion concentration in the external solution decreased but did not completely overcome inhibition of sodium efflux by strophanthidin. It is concluded that potassium or cesium ions do not compete with these drugs for a particular site on the ion transport complex. The same structural features of the drugs are necessary for inhibition of ion transport in frog muscle as are required for inhibition of ion transport in other tissues, inhibition of sodium-potassium–stimulated ATPases, and toxicity to animals.

INTRODUCTION

Muscles enriched with sodium will take up cesium ions from the external solution and extrude sodium ions (Beaugé and Sjodin, 1968). Although the mechanism of this ion movement is unknown, both the cesium uptake and part of the sodium efflux are sensitive to the cardiotonic steroids such as ouabain and strophanthidin (Sjodin and Beaugé, 1967; Beaugé and Sjodin, 1968). As most studies of ion transport in frog muscle have used only high concentrations of

these drugs, 10^{-5} M, to inhibit the ion fluxes it is of interest to determine the effects of lower dosages of these drugs. Also, certain structural requirements for the drugs have been determined by measuring inhibition of ion transport by the drug in red blood cells and the lethal dosage in cats for a variety of

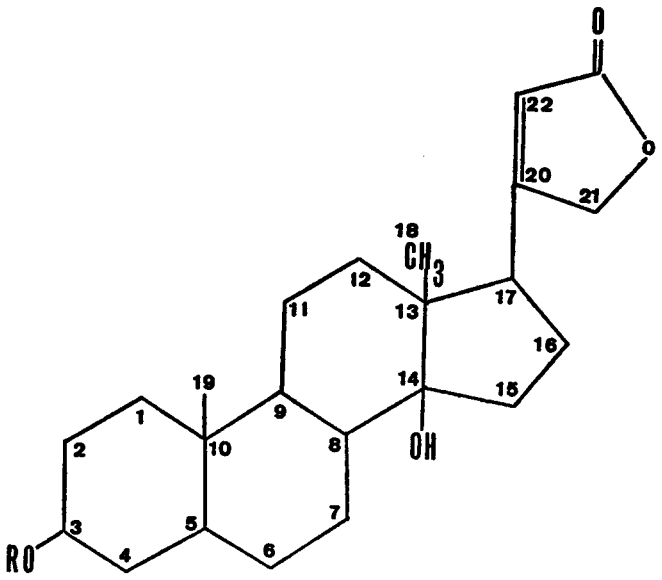

FIGURE. 1. Basic cardenolide structure.

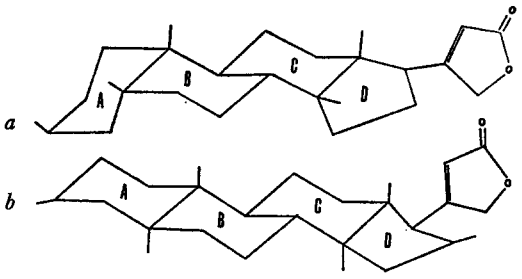

FIGURE 2 a. Cardiac drugs.

FIGURE 2 b. Other hormones.

these drugs (Kahn and Acheson, 1955; Kahn, 1957; Glynn, 1957; Machova, 1961; Chen, 1963; Tamm, 1963). Machova found a direct relationship between the mean lethal dosage in cats and the concentration required for 50% inhibition of potassium ion uptake in red blood cells. The ability of several active cardiac drugs to inhibit ion transport in frog muscles is compared in this work.

Several structural requirements found to be important for the activity of the drugs in other systems are (*a*) that the rings of the cyclopentanophenanthrene nucleus are joined A/B-*cis*, B/C-*trans*, and C/D-*cis*; (b) that there is a hydroxyl group β at C-14 and either a hydroxyl group or a glycosidic link β at

C-3; and (*c*) that the α,β-unsaturated five-membered lactone or a hexadienolide ring is joined β at C-17. Fig. 1 shows the basic structure of the drugs with their numbering system. The cardiac drugs are shaped differently from the

TABLE I

CARDENOLIDES TESTED*, ‡

Drug	R	C-19	Joining of A/B rings§	Substituents
Sarmentogenin	H	CH_3	5-β	11-α-OH
Periplogenin	H	CH_3	5-β	5-β-OH
Digitoxigenin	H	CH_3	5-β	—
Uzarigenin	H	CH_3	5-α	—
Strophanthidin	H	CHO	5-β	5-β-OH
Cymarin	D-Cymarosyl	CHO	5-β	5-β-OH
Ouabain	L-Rhamnosyl	CH_2OH	5-β	1-β-OH, 5-β-OH, 11-α-OH

* Adapted from Fieser and Fieser (1959).

‡ Refer to Fig. 3 for the location of the substituents.

§ 5-β, *cis*; 5-α, *trans*.

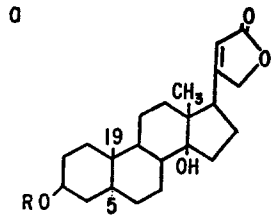

FIGURE 3 a. The basic cardenolide structure

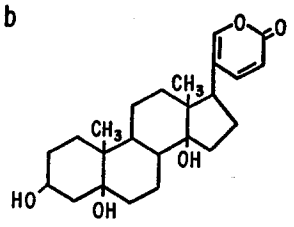

FIGURE 3 b. Telocinobufagin. Refer to Table 1 for the structural details of the drugs tested.

other common hormones such as the adrenal and sex hormones. In the cardiac drugs the *cis* fusion of rings A/B and C/D gives the drugs a very bent configuration while the other hormones have all their rings fused *trans* (Fig. 2). The crystal structure of the simplest of the cardiac drugs, digitoxigenin, has just been determined and illustrates clearly this bent configuration of the molcule.[1] In the cardiac drugs the 3-β-hydroxyl group and 1-β and 5-β groups are axial

[1] Karle, I. L., and J. Karle. 1969. The crystal structure of digitoxigenin, $C_{23}H_{34}O_4$. *Acta Cryst., Sect. B.* In press.

with respect to their rings. The five-membered lactone ring of the cardenolide is β at C-17 and the plane of the lactone is almost perpendicular to that of the D ring of the steroid.[1] The hexadienolide ring, a six-membered unsaturated lactone, is attached β to C-17 in the bufadienolides such as telocinobafagin. The cardenolides are more active as monosides of the C-3 hydroxyl group than are the free genins or oligosaccharides. The bufadienolides are more active as the aglycones. Several other molecular characteristics have been found to increase or decrease the activity of the drugs but the pertinent ones will be mentioned while discussing the drugs used in this research. These drugs are listed in Table I and Fig. 3. A similarity in the structural requirements of the drugs for inhibition of transport in different systems and inhibition of the sodium-potassium–stimulated transport adenosine triphosphatase (ATPase) would indicate that these transport systems were similar (Skou, 1965).

METHODS AND MATERIALS

Preparation of Muscles Sartorius muscles from freshly killed frogs, *Rana pipiens*, were carefully dissected and mounted as described by Sjodin and Henderson (1964). Summer frog muscles (April to October) were enriched with sodium by storage for 16–20 hr at 4–5°C in a potassium-free Ringer solution. Winter muscles were enriched similarly for 24 hr with only one muscle per 5 ml Ringer solution, then switched to fresh potassium-free Ringer solution and stored in the cold another 16–20 hr with two muscles per 5 ml. The potassium-free Ringer solution for winter muscles contained 5 mg streptomycin per liter to reduce bacterial contamination. In the sodium efflux experiments high specific activity ^{22}Na (50–100 μl of a 1 mc/ml $^{22}NaCl$ solution) was added to the muscles during the last 20 hr of cold storage. These methods were adapted from Carey and Conway (1954), Sjodin and Henderson (1964), and Beaugé and Sjodin (1968). As cesium uptake and sodium efflux depend on the internal concentration of sodium, all muscles were enriched with sodium in order to have them in an approximately equal condition (Beaugé and Sjodin, 1968; Sjodin and Beaugé, 1968). The dependence of the strophanthidin-sensitive sodium efflux on internal sodium concentration is not so steep for high sodium muscles as it is for fresh or only slightly sodium-enriched muscles (Sjodin and Beaugé, 1968).

Materials The basic Ringer solutions are described in Table II. Strophanthidin and ouabain were purchased from Sigma Chemial Co. (St. Louis, Mo.) and cymarin and digitoxigenin were purchased from Boehringer Mannheim Corp. A crystalline preparation of strophanthidin was a gift of Dr. A. Hofman of Sandoz (Pharmaceutical Department, Sandoz Ltd., Basel, Switzerland). Dr. T. Reichstein kindly made a gift of periplogenin, sarmentogenin, and strophanthidin (Institut für Organische Chemie, Universität Basel, Switzerland). Dr. K. Meyer made a gift of uzarigenin and telocinobufagin (Pharmazeutisches Institut der Universität, Basel, Switzerland). All drugs were prepared as 20 mM and 2 mM solutions in ethanol and stored in the cold until used. Uzarigenin and sarmentogenin were not completely soluble at 20 mM. These solutions were allowed to settle and 50 μl aliquots of the saturated solutions were used per 100 ml Ringer solution. The concentrations of all the drugs, exceps telocino-

bufagin, were determined by their absorbance at 220 nm for solutions of 10^{-5}–10^{-6} M. The molecular extinction coefficient, ϵ, for many cardiac glycosides and genins is similar, being about 1.6×10^4 for strophanthidin in water (Parsons, 1965; Dorfman, 1953).

Procedure for Cesium Uptake The procedure for measuring cesium uptake with ^{134}Cs as a tracer was the same as described by Beaugé and Sjodin (1968). Cesium uptake was used rather than that of potassium or rubidium because cesium uptake is the most sensitive to the action of strophanthidin (Beaugé and Sjodin, 1968). After control uptake rates had been established (2–2.5 hr), the muscles were placed in a similar

TABLE II
RINGER SOLUTIONS*

Solution	Concentration				
	NaCl	KCl	CsCl	Sucrose	Tris-Cl (85% neutral)
	mM	mM	mM	mM	
A	105	2.5	—	—	1
B	105	7.5	—	—	1
C	105	12	—	—	1
D	80	2.5	—	36	1
E	105	—	2.5	—	1
F	80	—	2.5	32.4	1
G	80	—	5.0	28.8	1
H	80	—	15	14.4	1
I	80	—	25	—	1
J	105	—	—	—	1
K	—	—	—	—	116
L	80	—	—	36	1

* pH adjusted to 7.2–7.3 with HCl. All solutions contained 2 mM $CaCl_2$.

Ringer solution which contained the drug being tested. The rates of cesium uptake are linear for at least 20 hr in the absence of a drug (Beaugé and Sjodin, 1968). The control solutions contained amounts of ethanol equal to those in the drug solution. Except in two experiments with 10^{-4} M drug, the most ethanol used was 50 μl per 100 ml Ringer solution, about 8 mM. This concentration had no effect on uptake. Ethanol does inhibit a brain sodium-potassium–stimulated ATPase but only about 10–20% at 100 mM (Israel and Salazar, 1967). Since the experiments with 10^{-4} M drug contained about 80 mM ethanol it is possible that the rates were inhibited 10% but control muscles were placed in an equal amount of ethanol so that the comparative inhibition was due to the drug. Inhibited uptake was followed for 2.5 hr. The estimation of extracellular space and cation analysis followed the methods of Beaugé and Sjodin (1968) and Sjodin and Henderson (1964). In the experiments comparing the effects of increasing cesium concentration on strophanthidin inhibition, Ringer solutions F, G, H, and I were used, and the amount of isotope added to the uptake solutions was increased.

Procedures for Sodium Efflux The method for sodium efflux has been described by Sjodin and Henderson (1964). For the first hour efflux for both muscles was followed into potassium-free Ringer solution (J or in some cases L); then one muscle was tested in a potassium plus drug-Ringer solution and the control muscle in a similar solution without drug. The cation analysis was by the same method used for cesium uptake. Some experiments were done with cesium slution (I) instead of potassium solution to stimulate the sodium efflux.

RESULTS

Inhibition of Cesium Uptake

Fig. 4 shows typical data from an experiment in solution E using 10^{-6} M strophanthidin to inhibit cesium uptake, 79% in this case, in both muscles

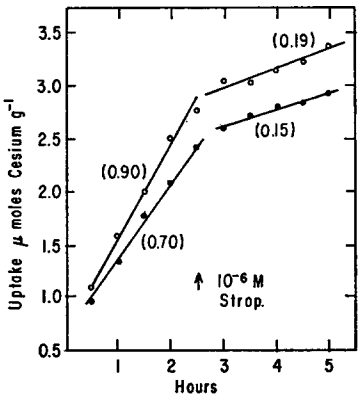

FIGURE 4. Uptake from 2.5 mM Cs Ringer solution was followed for 2.5 hr. Both muscles were changed to a similar solution containing 10^{-6} M strophanthidin and uptake measured for 2.5 hr. The numbers in parentheses are the uptake rates in micromoles per gram wet weight per hour.

after 2.5 hr control uptake. Similar experiments were done at other concentrations in order to determine the dose-activity curve for strophanthidin (Fig. 5 a). Although there is a slight increase in activity at 10^{-10} M this is probably not significant as 10^{-11} and 10^{-12} M had no effect. The average internal sodium concentration for the summer muscles was 23.3 ± 0.8 μmoles/g wet weight. Winter muscles revealed a slightly different sensitivity towards strophanthidin. When the cold storage time was increased to about 40 hr in potassium-free solution, the internal sodium concentration was raised and this resulted in the drug sensitivity being similar to that of summer muscles. Table III shows the average internal sodium concentrations from a number of experiments. The half-inhibition of cesium uptake for summer muscles or very high sodium winter muscles occurred with 1.5–2 × 10^{-7} M strophanthidin.

A comparison of the activity of strophanthidin, cymarin, ouabain, and sarmentogenin is shown in Fig. 5 b. Cymarin has a steroid structure similar to that of strophanthidin except that it has a D-cymarose in a glycosidic link to the 3-β-hydroxyl group. In ouabain the sugar is L-rhamnose which is also

linked to the 3-β-hydroxyl group. Ouabain has 1-β- and 11-α-hydroxyl groups not found in cymarin. Also the C-19 group is an alcohol rather than a carbonyl. The testing of sarmentogenin to determine whether the 11-α-hydroxyl group

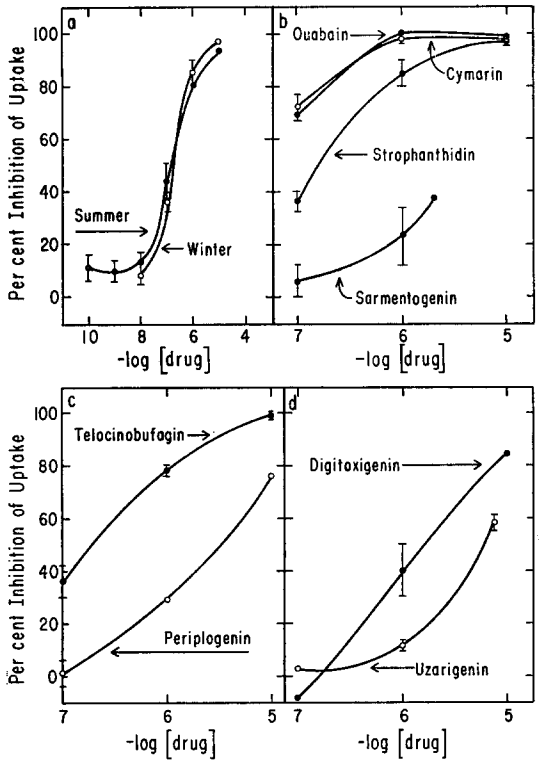

FIGURE 5 a. The points are average inhibitions of cesium uptake by strophanthidin. The summer muscles were stored 16–20 hr in potassium-free Ringer solutions at 5°C while the winter muscles were similarly treated for about 40 hr. Uptake was measured from a 2.5 mM cesium solution. In this and other figures, bars represent SE. b. The muscles were stored 40 hr at 5°C in K-free Ringer solution. Each point is the average uptake by two muscles except 10^{-7} M strophanthidin which is averaged from four muscles. c. Inhibition of cesium uptake from 2.5 mM cesium Ringer solution was measured with winter muscles stored 40 hr at 5°C in a K-free Ringer solution. Each point is the average of two muscles. d. A comparison of the inhibition of cesium uptake from 2.5 mM Cs Ringer solution by the *cis*-A/B configuration, digitoxigenin, and the *trans*-A/B form, uzarigenin. Each point is the average from two winter muscles stored 40 hr in K-free Ringer solution at 5°C.

is the major reason for the increased activity of ouabain seems to indicate that it is not an important change. Sarmentogenin is also lacking the 5-β-hydroxyl group and either an alcoholic or carbonyl group at C-19 which may account for its lower activity and solubility.

Fig. 5 c compares periplogenin and telocinobufagin. In these two drugs the

steroid is the same except that in telocinobufagin there is a six-membered unsaturated lactone ring. Telocinobufagin is 15 to 20 times more active than periplogenin. with a five-membered lactone, and about as active as strophan-

TABLE III
INTERNAL SODIUM CONCENTRATIONS

Hr*	Muscles	Treatment	Na
			μmoles/g wet weight
20	29‡	Strophanthidin	23.3±0.8§
20	8	"	24.0±1.0
40	12	"	33.8±1.5
20	19	Cymarin	25.0±1.3
40	6	"	38.4±1.8
20	10	Periplogenin	22.9±1.4
40	6	"	33.1±3.0
20	18	Digitoxigenin	21.2±1.7
40	6	"	36.5±4.4

* The number of hours the muscles were stored in potassium-free Ringer solution at 5°C.
‡ These muscles were from summer frogs while the rest of the table is for winter muscles.
§ The values are ± SE.

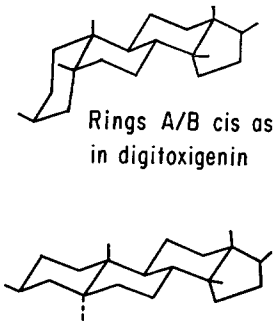

FIGURE 6. An illustration of the difference between the *cis*- and *trans*-junction of the A and B rings of digitoxigenin and uzarigenin.

thidin. The difference between the isomers, digitoxigenin-uzarigenin, is that the A/B rings are joined *trans* rather than *cis* in uzarigenin making the molecule more open than digitoxigenin (Fig. 6). This change results in a slight loss in activity for uzarigenin (Fig. 5 d). Digitoxigenin, periplogenin, and uzarigenin are less active than strophanthidin. Periplogenin has only a C-19 methyl group rather than a carbonyl group and is 10 times less active. Digitoxigenin is lacking both the 5-β-hydroxyl group and C-19 carbonyl group but is slightly more active than periplogenin.

Recovery from Inhibition

The reversibility of inhibition by cymarin, ouabain, and strophanthidin was compared because cymarin and ouabain were more active than strophanthidin. In the experiments summarized in Table IV control uptake was followed

TABLE IV

REVERSIBILITY OF DRUG INHIBITION

The rates for each drug treatment averaged from four winter muscles stored 40 hr at 5°C in K-free Ringer solution. The recovered rate is to be compared with the initial rate measured on the same muscle. All rates are ± SE.

Drug 10^{-5} M	Control rate	Inhibited rate	Recovered rate after 3.5 hr
		$\mu mole.\ g^{-1}.\ hr^{-1}$	
Strophanthidin	1.15±0.14	0.09±0.02	0.60±0.07
Ouabain	1.03±0.06	0.10±0.02	0.29±0.02
Cymarin	1.26±0.10	0.11±0.02	0.11±0.02

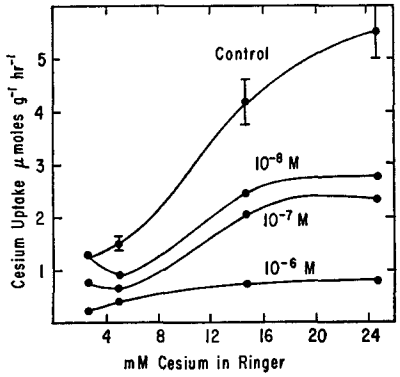

FIGURE 7. Cesium uptake in the presence and absence of strophanthidin was measured in various cesium Ringer solutions. Uptake was measured for 2.5 hr for the control rates and inhibited rates. The summer muscles were stored 20 hr at 5°C in K-free Ringer solution. The control points are averages of six muscles except for 15 mM Cs which is from three muscles. The other points are averages of two muscles except 15 mM points which are from one muscle.

for 2–2.5 hr, inhibited uptake for another 2 hr, and recovery for 3.5 hr. A longer recovery time, 5–8 hr, would have allowed the strophanthidin-treated muscles to recover completely (Beaugé and Sjodin, 1968). In this comparison the strophanthidin-treated muscles recovered to about 50% of their initial rates, ouabain-treated ones to 25% of their initial rates, and cymarin-treated ones not at all. Since cymarin and ouabain are about equally potent inhibitors of cesium uptake, their different behavior here might be due to the different sugars.

Effect of Raising the External Cesium Concentration

A series of experiments measuring the inhibition of cesium uptake by strophanthidin at different external cesium concentrations is shown in Fig. 7. As the cesium concentration was raised there was some reduction in the inhibition, but Table V shows that the per cent inhibition with 10^{-6} M was about 80% at all cesium concentrations. Even at the lower drug concentrations the inhibition was not removed by 25 mM cesium.

Inhibition of Sodium Efflux

The ability of the drugs to inhibit sodium efflux was compared using sodium-rich muscles labeled with ^{22}Na (Fig. 8). In this experiment efflux from a pair of

TABLE V

PER CENT INHIBITION OF CESIUM UPTAKE BY STROPHANTHIDIN*

	Concentration of strophanthidin Per cent inhibition		
External cesium	10^{-6} M	10^{-7} M	10^{-9} M
mM			
2.5	82	40	0(+9)‡
5.0	77	49	36
15	82	58	37
25	84	60	50

* The data are from Fig. 10.

‡ In this case 10^{-8} M stimulated cesium uptake 9%.

muscles into potassium-free Ringer solution (J) was followed for 1 hr. Then efflux from one muscle was followed in 2.5 mM potassium Ringer solution (A) and compared with efflux from the other muscle into a similar solution containing 10^{-7} M strophanthidin. In this case there was a 25% inhibition of the potassium-stimulated efflux. A concentration of 10^{-5} M strophanthidin blocked all the potassium-stimulated sodium efflux. A dose-activity curve for strophanthidin inhibition of sodium efflux was prepared. The curve in Fig. 9 was steeper than that for cesium uptake, going from 100% inhibition at 10^{-6} M to 0% at 10^{-8} M strophanthidin. In order to use cesium to stimulate the sodium pump the cesium concentration was raised to 25 mM and consequently the external sodium concentration was lowered to 80 mM for an osmotically balanced solution. To insure that lowered sodium concentration did not affect the sensitivity, the inhibition produced by strophanthidin was measured with only 80 mM sodium outside (solutions L and D). The per cent inhibitions were not significantly different even though the absolute rates were somewhat higher in the lower sodium solutions.

The data in Table VI show the inhibition of sodium efflux by cymarin, strophanthidin, and periplogenin when either solution A (2.5 mM potassium) or solution I (25 mM cesium) was used to stimulate the pump. Also included

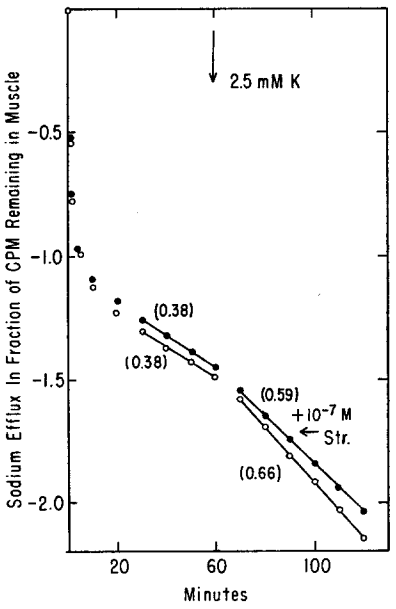

FIGURE 8. Sodium efflux was measured into potassium-free Ringer solution for 1 hr and then into 2.5 mM potassium Ringer solution for another hour. During the time in potassium solution one muscle was also exposed to 10^{-7} M strophanthidin. There is a 25% inhibition of the potassium-stimulated sodium efflux. The numbers in parentheses are the rates of sodium efflux (hr^{-1}). The summer muscles were stored in potassium-free Ringer solution at 5°C for about 20 hr.

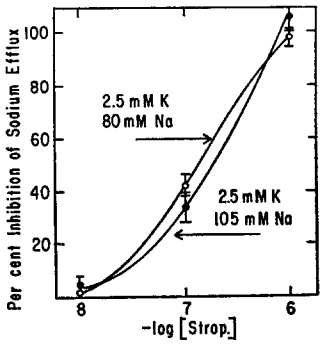

FIGURE 9. The per cent inhibitions of the K-sensitive sodium efflux were measured under conditions similar to those in Fig. 8. One set used 80 mM sodium solutions, the other 105 mM sodium Ringer solutions. Each point is the average from two pairs except 10^{-7} M strophanthidin in 105 mM sodium solution which is the average of four pairs.

for comparison are the same drugs' inhibition of cesium uptake. The drugs fall into the same relative order for inhibition of sodium efflux or cesium uptake.

Table VII compares the inhibitions of both assays by 10^{-6} and 10^{-7} M strophanthidin in 25 mM cesium Ringer solution. Each experimental point was the average of two experiments. As was seen in the dose-activity curve

for strophanthidin the sensitivity curve for sodium efflux inhibition was steeper than that for cesium uptake. The higher concentration, 10^{-6} M, inhibited sodium efflux more than cesium uptake while 10^{-7} M inhibited the efflux less than the uptake.

TABLE VI

COMPARISON BETWEEN THE ABILITY OF VARIOUS DRUGS TO INHIBIT SODIUM EFFLUX AND CESIUM UPTAKE

		Per cent inhibition	
		Sodium efflux	Cesium uptake
Drug	Concentration	Solution A 2.5 mM K	Solution E 2.5 mM Cs
	M		
Cymarin	10^{-6}	100% of K* stimulated (21% of K-free rate)	98±2%
Strophanthidin	10^{-6}	100% of K stimulated	85±5%
Periplogenin	10^{-6}	34% of K stimulated	29±1%
		Solution I 25 mM Cs	Solution E 2.5 mM Cs
Cymarin	10^{-7}	58% of Cs stimulated	72±5%
Strophanthidin	10^{-7}	39% of Cs stimulated	36±4%
Periplogenin	10^{-7}	35% of Cs stimulated	(1±5%)

* This drug inhibited all the potassium-stimulated rate and reduced the efflux to a rate 21% below the rate into the potassium-free solution without drug.

TABLE VII

INHIBITION OF CESIUM UPTAKE AND SODIUM EFFLUX IN 25 mM CESIUM RINGER SOLUTION

	Per cent inhibition	
Strophanthidin	Cesium uptake	Sodium efflux
M		
10^{-6}	84	100 of Cs stimulated, 22 of Cs-free
10^{-7}	60	39

Effect of Increasing External Potassium Concentration

The inhibition of sodium efflux at three outside potassium concentrations was measured to determine whether potassium ion competes with the drug for the enzyme site. Fig. 10 compares the rates of sodium efflux, not corrected for the potassium-free efflux rates, in the various potassium Ringer solutions. One can see that at low potassium concentrations, 10^{-5} and 10^{-6} M strophanthidin reduced the sodium efflux to rates lower than those found in potassium-free

solutions. The lowest concentration tested, 10^{-8} M, is not shown as it was not significantly different from the control. The other concentrations show inhibition at all potassium concentrations. Although some interaction was observed the results do not indicate competitive inhibition. The presence of a large potassium-free sodium efflux and the fact that high concentrations of the drug can inhibit some of this efflux, make the mechanism of transport more difficult to interpret.

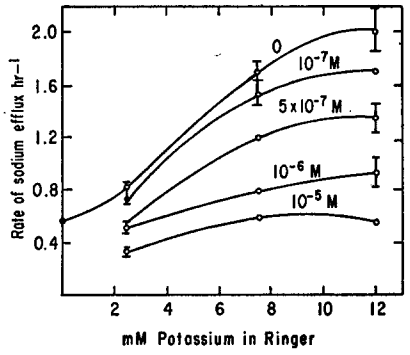

FIGURE 10. Total sodium efflux was measured into potassium Ringer solutions with strophanthidin. The potassium-free rate is from 68 muscles while the other drug-treated rates are averages from 2 pairs. The control curve is an average of 16 pairs for 2.5 mM K, 10 for 7.5 mM K, and 8 for 12 mM K solutions.

DISCUSSION

Several slightly different cardiac drugs were used in these experiments in the hope that a knowledge of the structural requirements for drug activity might help to identify the nature of the drug-sensitive site on the membrane. This work examined several structural modifications of the drugs with respect to inhibition of ion transport in frog muscle. The *trans*-A/B form, uzarigenin, showed reduced activity compared with the *cis*-A/B isomer, digitoxigenin; the doses for 50% inhibition being 6.3×10^{-6} M for uzarigenin and 1.6×10^{-6} M for digitoxigenin. The lethal dosage in cats for uzarigenin is about twice that needed for digitoxigenin (Tamm, 1963). Kupchan and coworkers (1967) compared several cardiac steroids for their cytotoxic activity and ATPase inhibition. In both assays activity of the drug was reduced with the change from *cis*-A/B to *trans*-A/B. In frog muscle the bufadienolide, telocinobufagin, was a more potent inhibitor of transport than the similar cardenolide, periplogenin. Telocinobufagin was also found to be four times more potent for inhibiting transport in red blood cells than periplogenin (Kahn, 1957). Another bufagin, hellebrigenin, has been found to be more active against a sodium-potassium ATPase than its cardenolide isomer, strophanthidin (Ruoho et al., 1968). However, compounds with saturated lactones

were always less potent inhibitors of transport than their corresponding glycosides with unsaturated lactones and often less potent than the free genins of the same compounds (Kahn, 1957).

Another variation examined was the C-19 methyl group compared with the C-19 aldehyde or hydroxymethyl groups. The C-19 aldehyde group resulted in a more potent compound than one with a C-19 methyl group as seen in the comparison of activity of strophanthidin and periplogenin. The same relationship was found for lethal dosages in cats (Chen, 1963; Tamm, 1963). A C-19 hydroxymethyl group as on ouabain results in a more active compound than one with the methyl group.

The importance of the 5-β-hydroxyl group and of the glycosidic sugars was examined. The 5-β-hydroxyl groups in strophanthidin, cymarin, periplogenin, and telocinobufagin result in increased potency for these drugs (Chen, 1963). Although 11-α-hydroxyl groups can give increased activity to the compound, the lack of oxygen-containing groups at C-19 and C-5 in sarmentogenin results in a weaker compound. In the cardenolides the monosides are usually the most active (Tamm, 1963; Chen, 1963). Cymarin and ouabain show this characteristic when cymarin is compared with strophanthidin. The presence of the cymarose gives cymarin increased activity especially at lower concentrations. Cymarin behaves much like ouabain which also is a monoside with rhamnose. Muscles treated with the glycosides, ouabain and cymarin, remained inhibited longer than the ones treated with the free genin, strophanthidin, when they were resuspended in drug-free control solutions. A comparison of the inhibition of sodium efflux by strophanthidin, cymarin, and periplogenin with their inhibition of cesium uptake revealed no large differences in their relative activities in both assays.

The results of the structural comparisons show the same structure-activity relationships which have been found in other systems such as red blood cell sodium-potassium transport. The most important area of the drug appears to be the unsaturated lactone and its β-link to C-17 of the D ring of the steroid. Sugars at the C-3 position also increase the toxicity of the drugs but other changes, especially at C-19, are not as important.

Although the concentrations of the drugs were measured in solutions of 10^{-6}–10^{-5} M before an experiment, no attempt was made to devise a system for determining lower concentrations of the drugs, or to measure the concentration remaining in the solution after an experiment. Such measurements of the drug concentrations after an experiment might indicate that one reason for lower activitiy of a drug was that this drug was less well-absorbed by the muscle than the other drugs. Also, from the concentration of drug taken up by the muscle one might estimate how many active or drug-sensitive sites were on the muscle. However, since the whole sartorius muscle was used rather than a single fibril and because the extracellular space of the muscle is large, 22%,

the estimate would not be very reliable. There also appears to be a certain amount of unspecific binding of these drugs which would increase the error in any estimate of the number of drug-sensitive sites (Kupchan et al., 1967).

The early work by Glynn (1957) implied that scillaren A inhibition could be removed by increasing the potassium concentration. More recent experiments have shown that potassium does not compete with the drugs for sites. In fact, the amount of inhibition obtained depends on both the external potassium concentration and the internal and external sodium concentrations with external sodium ion increasing the observed inhibition by the drugs (Whittam and Ager, 1962; Repke and Portius, 1963; Kinsolving, Post, and Beaver, 1963; Schatzmann, 1963, 1965; Auditore, 1964, 1965; Baker and Connelly, 1966).

Matsui and Schwartz (1966) have examined the competition between potassium ions and the drugs on a cardiac sodium-potassium–dependent ATPase. They found that the interaction was noncompetitive and depended on the sodium to potassium ratio in the solution. At a constant sodium concentration, the per cent inhibition of the ATPase by ouabain decreased with increasing potassium concentration while conversely, at a constant potassium concentration the per cent inhibition increased with increasing sodium concentration. The resulting kinetics were difficult to determine because the sodium and potassium ions are competitive inhibitors of their respective activating sites. In their experiments when the ratio of sodium to potassium in the medium was kept constant at 10 to 1 the double reciprocal plots, rate^{-1} vs. $[K]^{-1}$, became linear and indicated noncompetitive inhibition. Matsui and Schwartz concluded that the ouabain-binding site was different from the potassium site.

In these experiments the interaction between the drugs and potassium ion with respect to sodium efflux indicated that potassium ion could not prevent completely inhibition by strophanthidin. The same situation was true for inhibition of cesium uptake when increased cesium concentration did not completely remove strophanthidin inhibition. This indicated that the drugs were not truly competitive with potassium ion for a site on the transport enzyme. Since there is some sort of interaction one might suppose that the attachment of potassium ion or the drug to the enzyme changes the affinity of the enzyme toward the other.

SUMMARY

These studies indicate that the same structural details of the cardiac drugs, as are required to inhibit sodium-potassium–stimulated ATPase in cell membranes, and to cause heart fibrillation and death in cats and other animals, are important for their ability to inhibit sodium-potassium transport across cell membranes. The comparison of several cardiac drugs with different sub-

stituents on the basic steroid showed that the *cis*-A/B configuration of the drug was a more active inhibitor of transport than the *trans*-configuration, a bufadienolide was more active than the similar cardenolide, the presence of a C-19 aldehyde or hydroxymethyl group resulted in a more active compound than the presence of a C-19 methyl group, a 5-β-hydroxyl group resulted in a more active compound than one with a 5-β-hydrogen, and monosides of the cardenolides were more active than the free genins. The most important area of the drug is the unsaturated lactone and its β-link to C-17. These drugs act on the outside of the membrane but not at the potassium site since potassium and the other ions do not show true competition with the drugs. The attachment of the drugs to the membrane seems to be slowly reversible in the case of the genins and very slowly or not at all reversible in the case of the glycosides.

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