THE INHIBITORY EFFECT OF STROPHANTHIDIN ON SECRETION BY THE ISOLATED GASTRIC MUCOSA

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ABSTRACT

The unidirectional fluxes of Na^+ and Cl^- were measured across the isolated gastric mucosa of the bullfrog (*R. catesbiana*). The addition of strophanthidin, a cardiac aglycone, resulted in marked reductions of the spontaneous potential and short-circuit current. Associated with these changes, the isolated gastric mucosa ceased secreting chloride and hydrogen ion. Although the active component of chloride transfer was inhibited, the exchange diffusion component seemed to increase. No significant changes in membrane conductance or sodium flux were noted.

Possible mechanisms of strophanthidin inhibition were discussed in view of its effect on chloride transport across the gastric mucosa and on sodium and potassium transfer in other tissues. It was concluded that the cardiac glycosides may not be specific inhibitors of sodium and potassium transport. This non-specific inhibition suggests that active chloride transport is affected by strophanthidin directly and/or anion secretion is dependent upon normal functioning of cation transport systems in the tissue.

INTRODUCTION

The cardiac glycosides have been studied in considerable detail with respect to their "selective" inhibitory effect upon the movement of sodium and potassium across biological membranes. The purposes of this work were: first, to study their effect upon the active transport of other ions such as hydrogen and chloride across the gastric mucosa; and second to observe the effect of these agents upon the electrical properties of the gastric mucosa.

The sturdy tissue of the frog is ideal for obtaining information about the electrical properties and associated ion transfer across the stomach (1). The isolated gastric mucosa actively secretes chloride and hydrogen ion and can maintain transepithelial potential differences for long periods; the mucosal surface being negative with respect to the serosal surface. Subsequent studies on the isolated gastric mucosa have provided detailed information about the effect various metabolic inhibitors and pharmacological agents have upon the transfer of hydrogen ion and chloride (2–4). The effects of strophanthidin were observed and then contrasted with these results.

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METHODS

The stomach of the bullfrog, *R. catesbiana*, was used. After pithing, the stomach was isolated and stripped of its muscular coat by blunt dissection. The gastric mucosa was clamped between two lucite chambers; both chambers were subdivided into two compartments (2). Each of the four compartments of the gastric chamber assembly contained 20 cc. of electrolyte solution separated from its opposing compartment by 5 $cm.^2$ of tissue. The terms mucosa and serosa were used rather than secretory and nutrient to describe the direction of transfer.

Solution.—The solution used was buffered saline of the following composition: NaCl 87 mM, $NaHCO_3$ 20 mM, KCl 2.5 mM, Na_2HPO_4 1.3mM, NaH_2PO_4 0.3 mM, $CaCl_2$ 1.25 mM, $MgSO_4$ 0.5 mM, and glucose 5 mM. 95 per cent O_2 and 5 per cent CO_2 were used to gas the solution in each compartment, the initial pH being 7.3. Strophanthidin (Bios Laboratories, Inc.) dissolved in ethanol was subsequently added to buffered saline, and a calculated aliquot of this solution added to each compartment. The same concentration of ethanol when added had no observable effect on short-circuit current or potential. The fluxes of sodium and chloride were determined simultaneously with Na^{22} and Cl^{36}. The details of the radioactivity assay and flux calculations have been described previously (5).

Electrical.—The means by which the potential difference across the membrane was measured and adjusted to any desired level have been described (5). When flux measurements were made across the gastric mucosa the potential was maintained at zero: this is the short-circuited state (6). The electrical current data and ionic fluxes are expressed in μeq. $cm.^{-2}$ $hr.^{-1}$.

The gastric mucosa has been demonstrated to show transients in applied current or polarization effects when the membrane is short-circuited and they have been analyzed by Durbin and Heinz (4) in a previous study, but only the steady state values were used here. The total membrane conductance was obtained from the ratio of short-circuit current and spontaneous potential (1). In order to calculate membrane conductance during inhibition, the values of applied current in raising the potential back to its previous level were used. The partial conductance of anion can be calculated with the equation (7):

$$k_{ion} = \frac{zF^2}{RT} \cdot M^0$$

in which M^0 equals the flux of the ion in question at zero mv. If M^0 is expressed in μeq. $cm.^{-2}$ $hr.^{-1}$ then it is approximately equal to k_{ion} if the ion is moving by free diffusion alone. Membrane conductance and partial ionic conductance are expressed as millimhos $cm.^{-2}$.

RESULTS

The spontaneous potentials recorded across the isolated gastric mucosa varied from 36 to 48 mv. mucosal surface negative. All the flux measurements were performed during the short-circuited state (0 mv.). Under these conditions, Hogben (1) has established the following relationship between applied current and the transfer of hydrogen and chloride:

$$\text{Net } H^+ = \text{Net } Cl^- - I \text{ (short-circuit current)}.$$

Two of the three unknowns, short-circuit current and net chloride flux have been measured during these experiments; from these values a close approximation of net hydrogen ion secretion into the mucosal solution was calculated. In Table I the net chloride flux of 4.3 μ eq. less the mean short-circuit current of 2.95 μeq. leaves a deficit of 1.3. This indicates the mean rate of acid secretion was 1.3 μeq. cm.$^{-2}$ hr.$^{-1}$ during these experiments.

Hogben has also pointed out another significant relationship with regard to the transfer of chloride (1). The mucosa to serosa flux or presumed passive flux is unusually large to be accounted for by free diffusion alone. This conclusion was reached by comparing the chloride conductance with the total membrane conductance. If the mean partial chloride conductance is calculated

TABLE I

Strophanthidin Effect on Chloride Transfer by the Gastric Mucosa at 0 Mv. (12 One Hour Flux Periods)

	Before	After strophanthidin
S → M	7.68 (±0.6)	5.00 (±1.1)
M → S	3.36 (±0.6)	4.44 (±0.7)
Net Cl^-	4.32	0.56
Current	2.95 (±0.3)	0.25 (±0.3)
H^+	1.37	0.29
Membrane Conductance, *mmhos. cm.*$^{-2}$	1.89 (±0.05)	1.77 (±0.2)

Ionic flux and current expressed as μeq. cm.$^{-2}$ hr.$^{-1}$

S → M, serosa to mucosa flux, M → S, mucosa to serosa flux.

(±) Standard deviation.

from the mucosa to serosa flux at 0 mv., its value, 3.4 mmhos cm.$^{-2}$, is significantly larger than the mean total membrane conductance of 1.9 mmhos cm.$^{-2}$ In Table II the data of a typical experiment show that during each flux period the mucosa to serosa flux of chloride is greater than the steady state total membrane conductance; this indicates that a portion of chloride transferred across the mucosa is non-conducting and this transfer has been called exchange diffusion (1).

The addition of strophanthidin to the bathing solutions caused a drop in short-circuit current and spontaneous potential; the effect on the latter is shown in Fig. 1. The concentration of 10^{-5} M strophanthidin was used and approximately 2 to 3 hours were required for the spontaneous potential to approach zero. The immediate effect was as rapid with a lower dose (10^{-6} M) of the aglycone but approximately 4 hours were required for complete inhibition. Subsequent bathing and rinsing with strophanthidin-free solutions resulted in a gradual return of the spontaneous potential and short-circuit current.

In every experiment there was a transient rise in potential of 2 to 4 minutes' duration and 3 to 8 mv. amplitude within 1 minute of strophanthidin addition. The significance of this observation is obscure. The rise was not always observed with smaller doses (10^{-6} M).

The effect of strophanthidin on the flux of chloride is illustrated in Tables I and II. The decrease in short-circuit current is due directly to the inhibition

TABLE II

Typical Experiment Illustrating the Effect of Strophanthidin upon Na^+ and Cl^- Fluxes across Gastric Mucosa at 0 Mv.

Time	S → M flux			M → S flux		
	Na^+	Cl^-	Current	Na^+	Cl^-	Current
hrs.						
0–1	0.32	7.69	2.72	0.39	2.79	2.68
1–2	0.33	7.17	2.68	0.36	3.14	2.84
2–3	0.42	7.13	2.63	0.40	2.98	2.89
Mean.....	0.36	7.33	2.68	0.38	2.97	2.80
3	K = 1.71					
3.10	Strophanthidin added					
5–6	0.57	4.45	0.04	0.63	3.83	0.07
6–7	0.61	3.61	0.01	0.63	4.06	0.02
7–8	0.67	4.50	0.0	0.60	3.73	0.01
Mean.....	0.62	4.19	0.02	0.62	3.87	0.03
8	K = 1.73			K = 1.68		

Ionic flux and current expressed as μeq. $cm.^{-2}$ $hr.^{-1}$

S = serosa; M = mucosa.

K = membrane conductance and is expressed as mmhos $cm.^{-2}$

of the electromotive chloride transport, since the serosa to mucosa flux decrease was observed in every flux period. When hydrogen ion secretion is calculated, 0.29 μeq. is the difference between net flux of chloride and short-circuit current. This figure of 0.29 may or may not be actual H^+ secretion, nevertheless it is significantly less than 1.3 and indicates that the secretion of both hydrogen ion and chloride by the isolated gastric mucosa is inhibited by strophanthidin addition.

In contrast to the decrease in the serosa to mucosa flux the mean flux of chloride from mucosa to serosa increased from 3.4 to 4.4 μeq. $cm.^{-2}$ $hr.^{-1}$ A comparison of the mucosa to serosa flux of chloride was made in each experiment

in order to evaluate the significance of this 1.0 μeq. difference. The experiment described in Table II is typical and reveals that in every period the chloride flux increases by at least 25 per cent after strophanthidin. The strophanthidin effect on the mucosa to serosa flux is distinctly different from that observed with anoxia and with dinitrophenol and other metabolic inhibitors (3). With the latter agents, the chloride flux decreases, and this decrease was attributed to inhibition of the exchange diffusion component. Metabolic inhibitors also

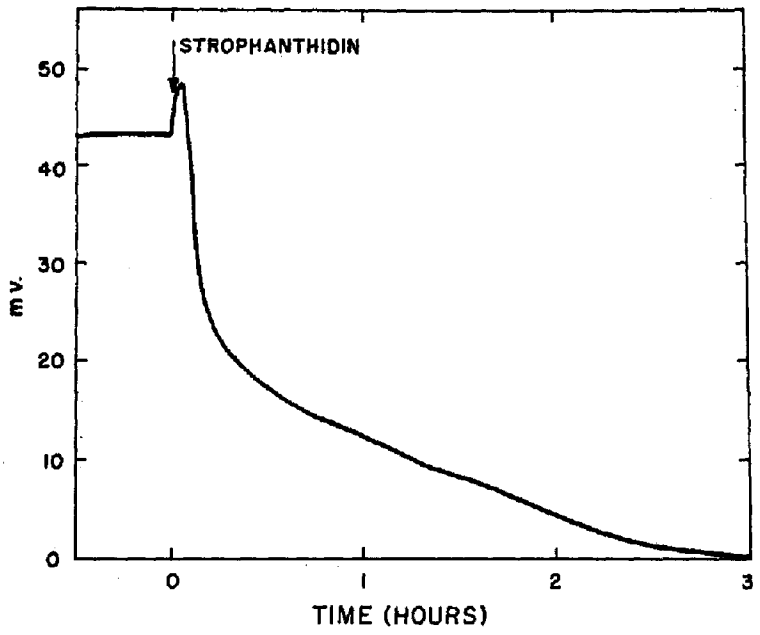

FIG. 1. The effect of strophanthidin addition (10^{-5} M) upon the transmucosal potential generated by the isolated frog gastric mucosa.

caused a more marked drop in the active serosa to mucosa flux than the decrease observed with the aglycone in these experiments.

No appreciable changes were observed in the total membrane conductance as presented in Tables I and II. The flux of sodium increased after the addition of strophanthidin as revealed in the data presented in Table II; smaller increases were observed in the other experiments. These two observations made on the inhibited tissue, showing no change in total membrane conductance and no decrease in sodium flux, suggest that the increase in the mucosa to serosa chloride flux is nonconducting and may represent an increase in the exchange diffusion component.

DISCUSSION

Following the demonstration of digitalis inhibition of K^+ reentry into the cardiac fiber (8), there have been numerous studies on the effects of the cardiac glycosides upon sodium and potassium transfer across skeletal muscle and red cells. The present data obtained with the isolated gastric mucosa demonstrate that the active secretion of both hydrogen ion and chloride is inhibited by strophanthidin. Hydrogen ion secretion by the chicken kidney is reduced when strophanthidin is infused into the leg vein (9). Recent study on the intestine has revealed that active secretion of bicarbonate is inhibited *in vivo* (10). The biochemical mechanism for bicarbonate secretion may be similar to but in the opposite direction from hydrogen ion secretion by the gastric mucosa. Hydrogen ion has been implicated with sodium and potassium exchange mechanisms and it is not difficult to visualize glycoside inhibition of hydrogen secretion. However, the inhibition of active chloride secretion warrants further discussion.

The gastric mucosa of the frog contains two cell types, the cells lining the gastric tubule which are analogous to the mammalian parietal cell, and the surface epithelial cell which secretes mucus (2). At present, no clear evidence is available indicating at which site either chloride or hydrogen is secreted. There is no evidence that the acid fraction and the current fraction of the net chloride transport result from the same mechanism or separate mechanisms. Durbin and Heinz have presented evidence that the generated current and acid secretion involve different mechanisms (4). The inhibitor, strophanthidin, is not selective and inhibits both net active chloride secretion and hydrogen ion secretion.

One of the most interesting facets of chloride transfer by frog gastric mucosa is the observation that three mechanisms are involved: passive diffusion, exchange diffusion, and active transport. The passive component cannot be quantitated but is assumed to be a major fraction of total membrane conductance (3). The bulk of chloride transfer can be presumed to result from the two remaining mechanisms: exchange diffusion and active transport. Each of these means of transport may reside in the same cells or different cells. However, Heinz and Durbin (3) have considered that both of these mechanisms are mediated by the same carrier after observing that metabolic inhibition lowers both components and that lowering the chloride in the mucosal solution, paradoxically reduces chloride transfer from serosa to mucosa.

If we accept this formulation and then assume that strophanthidin acts directly on the chloride transport system it may be possible to localize this action. The exchange diffusion component of chloride transfer is intact, and possibly increases. Thus the inhibition of chloride secretion cannot be due to (*a*) carrier breakdown or failure of synthesis, (*b*) blockage at the carrier site, (*c*) a significant permeability change to freely diffusing ions since the total

membrane conductance is not significantly altered. It could be acting at the step where energy is utilized during active chloride secretion.

The above assumptions were based on the existence of the same carrier for active transport and exchange diffusion. If this model does not represent the actual situation, active transport and exchange diffusion of chloride may occur at different sites either in the same cell or in different cells. The aglycone inhibits Cl^- transfer at the active transport site, but does not influence the exchange diffusion site. The latter mechanism is assumed not to require energy although it is sensitive to metabolic inhibition.

The observed effect on active chloride secretion may be secondary to an alteration in the cationic content of the secreting cell as K^+ leaks out and Na^+ diffuses in. Secretion by the sublingual gland involves an active chloride transport mechanism, and this secretion is greatly reduced when the gland is perfused with potassium-free saline (11). The addition of ouabain causes K^+ to diffuse from the inner surface of the frog skin (12). Since both the reduction in chloride secretion and the depletion of cellular potassium probably occur simultaneously, it is difficult to conclude that the action of strophanthidin is on the cation transport mechanisms alone.

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