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Inhibition of Active Transport by Ouabain in the Canine Stomach (38299)

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Cardiac glycosides are known to inhibit cation transport and the activity of Na^+–K^+-dependent ATPase in most tissues (1). In oxygenated *in vitro* frog gastric mucosa Davenport (2) reported that ouabain inhibited active transport of H^+ and Na^+, and Cooperstein (3) observed inhibition of H^+ and Cl^- transport with strophanthidin. In rat gastric mucosa Sernka and Hogben (4) found inhibition of active H^+ and Cl^- transport by ouabain.

The purposes of our study were (a) to explore the effects of ouabain on active transport and the electrical properties of an *in vivo* canine stomach preparation and (b) to compare these findings with flux measurements in an *in vitro* canine stomach preparation. This comparison suggests that simpler measurements in the *in vivo* preparation permit prediction of findings in the *in vitro*.

Materials and Methods. In vivo *preparation.* Six fasted mongrel dogs (13–22 kg) of either sex were anesthetized with intravenous chloralose and ethyl carbamate (1 ml/kg of a solution containing 9.25 g chloralose and 92.5 g ethyl carbamate in 150 ml normal saline). Our *in vivo* chambered stomach preparation has been described previously (5).

The chamber divided the stomach flap into two sides. Each side was bathed with 10 ml of isotonic saline maintained at 37°. The effluent from one side was collected and titrated (Radiometer autoburette) at 15 min intervals to determine the gastric acid output. The other side of the chamber was connected to calomel electrodes and Ag-AgCl electrodes to determine transmural potential difference (PD) and current (I) necessary to reduce the PD to zero, respectively. These measurements were determined with a Shanbour voltage-clamp system (6). The relative resistance (R) was then calculated as the ratio of PD to I. The pH of this side of the chamber was kept above 2.5 by constantly flushing the mucosa with normal saline at 34–37°.

Histamine base (1.2–2.0 μg/kg min) was infused through a femoral vein for 90 min. Then, with continuous histamine infusion, ouabain (Lilly) was injected as a bolus dose (50 μg/kg) into the other femoral vein. Throughout both control and postouabain periods, measurements of PD, I, and acid output were obtained at intervals noted above. Only those animals which achieved an acid secretory rate above 60 μEq/15 min were used in the study.

In vitro *preparation.* In twelve anesthetized dogs, a strip of the fundic portion of the stomach was excised. After dissecting away the muscle coat and connective tissue, paired segments of gastric mucosa were mounted in flux chambers thermoregulatedat 37 ± 2° and gassed with 100% oxygen (7). For sodium flux studies, 10 mM TES buffer (pH 7.4) containing 25 mM glucose bathed both mucosal and serosal sides of the tissue; for chloride flux studies, the mucosal solution was replaced with a 10 mM glycine buffer (pH 3.0). Except for pH, these buffers had identical ion compositions. When mucosal solutions are at neutral pH (resting stomach), active transport of sodium predominates, but, when the mucosal solution becomes acidic (secreting stomach), active chloride transport predominates (8). Calomel electrodes (Radiometer K 401) were used to detect PD, and I_{sc} was delivered via carbon electrodes using a Shanbour voltage-clamp system (6). Resistance was calculated as the ratio of periodic and momentary open-circuit PD to I_{sc}. To determine unidirectional fluxes, either radioactive ^{22}Na or ^{36}Cl was

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added to opposite sides of paired mucosal segments with collection of unlabeled chamber solutions every 5, 10, or 20 min under constant short-circuiting conditions. Sampled chambers were filled with the appropriate starting solutions. Unidirectional fluxes of sodium and chloride were calculated from tracer fluxes in the absence and presence of serosal ouabain (3.4×10^{-6} *M*), using a liquid scintillation counter. This concentration of ouabain was made twice the *in vivo* concentration, assuming that the bolus dose became uniformly distributed in a plasma volume equal to 4% body weight.

The mean (± standard error) was determined for net fluxes and potential differences. Significance was evaluated using a paired student's *t* test.

Results. In vivo preparation. The effects of ouabain on PD (serosa positive to mucosa) are shown in Fig. 1. Histamine infusion increased acid secretion and decreased PD within the first 60 min of infusion. During the second 60 min (60–120) of infusion there were no significant differences in acid secretion or PD values. Within 30 min after ouabain, PD decreased significantly ($P < 0.05$) by 9 ± 2 m V. No significant change in acid output occurred with ouabain.

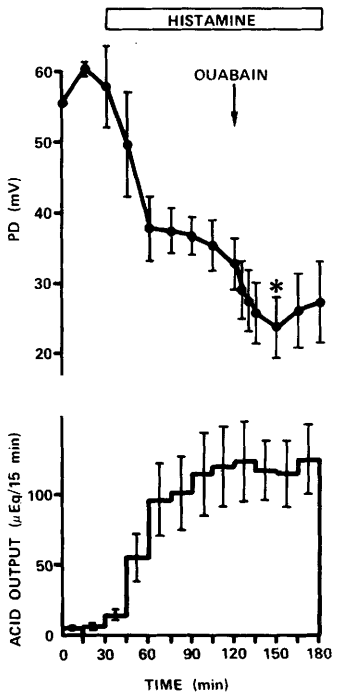

FIG. 1. Effects of ouabain *in vivo* on potential difference (PD) and acid output. Means ± standard errors are presented. Asterisk denotes significance ($P < 0.05$), $N=6$.

Table I shows the percentage change in calculated *R* in each experiment 30 min after injection of ouabain. There was no significant change and in no case was there a decrease in *R* with ouabain.

In vitro *preparation.* Addition of ouabain to the serosal solutions of gastric mucosae greatly accelerated the slow, normal decline in chloride transport from serosa to mucosa. The inhibitory response was not apparent at 5 min, variably present at 10 min, and significant ($P < 0.05$) at 20 min and later (Fig. 2). Net chloride movement was reduced nearly to zero at 20 min and fully abolished after 20 min. For statistical comparisons, chloride transport for each time period after ouabain administration was paired to its value just prior to ouabain addition. In six other experiments ouabain decreased ($P < 0.05$) net sodium flux (mucosa to serosa) within 40 min, at which time net flux was reduced nearly to zero (Fig. 3). In both sets of experiments PD declined as active ion transport was inhibited by ouabain.

Discussion. The PD across the resting dog stomach is generated primarily by the active transport of Na^+ from mucosa to serosa (8). This active transport generates the resting PD in which the serosa is positive with respect to the mucosa.

With histamine stimulation of gastric secretion, H^+ is transported actively from serosa to mucosa. Simultaneously, mucosal acidification inhibits sodium transport and uncovers serosal to mucosal chloride transport (8). These transport changes cause the PD to fall as the acid output increases with histamine.

The effect of ouabain on PD could have been due to (a) increased active secretion of H^+, (b) increased mucosal permeability, (c) decreased active transport of Na^+ from mucosal to serosal sides, or (d) decreased active secretion of Cl^-.

TABLE I. Change in Resistance by Ouabain.

Dog No.	% Increase in R[a]
1	8.5
3	0.7
4	4.5
7	9.5
8	4.1
9	62.0

[a] Measured 30 min after ouabain.

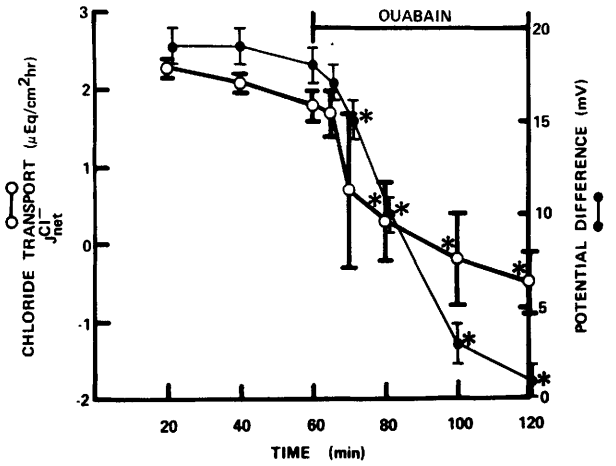

FIG. 2. Effects of ouabain *in vitro* on chloride transport and potential difference (PD). Means ± standard errors are presented. Asterisks denote significance ($P < 0.05$), $N=6$.

Simultaneous *in vivo* measurements of acid secretion and PD showed no change in acid output with ouabain, eliminating increased H^+ secretion as a likely explanation of the effect of the drug on PD. When mucosal permeability increases, passive ionic diffusion is enhanced and the membrane electrical conductance increases. Thus, electrical resistance, the reciprocal of conductance, would be expected to decrease. We did not observe any decrease in R with ouabain (Table I). Assuming that ouabain affects only active transport mechanisms for sodium and chloride, passive movements of these ions should remain unchanged. This was confirmed in our measurement of mucosal to serosal chloride flux and serosal to mucosal sodium flux before and after ouabain. These passive ion fluxes were unchanged by ouabain, indicating no change in permeability of the gastric mucosa. Ouabain also had no effect on the unidirectional fluxes of urea or resistance under open-circuit conditions (unpublished observations).

Kitihara *et al.* (8) observed that sodium transport functions at neutral pH, but declines with increasing mucosal acidity, whereas chloride transport in the thicker stomach wall preparation is more apparent at a low pH. Our *in vitro* studies on the much thinner dissected gastric mucosal preparation confirm that sodium transport predominates in a neutral TES buffer and that chloride transport prevails in an acidic glycine buffer, pH 3. This is important to the present study in that a low mucosal pH was developed with histamine-stimulated acid secretion *in vivo*, thereby diminishing sodium transport. With the low pH, active transport of chloride from serosa to mucosa was augmented, and the PD was maintained by this active secretion of chloride into the lumen. With the addition of ouabain, chloride transport was inhibited and the PD fell. This inhibition of chloride transport by ouabain in the absence of histamine resembled that obtained using isolated rat gastric mucosae in the presence of 1 m*M* histamine (4). Thus, the acid secretion of the *in vivo* stomach creates a condition favorable to active transport of chloride, which is

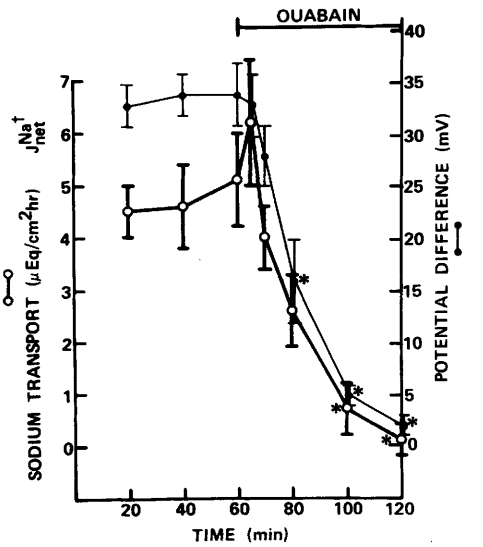

FIG. 3. Effects of ouabain *in vitro* on sodium transport and potential difference (PD). Means ± standard errors are presented. Asterisks denote significance ($P < 0.05$), $N=6$.

subsequently inhibited by ouabain.

Correlation of *in vitro* transport studies with the *in vivo* observations supports these conclusions. Active transport of chloride from serosa to mucosa was inhibited significantly by serosal ouabain at 20 min. Active transport of sodium was not significantly inhibited by serosal ouabain until after 40 min. The time sequence of PD inhibition *in vivo* correlates better with the *in vitro* effect of ouabain on chloride transport than the effect on sodium.

Summary. Ouabain (50 μg/kg) decreases the PD of the histamine-stimulated canine gastric mucosa *in vivo*. Calculated electrical resistance (PD/*I*) was not decreased, and the rate of acid secretion was unaltered by ouabain. These findings support the inference that ouabain inhibited active chloride transport to diminish PD. This inference was verified by *in vitro* experiments in which ouabain inhibited the net flux of chloride in the acid-bathed mucosal segment.

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