PRELIMINARY NOTES

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Inhibition by amytal of water movements in gastric mucosa

It is known that in frog gastric mucosae *in vitro* there exists a spontaneous net water flux concomitant with acid secretion, Cl^- transport and anion diffusion from the serosal to the mucosal surface[1]. Replacement of Cl^- by SO_4^{2-} in the solutions reduces the ionic transport and H^+ secretion without a simultaneous effect on the net water flux, which results in hypotonic secretion[2,3]. Thus, in SO_4^{2-} solution, the ionic concentration of the secretion is insufficient to explain the net water flux across the mucosa in terms of the osmotic difference between the secretion and the solutions in contact with the serosal surface. As a possible explanation for this phenomenon, the existence of a compartment accessible to the diffusion of sucrose and inulin, has been demonstrated which possesses a K^+ to Na^+ relationship similar to that of the cellular compartment. This compartment, which would be in contact with the mucosal surface, could be the site where primary ionic accumulation occurs and which could provoke hypotonic secretion as a secondary effect[4]. The present communication deals with the influence of amytal, an inhibitor of acid secretion, on the net water flux. We have used amytal since SACHS *et al.*[5] have shown that 2 mM amytal inhibits the acid secretion with a rise in resistance and a fall in short circuit current, without significant changes in transmucosal electrical potential difference.

Frogs, *Rana pipiens*, were kept in tap water at room temperature (22–24°) for at least 1 week before the experiments. The animals were pithed, and their stomachs were removed and opened along the small curvature. The mucosa was stripped by blunt dissection from the muscular coat. For the flux experiments, the mucosa was mounted between two Lucite chambers as previously described. These chambers are equipped with facilities for measuring the water flux, electrical potential difference and short circuit current[2]. Acid secretion was measured by the pH-stat method of DURBIN AND HEINZ[6]. The experiment was performed in three periods, and each period lasted 90 min. During the first and the last periods, the chamber in contact with the mucosal face was filled with secretory solution. In the second period, 2 mM amytal were added to the nutrient solution. For measurements of the water content, the mucosae were mounted in plastic tubes as described by FORTE AND NAUSS[7]. 2 ml of nutrient solution were introduced in each tube, and the tubes were immersed in 100 ml of the same nutrient solution. Under these circumstances, there were no hydrostatic or osmotic pressure gradients across the mucosa. One group of mucosa was incubated only for the first period of 90 min in nutrient solution; another group was incubated for the first and second periods, the first in nutrient solution and the second in nutrient solution with amytal; and a third group was incubated for the three periods as described in the flux experiments. The solutions used were similar to those previously described [8]. The nutrient solution buffered with $KH_2PO_4/NaHCO_3$ was oxygenated with a mixture of O_2-CO_2 (95:5, v/v) and the unbuffered secretory solution with pure O_2.

TABLE I

EFFECT OF AMYTAL ON THE POTENTIAL DIFFERENCE, SHORT CIRCUIT CURRENT, ACID SECRETION, NET WATER FLUX AND WATER CONTENT IN FROG GASTRIC MUCOSA

Each value is the mean ± S.E. from 10 experiments.

	Control period 0–90 min	*Amytal period 90–180 min*	*Control period 180–270 min*
Potential difference (mV)	28 ± 2	29 ± 2	26 ± 2
Short circuit current (μequiv/cm^2·h)	5.02 ± 0.29	3.62 ± 0.22	4.17 ± 0.40
Acid secretion (μequiv/cm^2·h)	0.42 ± 0.06	0.03 ± 0.01	0.12 ± 0.03
Net water flux (μl/cm^2·h)	11.7 ± 0.9	5.9 ± 0.5	13.0 ± 1.0
Water content (g/g dry wt.)	5.28 ± 0.16	5.56 ± 0.33	5.17 ± 0.22

Table I shows the experimental results. The effect of amytal on acid secretion, short circuit current and electrical potential difference are similar to those previously reported[5]. No significant difference exists between the mean values of electrical potential differences measured in solutions with or without amytal. Acid secretion was reduced almost immediately in amytal, reaching its minimal value after 30 min. This change is only partially reversible after removal of amytal. The effect on the short circuit current does not occur immediately, being apparent only after 30 min. The reduction in short circuit current that occurs after this time has been interpreted by SACHS *et al.*[5] to be the consequence of biochemical coupling between the rate of H^+ secretion and the mechanism responsible for the Cl^- transport. The partial recovery of the short circuit current after removal of amytal, when H^+ secretion remains partially inhibited, is also in agreement with the existence of this coupling between the mechanisms responsible for both H^+ secretion and Cl^- transport. Amytal reduces the water efflux to the secretory solution from 11.7 to 5.9 μl/cm^2·h and increases the water content of the mucosae from 5.28 to 5.56 g/g dry wt. of the mucosae. Both changes are reversible, the values returning to 13.0 μl/cm^2·h and 5.17 g/g dry wt., respectively, 90 min after the removal of amytal, as shown in Table I.

These effects on the water efflux and water content of the mucosa suggest that water follows, at some stage of its movement, the H^+ production and Cl^- transport. When these mechanisms are inhibited, water accumulates in the cellular compartment from which the ionic transport originates. At the onset of secretion, by removal of amytal, the water efflux increases, and the water content of the mucosa recovers its initial value. The site where the mechanisms responsible for the H^+ secretion and Cl^- transport are coupled with the water flux must be located at the point of entry into the compartment in which the primary secretion is accumulated. Both SO_4^{2-} and amytal inhibit H^+ secretion and Cl^- transport. However, net water flux continues in sulphate solution, but it is inhibited by amytal. This difference must be explained as follows. (a) When sulphate is used, electrolytes could accumulate in the compartment in contact with the mucosal surface, and the low permeability of this compartment to the efflux of sulphate towards the secretory solution produces a hypotonic secretion. (b) When the mechanisms responsible for the electrolyte transport and secretion are inhibited by the use of amytal, the accumulation in the compartment in contact with the mucosal surface is reduced and the water flux is also reduced. Under these conditions, total water content increases.

In summary, partial inhibition of the water flux by the use of 2 mM amytal in frog gastric mucosa is demonstrated. This is interpreted on the basis of the existence of a compartment in contact with the mucosal surface in which the primary secretion accumulates.

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