Acid secretion, potential difference, and resistance of elasmobranch stomach

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Acid secretion, potential difference, and resistance of elasmobranch stomach. Am. J. Physiol. 203(6): 1081–1093, 1962.—The present report is concerned with in vitro studies on gastric mucosa of the skate, Raja eglanteria, the electric ray, Narcine brasiliensis, and the shark, Negaprion brevirostris. Maximum secretory rates of from 0.65 to 2.7 μEq hr⁻¹ cm⁻² were found. An increase in the secretory rate from an initial low level was associated with an increase in potential difference (PD), a decrease in resistance, and an increase in the calculated short-circuit current. The average PD and resistance before the increase in the secretory rate was 2.2 mv (nutrient positive) and 268 ohms cm². After the increase they were 6.4 mv and 199 ohms cm². Thiocyanate (10⁻³ M) to nutrient resulted in a decrease of secretory rate to zero and an increase in PD and resistance. The results can be explained on the basis of the separate mechanisms theory of H⁻ and Cl⁻ ion secretion on the assumption that the resistance in the Cl⁻ ion limb of the circuit is considerably lower than that in the H⁺ ion limb of the circuit.

In previously published work (1, 2) it was found for dog and frog gastric mucosa that the onset of acid secretion was associated with a transient increase in the potential difference (PD) and a sustained decrease in the electrical resistance. The ratio of the PD to the resistance is the predicted or calculated short-circuit current (current flow when transmucosal PD is maintained at zero (3)). With the onset of secretion in both dog and frog gastric mucosa, there is an initial increase in calculated short-circuit current. The actual short-circuit current was measured for frog gastric mucosa, and it initially increased as predicted (2). It was also found that inhibition of secretion was associated with an increase in resistance and with changes in the PD. These and other findings can be interpreted on the basis of the author’s theory of separate mechanisms for the secretion of H⁺ and Cl⁻ ions (4), with the added postulate of a postulated cytoplasmic biochemical coupling between the mechanisms (2).

Hogben (5) reported for the elasmobranch stomach (the dogfish, Squalus acanthias, and one experiment on the skate, Raja erinacea) an essentially zero PD during secretion of hydrogen ions. A zero PD during secretion could be explained on the basis of an opposite orientation of the postulated hydrogen and chloride ion electromotive forces (Fig. 1). However, if further studies revealed a maintained zero transmucosal PD and no change in resistance with the onset and cessation of secretion, then the above theory would be eliminated from further consideration as an explanation for secretion in the elasmobranch gastric mucosa because so many ad hoc postulates would be demanded. It is therefore of importance to determine, in the elasmobranch stomach, if changes in the secretory state are accompanied by changes in the PD and in the resistance. This is the primary purpose of the present report.

METHODS

Eleven experiments were performed (4 with the electric ray, 1 with the yellow shark, and 6 with the clear nose skate) with an in vitro method described in detail elsewhere (2). Two pairs of electrodes were used, one pair for sending current across the mucosa, the other for measuring the PD. The current was measured with a microammeter, and the PD with a Keithley model 200B electrometer voltmeter. The resistance was determined as the change in PD per unit of applied current (10 μamp 2.8 cm², the area of the mucosa). The resistance was not dependent on the direction of the applied current.

A nutrient fluid with the following composition, in millimoles per liter, was used: 260 Na⁺, 10 K⁺, 5 Ca++, 2 Mg++, 250 Cl⁻, 30 HCO₃⁻, 2 phosphate, and 10 glucose. The fluid on the secretory side had the same cation concentration as the nutrient, but the Cl⁻ ion was the only anion. Both sides were usually gassed with 95% O₂-5% CO₂. In some experiments the nutrient fluid was replaced with secretory fluid for a portion of the

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FIG. 1. Equivalent circuit representation of separate mechanism theory. Eui represents the EMF due to an electrogenic mechanism. Eei, the EMF due to an electrogenic ion transport mechanism. E, the EMF due to an electrogenic H+ ion mechanism; \( R_G \) and \( R_H \), the resistance in the two limbs of the circuit. Positive current flows around the equivalent circuit loop in the direction indicated by arrows. Positive current flow, from the H+ ion site into the lumen, is due to the movement of H+ ions. Positive current flow inward from the lumen is due to the outward movement of Cl- ions.

experiment and air was used in place of the \( O_2-CO_2 \). During a portion of some of the experiments a nutrient solution was used that contained urea (0.3 M) in addition to the composition given above. The addition of urea did not have any significant effect.

The hydrogen ion secretory rate was measured in some experiments by the pH stat method, introduced into this field by Durbin and Heinz (6). In other experiments the hydrogen ion secretory rate was calculated from the change in pH of the secretory fluid. The pH decreased to as low as 2.8 (3 cc secretory fluid cm\(^2\) mucosa) in about 2 hr. Electroemetric titrations of the secretory fluid revealed no significant amounts of buffer in this fluid.

It is standard practice in studies on the stomach, under in vitro conditions, to use the mucosa after cutting away the external muscle layers. The muscle layers of these elasmobranch stomachs were more difficult to remove than in the case of the frog stomach. Muscle layers of the ray were more difficult to remove than those of the skate. Higher secretory rates were obtained with skates, in vitro conditions, to use the mucosa after cutting away the external muscle layers. The muscle layers of these experiments the spontaneous secretory rate was about 5.4 and 2.5 mv and a decrease in resistance of 70 and 81 ohms cm\(^2\), respectively.

In one experiment with secretory solution on both sides and with air as the gas, methacholine addition to the nutrient resulted in a small increase in PD and a small decrease in resistance, and the establishment of secretion at a level of 0.3 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\). Regular nutrient solution was then placed on the nutrient side and the gas was changed to 95% \( O_2 \)-5% \( CO_2 \). This was followed by a marked increase in hydrogen ion secretory rate, an increase in PD of about 6 mv, and a decrease in resistance of 90 ohms cm\(^2\) (Fig. 2C).

In three experiments (one shown in Fig. 2F) in which there was a sizable spontaneous secretory rate, methacholine produced an increase in the secretory rate, a decrease in resistance, and a small initial increase in the PD (range 0.5-1.5 mv) which was followed by a decreasc. In the only ray with a secretory rate greater than 0.12 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\), the spontaneous rate was 0.4 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\). After methacholine the secretory rate increased to 0.8 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\) for about 15 min and then decreased to a level less than 0.1 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\). The increase in secretory rate was accompanied by a change in PD of from +1.0 mv to 2.1 mv and a decrease in resistance of from 224 to 199 ohms cm\(^2\). As the secretory rate declined the PD decreased to +0.4 mv and the resistance increased to 280 ohms cm\(^2\).

In only one experiment was the addition of glucose (Fig. 2F) followed by an effect, and in this case it resulted in an increase in the secretory rate and changes in PD and resistance. In two experiments (one shown in Fig. 2F) thiocyanate was added to the nutrient solution (final concn., \( 10^{-2} M \)), and it produced a decrease in the secretory rate to zero and an increase in the PD and resistance.

RESULTS

In six experiments (1 ray, 5 skates) adequate secretory rates were obtained. The maximum secretory rate ranged from 0.65 to 2.7 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\). Histamine had no clear effect, but methacholine did. Before methacholine the average PD for these experiments was -9.9 mv, with a range of from -2.2 to +12 mv, and after methacholine the average PD was +6.4 mv, with a range from +1 to +10.5 mv (+ = nutrient positive). In two of these experiments the spontaneous secretory rate was less than 0.1 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\) and addition of methacholine to the nutrient solution (final concn., \( 10^{-5} M \)) resulted in the establishment of secretory rates of 0.65 (Fig. 2D) and 1.0 \( \mu Eq \) hr\(^{-1}\) cm\(^{-2}\) (Fig. 2F), and an increase in the PD of 5.4 and 2.5 mv and a decrease in resistance of 70 and 81 ohms cm\(^2\), respectively.

FIG. 2. Scales for the PD, resistance, and secretory rate are on the left. M indicates addition of methacholine to nutrient (final concn., \( 10^{-5} M \)); SCN, addition of thiocyanate to nutrient (final concn., \( 10^{-2} M \)); G, addition of glucose to nutrient (final concn., \( 10^{-2} M \)).
In two experiments the effect of cutting off the oxygen supply was determined (one shown in Fig. 2E). Secretory solution was placed on both sides and the preparation was gassed with air. Circulation of fluid through the chambers was interrupted during the period indicated in the figure. This was followed by a decrease in the PD and an increase in resistance. Recirculation of aerated fluid resulted in the return of both PD and resistance to their former levels.

The PD/resistance ratio, the calculated short circuit current, increased when the secretory rate increased from an initial low level, as is evident in Figs. 2A, D, F, and G. The average value for the calculated short-circuit current at the low level of secretion in these four experiments was 21.5 μamp cm⁻² (range 9-26) and, after the increase in secretory rate the average maximum value was 49.8 μamp cm⁻² (range 29-69). In these four experiments the average resistance was 199 ohms cm² (range 177-235). The average decrease in resistance was 66 ohms cm² (range 33-95).

In five experiments (1 shark, 1 skate, and 3 rays, including the ray in which the whole stomach was used) the total range of the PD was between -4.3 and +7.6 mv and was within 2 mv of zero during 80% of the time. The maximum hydrogen ion secretory rate was less than 0.13 μEq hr⁻¹ cm⁻². Addition of histamine, methacholine, glucose, or adrenaline to the nutrient solution over a wide range of concentration had no clear effect in the five experiments.

DISCUSSION

It is clear from the six experiments in which appreciable secretory rates were obtained that changes in secretory rate are accompanied by changes in the PD and in the resistance. Both the PD and the secretory rate were about one-third of those of the frog stomach (2). The results are qualitatively similar to those on both the dog and frog stomach.

The present results can be explained on the basis of the author’s separate mechanism theory of HCl production (Fig. 1) by assuming that R₃, the resistance in the Cl⁻ limb of the circuit, is considerably lower than Rₛ, the resistance in the H⁺ ion limb of the circuit (2). For example, assume that R₁ = 200 ohms cm², Rₛ = 1,000 ohms cm², the PD = +5.0 mv, and the current flow = 50 μamps cm² (corresponding to a secretory rate of 1.86 μEq hr⁻¹ cm⁻²). The calculated values for E₁ and E₂ would then be 15 mv and 45 mv, respectively. One might then anticipate a resting PD of about 15 mv, since, in the absence of H⁺ ion secretion, Eₛ would not be present. However, this assumes that the gastric stimulant has no effect on the Cl⁻ ion transport mechanism. As discussed elsewhere (2), in order to explain an increase in PD and short-circuit current, occurring with the onset of secretion, it is necessary within the framework of the separate mechanism theory to postulate a stimulation of the Cl⁻ ion transport mechanism, i.e., a decrease of R₁ and/or an increase in E₁. On this basis, E₁ could be considerably less in the resting than in the secreting stomach, and hence the PD of the resting stomach of the skate could be considerably lower than 15 mv.

The results in the present experiments are somewhat different than those reported by Hogben (5). However, Hogben used a different species of elasmobranch, and it is possible that there is a species difference. Hogben, on the other hand, did not explore the effect of changes of the secretory rate on the PD and on the resistance. Further experiments on the dogfish are needed to determine whether the relationships between the secretory rate and the other parameters, during periods of marked changes in secretory rate, are similar to those in the present study.

REFERENCES