PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **151**, 523-525 (1976)

Immediate Short-Time Effects of Ethacrynic Acid on Frog Gastric Mucosa in Chloride-Free Solutions (39249)

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Previous work (1) showed that the addition of ethacrynic acid to a concentration of 1 m*M* in a normal Cl^- nutrient solution bathing the frog gastric mucosa *in vitro* produced an immediate decrease in resistance followed by an increase in resistance and a decrease in H^+ secretory rate. The latter effect was irreversible and the former reversible. The initial short-time effect, lasting generally no more than 3 min after the addition of ethacrynic acid to the nutrient solution, concerns us here. In either low K^+ (4 m*M*) or high K^+ (79 m*M*) in the nutrient solution, ethacrynic acid initially caused a decrease in resistance accompanied by a decrease in transmucosal potential difference (PD). An explanation of these data suggested that the effect of ethacrynic acid predominated on the lowest resistance pathway of the nutrient membrane, the K^+ pathway, resulting in an increase of K^+ permeability. In the presence of Ba^{2+}, which markedly increases the resistance of the K^+ pathway (2) and increases at least to some degree the resistance of the Cl^- pathway (3), ethacrynic acid in the nutrient solution also decreased the resistance but produced both positive and negative changes in PD. In this case it appeared that ethacrynic acid affected predominantly sometimes the K^+ pathway and at other times the Cl^- pathway.

The finding above of a predominant effect in gastric mucosa on the K^+ pathway of the nutrient membrane is contrary to a dominant effect on the Cl^- pathway as speculated from the results of this drug on the renal tubule (4–7). Therefore, it seemed reasonable to study the extent of the effect on the K^+ pathway in the absence of Cl^-. The results of experiments carried out in sulphate (Cl^--free) media are reported in this paper.

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described in detail elsewhere (8). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was determined as the change in PD per unit of applied current. In Cl^--free preparations, the nutrient bathing solution contained (m*M*): Na^+, 101; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; $SO_4{}^{2-}$, 41.3; $HCO_3{}^-$, 25; phosphate, 1; glucose, 10; and sucrose, 40; and the secretory solution: Na^+, 100; $SO_4{}^{2-}$, 50; and sucrose, 100. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. The pH of the secretory solution was maintained at 4.90. After the control part of the experiment, ethacrynic acid was added to the nutrient solution to yield a concentration of 1 m*M* in that solution.

Results. Figure 1 shows the results of adding ethacrynic acid to the nutrient solution. The addition led to a decrease in resistance, which was accompanied by a small increase in PD. Soon after, the resistance started to increase again and the PD commenced to decrease. Washing both sides of the mucosa with regular solutions restored the parameters generally to near control values.

Table I summarizes the results in the initial phase in which the effects induced by ethacrynic acid usually occurred in less than 3 min. Columns II and IV give, respectively, the average resistance and PD values for mucosae bathed in regular sulphate solutions without ethacrynic acid. Columns III and V give, respectively, the average percentage changes in the individual experiments after the addition of ethacrynic acid to the nutrient solution. The relative decrease in resistance is the maximum observed change in magnitude and the relative increase in PD corresponds in time to this observed change in resistance. The maximum decrease of about 51 Ω-cm^2 compares to a decrease of 75 Ω cm^2 in Cl^- solutions containing 4 m*M* K^+ and 48 Ω cm^2 in Cl^- solutions containing 79 m*M* K^+ (1). The corresponding increase in PD of about 1.5 mV compares to a decrease of 1 mV in Cl^- solutions containing 4 m*M* K^+ and 2 mV in Cl^- solutions containing 79 m*M* K^+ (1).

Discussion. It is not possible, as in the case of Cl^- solutions bathing the frog gastric mucosa, to explain completely the data of the immediate short-time effects in SO_4^{2-} media at present. In Cl^- media it is well established that the low resistance pathways of the nutrient membrane are the Cl^- and K^+ pathways. This information coupled with the fact that Cl^- is actively transported strongly suggests that ethacrynic acid causes an increase of K^+ permeability of the nutrient membrane in the absence of Ba^{2-} (1).

In SO_4^{2-} media there is little known about the relative resistance of ionic pathways except that the K^+ pathway has a low resistance. The electrical circuit, shown in Fig. 2 and representing ionic pathways on the nutrient membrane, has its basis in a model proposed by Rehm (9). The PD across the nutrient membrane is given by

$$PD = \frac{-R_K R_{SO_4} E_{Na} + R_{SO_4} R_{Na} E_K + R_{Na} R_K E_{SO_4}}{R_K R_{Na} + R_{SO_4} R_{Na} + R_{SO_4} R_K}.$$

From Fig. 2 and this equation, it can readily be inferred that, if the addition of ethacrynic acid gave primarily a decrease of the resistance of the Na^+ pathway, then the PD would decrease contrary to the experimental result that initially the PD increases. However, without additional knowledge of the relative resistances of the frog gastric mucosa in SO_4^{2-} media, it cannot be inferred whether ethacrynic acid affects predominantly the SO_4^{2-} or the K^+ pathway.

Summary. The presence of ethacrynic acid in the nutrient sulphate solution produces, generally in less than 3 min, a de-

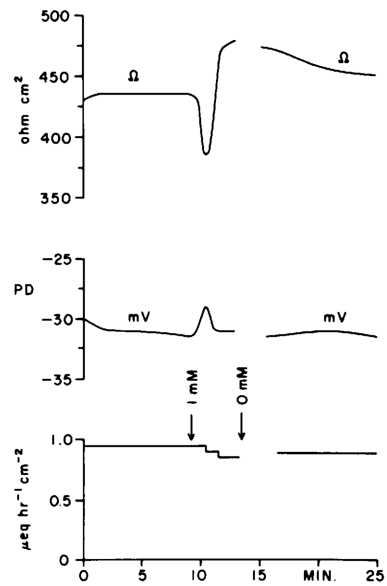

FIG. 1. Effects of the addition of ethacrynic acid to the nutrient solution on the electrophysiological parameters. At the time indicated by the first arrow, ethacrynic acid was added to a concentration of 1 m*M* in the nutrient solution, and at the time indicated by the second arrow, no ethacrynic acid was present in the solution.

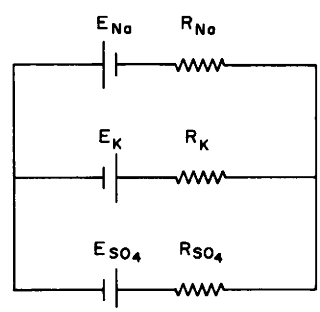

FIG. 2. Equivalent circuit showing the Na^+, K^+ and SO_4^{2-} pathways across the nutrient membrane.

TABLE I. EFFECTS OF ETHACRYNIC ACID ON RESISTANCE AND PD IN FROG GASTRIC MUCOSA DURING THE INITIAL PHASE.

I Number of experiments	II R (Ω − cm^2)	III ΔR/R (%)	IV PD (mV)	V ΔPD/PD (%)
7	465 ± 72	−11 ± 1 ($P < 0.01$)	−31 ± 4	5 ± 4[a] ($P < 0.02$)

[a] In each case this number denotes the mean increase or decrease ± standard deviation.

crease in resistance accompanied by an increase in transmucosal PD. These results strongly suggest that the dominant effect of ethacrynic acid is not on the Na^+ pathway of the nutrient membrane, but without further knowledge of the resistance of other ionic pathways, it cannot be inferred whether the effect predominates on the K^+ pathway as in Cl^- media.

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Received June 16, 1975. P.S.E.B.M. 1976, Vol. 151.