Determination of Electrogenicity of Frog Gastric Mucosa in Chloride-Free Solutions by Using Barbiturates (35923)

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Rehm and LeFevre (1) found a straight-line relationship of negative slope in chloride-free media between the transmembrane potential difference (PD) and the H^+ secretory rate following inhibition of the frog gastric mucosa with 2,4-dinitrophenol (DNP). Schwartz and MacKrell (2) confirmed the results using the volatile anesthetic, methoxyflurane (Penthrane). These inhibitors unlike thiocyanate (3, 4) affect only the H^+ mechanism in Cl^--free solutions and hence can be used for studies of electrogenicity in these media. In addition, these experiments permit estimates of electrical parameters of the H^+ secretory mechanism.

Generally the available evidence in the literature for electrogenicity of the transport of ions such as Na^+, K^+, and Ca^{2+} is based on the difficulties of explaining the origin of the PD in terms of a neutral mechanism. The above finding of linearity constitutes positive proof for an electrogenic mechanism in frog gastric mucosa. Because of the crucial nature of these experiments, the use of other inhibitors is warranted. In the work reported here, barbiturates were used to validate the electrogenic concept.

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described elsewhere (5). 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. After the control part of the experiment, barbiturate was added to the nutrient solution to yield concentrations up to 2 m*M*.

Results. Figure 1 shows the effects of adding secobarbital (Seconal) to the nutrient solution. At the times indicated by the arrows, the concentration of secobarbital in the nutrient solution was increased in steps of 0.2 m*M* up to 1.6 m*M*. Here the resistance increased as much as 67% above control levels as the H^+ secretory rate decreased towards zero. As depicted in Fig. 1, the absolute magnitude of the negative PD decreased towards zero with decreasing H^+ rate. Washing both sides with solutions without barbiturate restored the parameters to near control levels. Similar results were obtained with thiamylal (Surital). The graph on the right exhibits the linear relation between PD and H^+ rate. For each horizontal segment of the H^+ rate in the graph on the left, the average PD was determined. The plot on the right represents average PD versus H^+ rate. The existence of this linear relation permits, as shown below, estimates of the H^+ mechanism.

Discussion. Figure 2 represents an equivalent circuit for frog gastric mucosa. In addition to the H^+ and Cl^- limbs, there are also the following pathways: R_X and E_X, representing the transport of ions other than H^+ and Cl^- across the secretory surface of the tubular cells; R_S and E_S, the transport of ions across the surface epithelial cells; R_N and E_N, the transport of ions across the nutrient surface of the tubular cells; R_{SUB}, the resistance of the submucosa.

In Cl^--free solutions, the Cl^- limb shown

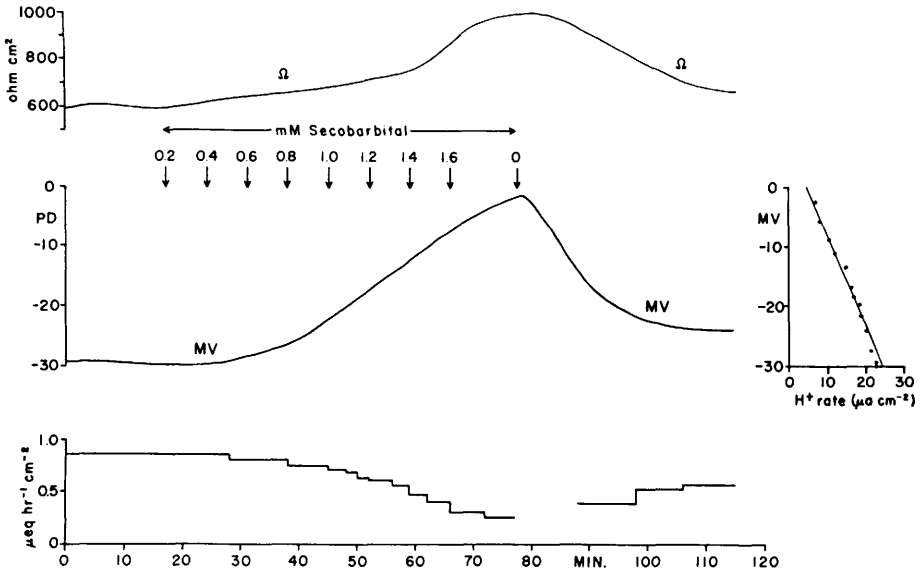

FIG. 1. Effects of secobarbital (Seconal) on resistance, PD, and H^+ secretory rate in chloride-free solutions. The resistance, PD, and H^+ rate are plotted versus time. At the times indicated by the arrows the concentration of secobarbital in the nutrient solution was increased in steps of 0.2 mM up to 1.6 mM and then decreased to zero. The graph on the right gives average PD versus H^+ rate.

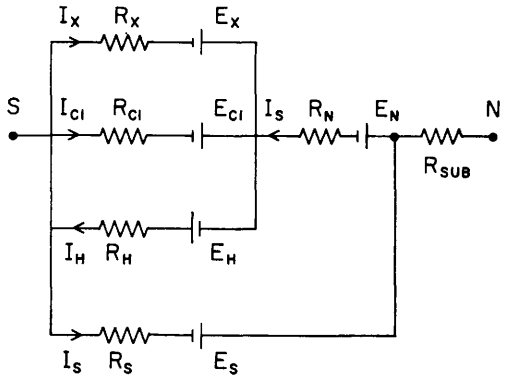

FIG. 2. Equivalent circuit diagram.

in Fig. 2 is absent. Analysis of the circuit with R_N assumed to be zero yields for PD_{NS}, the transmembrane potential difference measured from nutrient to secretory side,

$$PD_{NS} = \frac{R_X E_S + R_S E_N + R_S E_X}{R_S + R_X} - \frac{R_S R_X}{R_S + R_X} I_H.$$

If only R_H and E_H are affected by the presence of an inhibitor, PD_{NS} is linearly related to I_H with slope R_{SX} given by

$$R_{SX} = \frac{R_S R_X}{R_S + R_X}.$$

If R_M is the measured transmembrane resistance, it readily follows that

$$R_H = \frac{R_{SX}(R_M - R_{SUB})}{R_{SX} - (R_M - R_{SUB})}.$$

Furthermore, on the assumption that $R_N = 0$, we have $E_H - E_N = R_H I_H - PD_{NS}$.

Based on the alternating impedance studies of Teorell and Wersall (6), R_{SUB} may be taken equal to 30 ohm cm². The quantities R_H and $E_H - E_N$ can then be calculated from the equations above. In Table I, data are presented for one secobarbital experiment and one thiamylal experiment. The experimental values of PD and R_N in Columns III and IV, respectively, are average values for each horizontal segment of I_H. The quantity R_{SX} is obtained from the slope of the straight line relating PD_{NS} and I_H. The last two columns give the computed values for R_H and $E_H - E_N$. The first row of each experiment gives the experimental and computed values just prior to the addition of barbiturates to the nutrient solution. The descending rows indicate the changes in the parameters in the presence of increasing amounts of barbiturate and for increasing time periods. Since E_N would not be expected to change with decreasing H^+ rate, it follows that the decrease

TABLE I. Experimental Values of I_H, PD, and R_M and Calculated Values of R_H and $E_H - E_N$.

Expt. no.	I_H (μA cm^{-2})	PD (mV)	R_M (ohm cm^2)	R_{SX} (ohm cm^2)	R_H (ohm cm^2)	$E_H - E_N$ (mV)
1	23.0	—30.0	598	1560	893	50.5
Secobarbital	23.0	—29.4	627		967	51.6
	21.6	—27.5	651		1030	49.8
	20.2	—24.4	674		1100	46.6
	19.0	—21.6	690		1140	43.3
	18.2	—19.7	712		1210	41.8
	16.9	—18.4	720		1240	39.3
	16.2	—17.0	739		1300	38.1
	14.8	—13.4	752		1340	33.3
	12.6	—11.2	783		1460	29.5
	11.0	— 8.8	849		1720	27.8
	8.4	— 5.6	942		2200	24.0
	6.8	— 2.5	997		2540	19.8
2	23.0	—24.2	394	1180	526	36.3
Thiamylal	20.0	—20.7	437		621	33.1
	16.8	—16.2	464		686	27.7
	12.4	—11.8	497		773	21.4
	8.2	— 6.4	568		989	14.5
	4.9	— 2.4	643		1280	8.7

in $E_H - E_N$ results from a decrease in E_H. Thus Table I indicates that a decrease in H^+ rate is associated with a decrease in E_H and/or an increase in R_H.

Average values were obtained for R_H and $E_H - E_N$ just before the addition of barbiturate to the nutrient solution. Five experiments were performed with each barbiturate. Since there were no statistically significant differences in the results, the averages are based on the 10 experiments. The average values are $R_H = 730 \pm 210$ (SD) ohm cm^2 and $E_H - E_N = 44 \pm 10$ (SD) mV. These compare favorably with the combination of available previous data (1, 2) which gave average values of $R_H = 790$ ohm cm^2 and $E_H - E_N = 42$ mV.

Conclusion. Barbiturates can be used to reveal the linear relationship between the PD and the H^+ rate and to obtain estimates of electrical parameters of the H^+ secretory mechanism in Cl^--free solutions.

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