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Effects of Norepinephrine and Phentolamine on Frog Gastric Mucosa in Chloride Solutions (39745)

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In a review article, Holton (1) pointed out that α-agonists, as exemplified by norepinephrine, decrease acid gastric secretion and that this decrease is possibly secondary to vasoconstriction. The isolated frog gastric mucosa provides the opportunity to examine various catecholamines in the absence of vascular effects. Somewhat earlier than the article by Holton, Thorpe *et al.* (2) investigated the effect of catecholamines on the H^+ secretory rate of the isolated bullfrog gastric mucosa. They found that epinephrine and norepinephrine in concentrations as high as 5×10^{-3} *M* produced statistically insignificant decreases in histamine-stimulated acid secretion presumably because of the limited number of experiments. However, 5×10^{-3} *M* isoproterenol, a β-agonist, produced significant inhibition of both histamine and acetylcholine-stimulated H^+ secretion, namely decreases of 55 and 60%, respectively, in the H^+ secretory rates. This inhibitory effect of isoproterenol was blocked by propranolol, a β-antagonist. The authors interpreted their data to suggest that isoproterenol inhibits histamine- and acetylcholine-stimulated acid secretion by direct activation of mucosal β-adrenergic receptors. Recent work in our laboratory (3) lends support to this phase of their work.

It is evident that Thorpe *et al.* (2) had limited interest in α-adrenergic amines and that they did not measure the transmucosal resistance and the transmucosal potential difference (PD) concomitantly with the H^+ secretory rate. It therefore seemed of interest to study the α-adrenergic amines in greater detail. In this paper, the electrophysiological effects of the adrenergic amines, namely norepinephrine (Levophed), an α-agonist, and phentolamine (Regitine), an α-antagonist, are reported.

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described in detail elsewhere (4). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was obtained as the change in PD per unit of applied current. The H^+ secretory rate was determined by the pH stat method introduced by Durbin and Heinz (5). The pH of the secretory side was maintained at 4.90. The nutrient bathing solution contained (in m*M*): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; HCO_3^-, 25; phosphate, 1.0; and glucose, 10; and the secretory bathing solution contained: Na^+, 102; K^+, 4; Cl^-, 106. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. Histamine was added to the nutrient solution to a concentration of 10^{-4} *M*, thereby providing maximal stimulation of the gastric mucosa. After the control part of the experiment, norepinephrine or phentolamine was added in concentrations varying from 10^{-5} to 2 or 2.35 m*M*. Combinations of these amines were also investigated.

Results. Figure 1 shows the effects of adding 1 m*M* norepinephrine to the nutrient solution. At the time indicated by the first arrow, norepinephrine was added to a concentration of 1 m*M* in the nutrient solution. A comparison was made of the changes in the parameters in about 30 min following the addition of norepinephrine in the nutrient solution to the control values just prior to the addition. As shown in Fig. 1, the resistance increased about 53%, the PD decreased about 7%, and the H^+ secretory rate decreased about 48%. Washing both sides of the mucosa with regular solutions restored the parameters to near control values.

Table I summarizes the results of the

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effects induced by norepinephrine and phentolamine about 30 min after the addition of either amine. In the case of norepinephrine, a concentration of 0.1 m*M* in the nutrient solution gave a significant decrease in the H^+ secretory rate; a concentration of 1.0 m*M* gave a significant increase in resistance and a significant decrease in H^+ secretory rate; and a concentration of 2.35 m*M* gave a significant increase in resistance, a significant decrease in PD, and a significant decrease in the H^+ secretory rate. For phentolamine, as Table I shows, the same trends are evident as for norepinephrine. It is interesting to note that for both the α-agonist and the α-antagonist the decrease in H^+ secretory rate shows essentially the same dose dependency as a function of concentration. Experiments with either adrenergic amine at concentrations of 10^{-5} to 10^{-2} m*M* in the nutrient solution showed no significant changes in the electrophysiological parameters.

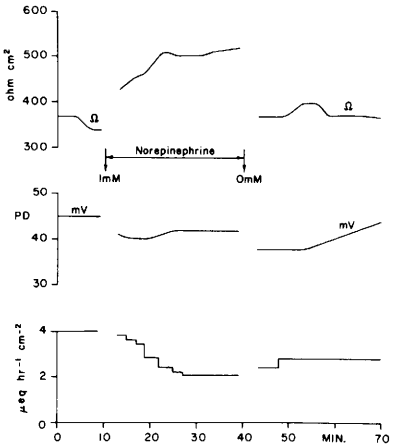

FIG. 1. Effects of norepinephrine on the resistance, PD, and H^+ secretory rate of frog gastric mucosa in chloride solutions. At the time indicated by the first arrow, norepinephrine was added to a concentration of 1.0 m*M* in the nutrient solution and, at the time indicated by the second arrow, the norepinephrine was removed. Resistance, PD, and H^+ secretory rate are plotted versus time.

As shown in Table I, for the lower concentrations in the nutrient solution, neither amine resulted in a significant change in the PD but, at the highest concentration of 2.0 or 2.35 m*M* in the nutrient solution, the PD decreased significantly. The β-antagonist, propranolol, behaves quite similarly with concentrations of 1.0 and 2.0 m*M* in the nutrient solution resulting in significant decreases in PD (3). In contrast, the β-agonist, isoproterenol, for concentrations of 0.10 and 0.25 m*M*, produced significant increases in PD and, for the concentration of 0.50 m*M* which results in a decrease of the H^+ secretory rate to zero, produced no significant change (3).

In order to test whether the two amines antagonize each other, experiments were performed in which small concentrations of either norepinephrine or phentolamine up to and including 0.1 m*M* were present in the nutrient solution for periods of 0.5 or 1 hr. Then the other amine was added to the nutrient solution in amounts yielding concentrations of 0.1 to 1.0 m*M* in that

TABLE I. EFFECTS OF NOREPINEPHRINE AND PHENTOLAMINE ON RESISTANCE, PD, AND H^+ SECRETION RATE OF FROG GASTRIC MUCOSA.

Adrenergic amine	Concentration(m*M*)	Number of experiments	R (Ω – cm²)	$\Delta R/R$ (%)	PD (mV)	ΔPD/PD (%)	$\dot{H}$ (μEq hr^{-1} cm^{-2})	$\Delta\dot{H}/\dot{H}$ (%)
Norepinephrine	0.1	5	179 ± 62[a]	17 ± 24	35 ± 6	−2.2 ± 14	4.6 ± 1.2	−22 ± 11
				($P > 0.30$)		($P > 0.60$)		($P < 0.02$)
	1.0	8	247 ± 82	80 ± 80	34 ± 9	8.1 ± 30	5.2 ± 1.7	−55 ± 29
				($P < 0.05$)		($P > 0.40$)		($P < 0.01$)
	2.35	7	245 ± 92	172 ± 76	34 ± 12	−13 ± 12	3.9 ± 1.5	−97 ± 8
				($P < 0.01$)		($P < 0.05$)		($P < 0.01$)
Phentolamine	0.1	6	257 ± 113	17 ± 18	24 ± 5	3.8 ± 15	3.8 ± 1.0	−21 ± 11
				($P > 0.05$)		($P > 0.50$)		($P < 0.01$)
	1.0	6	209 ± 92	103 ± 73	27 ± 8	10 ± 44	3.5 ± 1.3	−50 ± 15
				($P < 0.02$)		($P > 0.40$)		($P < 0.01$)
	2.0	5	191 ± 57	157 ± 64	30 ± 4	−28 ± 18	4.7 ± 1.2	−99 ± 2
				($P < 0.01$)		($P < 0.05$)		($P < 0.01$)

[a] In each case the number following the ± sign denotes the standard deviation. $\dot{H}$ refers to the H^+ secretory rate. The columns labeled R, PD, and $\dot{H}$, respectively, refer to the control solutions and the columns labeled $\Delta R/R$, ΔPD/PD, and $\Delta\dot{H}/\dot{H}$, respectively, refer to the changes produced about 30 min after the addition of the amine to the nutrient solution.

solution. For example, in four experiments with 0.1 m*M* phentolamine in the nutrient solution followed by a combination of 0.1 m*M* phentolamine and 1.0 m*M* norepinephrine, the latter produced a significant decrease of 93% in the H^+ secretory rate whereas, as Table I shows, 1.0 m*M* norepinephrine caused a decrease of only 55%. The results of the combination experiments indicated no blocking effects between norepinephrine and phentolamine.

Discussion. Agents or procedures which markedly increase the resistance of the H^+- and/or Cl^--active pumps on the secretory membrane produce a concurrent marked decrease in the H^+ secretory rate (6, 7). On the other hand, agents or procedures which increase the resistance of the ionic pathways on the nutrient membrane are usually asssociated with small decreases in the H^+ secretory rate (8, 9).

An analysis based on these facts will attempt to explain changes in PD arising from changes in resistance. For this purpose, we refer to Fig. 2. We first note that

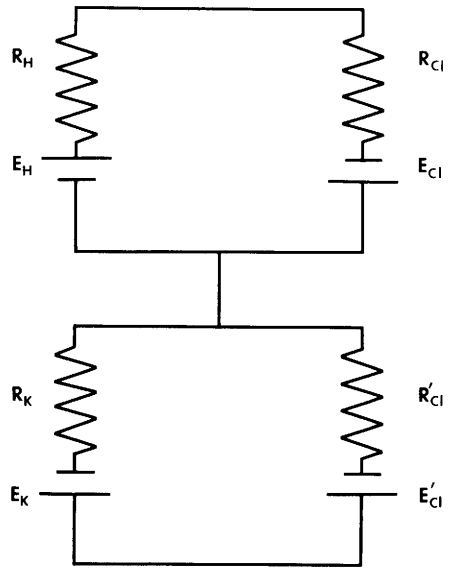

FIG. 2. Electrical circuit showing principal pathways of the secretory and nutrient membranes. Upper part refers to pathways of the secretory membrane, namely, the H^+ and Cl^- emf's and their respective resistances R_H and R_{Cl}. Lower part refers to low resistance pathways of the nutrient membrane, namely, the emf's E_K and E'_{Cl} due to ion gradients, and their respective resistances, R_K and R'_{Cl}.

$$PD_{NS} = PD_{NC} + PD_{CS}, \qquad [1]$$

where PD_{NS} is the potential difference of the nutrient side relative to the secretory side, PD_{NC} is the PD of the nutrient side relative to the cell, and PD_{CS} is the PD of the cell relative to the secretory side. We obtain

$$PD_{CS} = -E_H + \frac{E_H + E_{Cl}}{R_H + R_{Cl}} R_H = E_{Cl} - \frac{E_H + E_{Cl}}{R_H + R_{Cl}} R_{Cl} \qquad [2]$$

and

$$PD_{NC} = E_K + \frac{E'_{Cl} - E_K}{R'_{Cl} + R_K} R_K = E'_{Cl} - \frac{E'_{Cl} - E_K}{R'_{Cl} + R_K} R'_{Cl}. \qquad [3]$$

From the above equations we can discuss relationships between ionic resistances and transcellular PD. Since there was a uniform increase in resistance induced by both amines we will assume that changes in PD_{NS} and decreases in H^+ secretory rates observed are mostly due to the increase in resistance of one or more of the ionic pathways depicted in the equations above.

When any treatment results in an increase in resistance and a marked decrease in the H^+ secretory rate it is reasonable to assume that its effect is mostly on the secretory membrane. Therefore the effect must be on R_H and/or R_{Cl}. From Eqs. [1] and [2] one can see that a decrease in PD_{NS} should result if only R_{Cl} increases and an increase in PD_{NS} should result if only R_H increases. If we divide numerator and denominator of the fraction in the middle of Eq. [2] by R_H, we obtain

$$PD_{CS} = -E_H + \left(1 + \frac{R_{Cl}}{R_H}\right)^{-1} (E_H + E_{Cl}). \qquad [4]$$

Should any treatment result in an increase in both R_H by ΔR_H and R_{Cl} by ΔR_{Cl}, a new equation can be obtained:

$$PD'_{CS} = -E_H + \left(1 + \frac{R_{Cl} + \Delta R_{Cl}}{R_H + \Delta R_H}\right)^{-1} (E_H + E_{Cl}). \qquad [5]$$

The PD_{NS} would decrease ($PD'_{CS} < PD_{CS}$) if $(R_{Cl} + \Delta R_{Cl})/(R_H + \Delta R_H) > R_{Cl}/R_H$, that is, if R_{Cl} had a higher proportional increase than R_H. The PD_{NS} would increase if R_H increased proportionally more than R_{Cl}. Finally the PD_{NS} would not change if both R_{Cl} and R_H had equal proportional increases. It should be noted that for the secretory pathways an increase in R_H alone is equivalent in its effect on PD to a decrease of E_H and, similarly, R_{Cl} is equivalent to E_{Cl}. For brevity we refer to resistance only.

When increases in resistance are accompanied by small changes in H^+ secretory rate it is reasonable to assume that the effect is mostly on the nutrient membrane. From Eqs. [1] and [3] one may deduce that a decrease in PD_{NS} should result if only R'_{Cl} increases, while an increase in PD_{NS} would be observed if only R_K increases (9). Dividing numerator and denominator of the fraction in the middle of Eq. [3] by R_K, we obtain:

$$PD_{NC} = E_K + \left(1 + \frac{R'_{Cl}}{R_K}\right)^{-1} (E'_{Cl} - E_K). \quad [6]$$

With an increase of both R'_{Cl} by $\Delta R'_{Cl}$ and R_K by ΔR_K we obtain:

$$PD'_{NC} = E_K + \left(1 + \frac{R'_{Cl} + \Delta R'_{Cl}}{R_K + \Delta R_K}\right)^{-1} (E'_{Cl} - E_K). \quad [7]$$

The interpretation of the effects of increases in resistance on the PD of the nutrient membrane is more difficult since it will depend on the relative magnitudes of E'_{Cl} and E_K which have not been well established. If one assumes that $E'_{Cl} > E_K$ (9), the PD_{NS} would decrease ($PD'_{NC} < PD_{NC}$) if $(R'_{Cl} + \Delta R'_{Cl})/(R_K + \Delta R_K) > R'_{Cl}/R_K$, that is, if R'_{Cl}increased proportionally more than R_K. The PD_{NS} would increase ($PD'_{NC} > PD_{NC}$) if R_K increased proportionally more than R'_{Cl}. Should it be shown that E_K is actually greater than E'_{Cl} the opposite effects would be obtained. The PD_{NS} would not change if both R'_{Cl} and R_K increased proportionally the same.

The fact that both norepinephrine and phentolamine decreased the H^+ to near zero at concentrations of 2.35 and 2.0 mM, respectively, in the nutrient solution suggests that both drugs affect the ionic pumps on the secretory membrane. The increase in resistance could be due solely to an increase in R_{Cl}. If both R_{Cl} and R_H increased, R_{Cl} must have increased proportionally more than R_H. At the lower concentrations of 0.1 and 1.0 mM in the nutrient solution, the increase in R is not accompanied by a marked decrease in H^+ suggesting that the effect is mostly on the nutrient membrane. Since the PD_{NS} is not significantly changed, it would appear that the increase in R'_{Cl} and/or R_{Cl} is accompanied by an appropriate increase in R_K (if $E'_{Cl} > E_K$) and/or R_H. In contrast, for the β-adrenergic amines, propranolol appeared to show a principal effect on the Cl^- pathway of the nutrient membrane (if $E'_{Cl} > E_K$) and isoproterenol showed an effect on the K^+ pathway, aside from its effects on the H^+ and Cl^- pumps (unpublished observations in our laboratory).

Both amines (α-agonist and -antagonist) probably affect the nutrient and secretory ionic pathways. At the lower doses the effect is manifested only on the nutrient membrane, while the effect on the secretory membrane becomes evident only with the higher doses. The fact that both amines produced similar effects, without inhibiting each other when used in combination, suggests that their inhibitory effects on the frog gastric mucosa are of a nonspecific nature, and not through α-adrenergic receptors.

Summary. The α-adrenergic amines, norepinephrine and phentolamine, when added to the nutrient solution of frog gastric mucosa, produce a decrease of the H^+ secretory rate of about 20 to 100% for concentrations increasing from 0.1 to about 2.3 mM of either amine in the nutrient solution. Combinations of these amines produced no antagonistic effects, thereby providing no evidence for the existence of α-adrenergic receptors in frog gastric mucosa.

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