PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 256-261 (1978)

Effects of β-Adrenergic Amines on Frog Gastric Mucosa (40033)

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In a review article, Holton (1) states that both alpha and beta agonists in doses of the order of 1 mcg kg^{-1} min^{-1} inhibit acid secretion in conscious animals, that histamine-stimulated secretion is less easily depressed than other means such as pentagastrin stimulation, and that beta agonists in doses of the order of 0.1-0.5 mcg kg^{-1} min^{-1} increase histamine-stimulated secretion. However, much of the influence of adrenergic amines, *in vivo,* had been attributed to effects on mucosal blood flow (2). The isolated frog gastric mucosa enables the effects of catecholamines to be studied in the absence of a blood supply. Thorpe *et al.* (3) had, in fact, investigated the effects 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 m*M* produced statistically insignificant decreases in histamine-stimulated H^+ secretion. Recently Rose *et al.* (4) found that norepinephrine, when added to the nutrient solution of isolated gastric mucosae of Rana pipiens, produced a significant decrease of about 20% to about 100% respectively for concentrations increasing from 0.1 to about 2.3 m*M*. In the case of 5 m*M* isoproterenol, a beta agonist, Thorpe et al found that the H^+ secretory rate was significantly decreased by 55% and 60% respectively for histamine and acetylcholine-stimulated H^+ secretion. This inhibition of isoproterenol was blocked by propranolol, a beta antagonist. The authors suggested on the basis of their data that isoproterenol inhibited acid secretion by direct activation of mucosal beta-adrenergic receptors. Recent work in our laboratory (5) lends support to their experimental findings on beta adrenergic amines.

In the study of Thorpe *et al.* (3), however, only the H^+ secretory rate was measured. The present investigation considers the inhibitory effects of isoproterenol (Isuprel), a beta agonist; epinephrine (Adrenalin) a predominantly beta agonist; and propranolol (Inderal), a beta antagonist on the transmucosal resistance, the transmucosal potential difference (PD) and the H^+ secretory rate of the gastric mucosa of Rana pipiens in Cl^- media. In addition, blocking effects between propranolol and isoproterenol and between propranolol and epinephrine are reported.

Methods. The experiments were performed on gastric mucosae of Rana pipiens with an in vitro method described in detail elsewhere (6). 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 (7). 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, the beta adrenergic amine was added to the nutrient solution in concentrations varying from 0.1 m*M* up to as much as 4.7 m*M*. Combinations of these amines were also investigated. For each experiment a different frog was used.

Results. Electrophysiological effects of varying concentrations of beta adrenergic amines. Figure 1 depicts the results of a typical experiment in which the nutrient solution contained isoproterenol at a concentration of 0.25 m*M*. In 30 min after the

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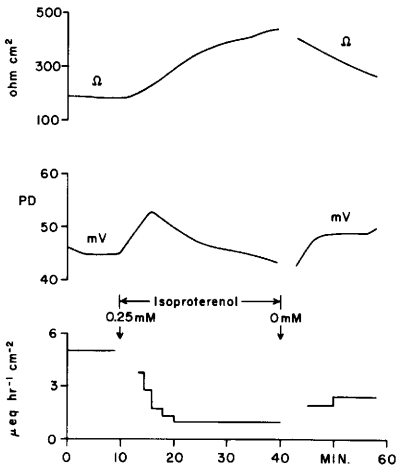

FIG. 1. Effects of isoproterenol on the resistance, PD and H^+ secretory rate of frog gastric mucosa in chloride solutions. At the time indicated by the first arrow, isoproterenol was added to a concentration of 0.25 m*M* in the nutrient solution and at the time indicated by the second arrow, the isoproterenol was removed. Resistance, PD and H^+ secretory rate are plotted versus time.

addition of isoproterenol, the resistance increased about 100% above control values while the H^+ secretory rate decreased about 80%. The PD at first increased to a maximum value but at the end of 30 min returned essentially to the control level. Washing both sides of the mucosa with regular solutions produced a partial recovery of the resistance and H^+ secretory rate towards control levels.

Table I summarizes the results of the effects induced by each of the three adrenergic amines about 30 min after the addition of a specified concentration of the amine to the nutrient solution, compared to control values. Except for 0.1 m*M* isoproterenol and 0.1 m*M* propranolol, all concentrations of each amine gave significant increases in resistance. Without exception, each amine showed significant decreases in the H^+ secretory rate at all concentrations. In fact, there was a dose dependency in that for each amine the H^+ secretory rate became increasingly smaller with increasing concen-

TABLE I. EFFECTS OF BETA ADRENERGIC AMINES ON RESISTANCE, PD AND H^+ SECRETORY RATE OF FROG GASTRIC MUCOSA.

Adrenergic amines	Conc. (m*M*)	No. of Expts.	R (Ω-cm^2)	$\Delta R/R$ (%)	PD (mV)	$\Delta PD/PD$ (%)	$\dot{H}$ (μeq h^{-1} cm^{-2})	$\Delta\dot{H}/\dot{H}$ (%)
Isoproterenol	0.10	6	183 ± 67[a]	8 ± 14 ($P > 0.1$)	34 ± 5	14 ± 6 ($P < 0.01$)	6.0 ± 2.1	−9.7 ± 5.8 ($P < 0.05$)
	0.25	9	193 ± 59	111 ± 77 ($P < 0.01$)	37 ± 8	12 ± 10 ($P < 0.01$)	4.2 ± 1.0	−62.0 ± 24 ($P < 0.01$)
	0.50	6	237 ± 101	279 ± 172 ($P < 0.01$)	42 ± 3	−6 ± 15 ($P > 0.4$)	4.1 ± 0.7	−100 ± 0 ($P < 0.01$)
Epinephrine	0.10	7	336 ± 123	17 ± 11 ($P < 0.01$)	44 ± 11	6 ± 9 ($P > 0.20$)	3.1 ± 0.7	−22 ± 12 ($P < 0.01$)
	1.00	14	203 ± 91	122 ± 112 ($P < 0.01$)	28 ± 8	32 ± 20 ($P < 0.01$)	4.6 ± 1.1	−70 ± 31 ($P < 0.01$)
	2.00	6	313 ± 130	69 ± 49 ($P < 0.02$)	34 ± 5	14 ± 8 ($P < 0.01$)	3.3 ± 1.1	−78 ± 35 ($P < 0.01$)
	4.70	3	127 ± 29	725 ± 141 ($P < 0.01$)	46 ± 6	−4 ± 16 ($P > 0.7$)	4.7 ± 0.7	−100 ± 0 ($P < 0.01$)
Propranolol	0.10	8	190 ± 101	5.1 ± 9.9 ($P > 0.4$)	30 ± 7	−4 ± 16 ($P > 0.5$)	3.6 ± 1.0	−13 ± 10.8 ($P < 0.01$)
	1.00	7	303 ± 99	19 ± 14 ($P < 0.02$)	41 ± 8	−11 ± 8 ($P < 0.02$)	2.8 ± 0.8	−28 ± 26 ($P < 0.05$)
	2.00	7	332 ± 166	47 ± 31 ($P < 0.01$)	35 ± 9	−12 ± 9 ($P < 0.05$)	3.0 ± 1.4	−51 ± 25 ($P < 0.05$)

[a] Values are means ± SD. $\dot{H}$ refers to H^+ secretory rate. R, PD, and $\dot{H}$ refer to nutrient solution without amine (control values). AR/R, ΔPD/PD, and $\Delta\dot{H}/\dot{H}$ refer to relative changes produced 30 min after the addition of the amine to the nutrient solution. Statistics were determined from differences and not from percentages changes.

tration of that amine in the nutrient solution.

In contrast, changes in PD showed some specificity in that the effects of the beta antagonist and the beta agonists differed with increasing concentrations. We note that for propranolol the PD changed insignificantly for 0.10 m*M* concentration but decreased significantly for the higher concentrations, thereby showing the same PD changes as for the alpha adrenergic amines (4). Contrary to this, the beta agonist, isoproterenol, showed an increase in PD for the lower concentrations of 0.10 and 0.25 m*M* and an insignificant change for the highest concentration of 0.50 m*M*. With the exception of 0.10 mM concentration of epinephrine resulting in an insignificant change in PD, the higher concentrations of epinephrine showed the same trend as isoproterenol. It was this difference in the change in PD between agonist and antagonist that suggested the possibility of blocking effects.

Effects of a combination of agonist and antagonist in the nutrient solution following an induction period with a single amine. Figure 2 illustrates a typical experiment in which 0.10 m*M* propranolol in the nutrient solution for 30 min was followed by a combination of 0.10 m*M* propranolol and 0.25 m*M* isoproterenol. As Fig. 2 shows, the changes in the parameters resulting from the combination of amines after 30 min in the nutrient solution were only minimal compared to control levels. The resistance increased by about 15%, the PD decreased by about 20% and the H^+ secretory rate decreased by about 15%. A comparison of Fig. 2 with Fig. 1 shows that isoproterenol alone produced marked changes in resistance and H^+ secretory rate compared to small changes by the combination of amines. Finally, there was a recovery period with regular solutions in which the parameters returned towards control levels.

Table II summarizes the effects induced by a combination of adrenergic amines after a period of 30 min in the nutrient solution following a single amine in that solution for 30 min. In experiments with 0.10 m*M* propranolol followed by a combination of 0.10 m*M* propranolol and 0.25 m*M* isoproterenol, the effects of the combination on resistance, PD and H^+ secretory rate were significantly less compared to the effects of 0.25 m*M* isoproterenol alone. We note that the 25% decrease in H^+ secretory rate due to the combination is significantly smaller ($P < 0.01$) than the 62% decrease in H^+ secretory rate due to isoproterenol alone. Also the insignificant change in resistance and PD due to the combination contrasts significantly ($P < 0.01$) with the 111% increase in resistance and the 12% increase in PD due to isoproterenol alone. However, if 0.25 m*M* isoproterenol alone in the nutrient solution was followed by the combination of 0.25 m*M* isoproterenol and 0.10 m*M* propranolol, the effects obtained by the combination after 30 min in the nutrient solution on resistance, PD and H^+ secretory rate were not significantly different from the effects on these parameters obtained by isoproterenol alone.

As Table II indicates, the combination of 0.1 m*M* propranolol and 1.0 m*M* epinephrine exhibited the same kind of behavior as the combination of 0.1 m*M* propranolol and 0.25 m*M* isoproterenol. If propranolol

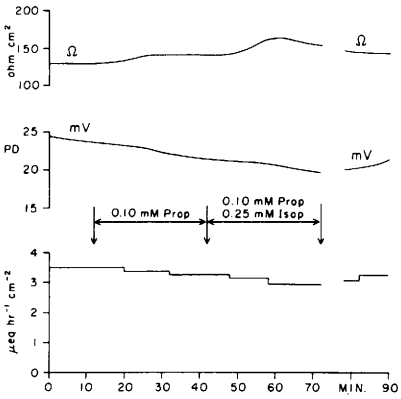

FIG. 2. Effects of propranolol (Prop) and isoproterenol (Isop) following an induction period with propranolol alone on the resistance, PD and H^+ secretory rate of frog gastric mucosa in chloride solutions. At the time indicated by the first arrow, propranolol was added to a concentration of 0.10 m*M* in the nutrient solution. At the time indicated by the second arrow, 30 min later, isoproterenol was added to a concentration of 0.25 m*M* in the nutrient solution which now had the combination of two amines. At the time indicated by the third arrow, the two amines were removed.

TABLE II. EFFECTS OF COMBINATION OF TWO ADRENERGIC AMINES ON RESISTANCE, PD AND H^+ SECRETORY RATE OF FROG GASTRIC MUCOSA FOLLOWING AN INDUCTION PERIOD WITH A SINGLE AMINE.[a]

Combination of Adrenergic Amines	No. of Expts.	R (Ω-cm^2)	$\Delta R/R$ (%)	PD (mV)	$\Delta PD/PD$ (%)	$\dot{H}$ (μeq h^{-1} cm^{-2})	$\Delta\dot{H}/\dot{H}$ (%)
0.10 m*M* Propranolol[a]	9	179 ± 100[b]	24 ± 32	29 ± 7	−10 ± 21	2.9 ± 1.4	−25 ± 26
0.25 m*M* Isoproterenol			($P > 0.1$)		($P > 0.1$)		($P < 0.01$)
0.25 m*M* Isoproterenol	6	248 ± 126	66 ± 46	25 ± 9	16 ± 163	3.5 ± 0.9	−73 ± 26
0.10 m*M* Propranolol			($P < 0.01$)		($P > 0.50$)		($P < 0.01$)
0.10 m*M* Propranolol	7	216 ± 118	42 ± 28	23 ± 12	42 ± 58	4.0 ± 2.0	−30 ± 22
1.00 m*M* Epinephrine			($P < 0.01$)		($P > 0.05$)		($P < 0.01$)
1.00 m*M* Epinephrine	6	181 ± 93	117 ± 93	26 ± 9	27 ± 15	4.1 ± 0.8	−66 ± 27
0.10 m*M* Propranolol			($P < 0.05$)		($P < 0.01$)		($P < 0.01$)
0.10 m*M* Epinephrine	4	271 ± 161	44 ± 21	38 ± 11	5 ± 14	3.2 ± 0.8	−65 ± 12
1.00 m*M* Propranolol			($P < 0.05$)		($P > 0.20$)		($P < 0.01$)
0.10 m*M* Propranolol	4	341 ± 122	77 ± 47	40 ± 6	25 ± 11	3.4 ± 0.9	−85 ± 13
1.00 m*M* Norepinephrine			($P < 0.02$)		($P < 0.02$)		($P < 0.02$)

[a] First member of combination was single amine in nutrient solution to which second member of pair was later added.

[b] Values are means ± SD. $\dot{H}$ refers to H^+ secretory rate. R, PD, and $\dot{H}$ refer to nutrient solution without amine. $\Delta R/R$, $\Delta PD/PD$, and $\Delta\dot{H}/\dot{H}$ refer to relative changes produced 30 min after the addition of the combination of amines in the nutrient solution following a 30-min period of a single amine in the solution.

was added first, blocking effects were evident whereas if epinephrine was added first, blocking effects were absent. All other combinations failed to reveal any antagonism. For example, previously it was found that the alpha agonist, norepinephrine, and the alpha antagonist, phentolamine, showed no blocking effects (4). From Table II, it is evident that there is no interference between the beta antagonist, propranolol, and the alpha agonist, norepinephrine.

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 (8, 9). On the other hand, agents or procedures which increase the resistance of ionic pathways on the nutrient membrane are usually associated with small decreases in the H^+ secretory rate (9, 10). Rose *et al.* (4) provided an analysis based on these facts to explain changes in PD arising from changes in resistance. We shall apply this analysis as a working model to the present results. Referring to Fig. 3 which shows the principal ionic pathways on the secretory and nutrient membranes, we shall summarize the main results of the analysis at this point.

When any treatment results in an increase in resistance and a marked decrease in H^+ secretory rate, it is, as indicated above,

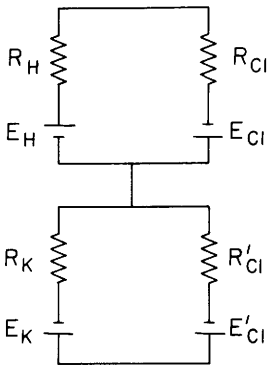

FIG. 3. Electrical circuit showing principal pathways of the secretory and nutrient membranes. Upper part refers to pathways of the secretory membrane, namely the H^+ and Cl^- emf's and their respective resistances R_H and R_{Cl}. Lower part refers to low resistance pathways of the nutrient membrane, namely the emf's E_K and E'_{Cl} due to ion gradients and their respective resistances R_K and R'_{Cl}.

reasonable to assume its effect to be mainly on the secretory membrane. Should changes in R_H by ΔR_H and R_{Cl} by ΔR_{Cl} both be involved, the changes in PD would be as follows. The PD decreases if $(R_{Cl} + \Delta R_{Cl})/(R_H + \Delta R_H) > R_{Cl}/R_H$, that is, if R_{Cl} has a higher proportional increase than R_H. The PD increases if R_H increases proportionally more than R_{Cl}. Finally, the PD does not change if both R_{Cl} and R_H have equal

proportional increases.

When any treatment results in increases in resistance and small changes in H^+ secretory rate, it is reasonable to assume that the effect is mainly on the nutrient membrane. The interpretation here is complicated by the fact that the relative magnitudes of E'_{Cl} and E_K are not well established. If one assumes that $E'_{Cl} > E_K$ (10), the PD decreases if $(R'_{Cl} + \Delta R'_{Cl})/(R_K + \Delta R_K) > R'_{Cl}/R_K$, that is, if R'_{Cl} increases proportionally more than R_K. The PD increases if the reverse is true. Should it be shown that $E_K > E'_{Cl}$, opposite effects would be obtained. The PD does not change if both R'_{Cl} and R_K increase proportionally the same.

The effects of the adrenergic amines on the ionic pathways can now be explained. For those concentrations in which the beta agonist, isoproterenol or epinephrine, shows both an increase in resistance and an increase in PD, it may be inferred that the principal effects must occur on the H^+ pathway of the secretory membrane and on the K^+ pathway of the nutrient membrane (if $E'_{Cl} > E_K$). On this basis, the Cl^- pathway could be affected on each membrane but to a lesser extent than the cationic pathways. At the highest concentrations of both amines at which secretion was abolished, there occurred an increase in resistance and no significant change in PD. These results imply that the H^+ and Cl^- pathways of the secretory membrane change proportionately about the same. For those concentrations in which the beta antagonist, propranolol, shows both an increase in resistance and a decrease in PD, it may, likewise, be inferred that the principal effects may occur on the Cl^- pathways of both membranes (if $E'_{Cl} > E_K$). The effects of propranolol on the PD and resistance compare with those of norepinephrine and phentolamine (4).

We shall now discuss briefly the blocking effects. Even though the concentrations were much higher than those needed in other tissues for studies of alpha and beta receptors, it is of interest that even at these high concentrations there seems to be a type of antagonism. Except for the large concentrations, the data are compatible with the concept of beta receptor sites as hypothesized by Thorpe *et al.* One might speculate that isoproterenol or epinephrine would inhibit primarily at these sites whereas propranolol would also act at non-specific sites. Thus neither isoproterenol nor epinephrine would interfere substantially with propranolol but the latter at the beta receptor sites would prevent the inhibitory action of isoproterenol or epinephrine. The existence of such sites needs to be established using the criteria for competitive antagonism and demonstrating affinity of the receptors as comparable with known beta receptors.

Summary. Beta adrenergic amines like alpha adrenergic amines decreased the H^+ secretory rate and increased the transmucosal resistance of the gastric mucosa of Rana pipiens. A concentration of 0.1 m*M* propranolol in the nutrient solution followed by a combination of 0.1 m*M* propranolol and either 0.25 m*M* isoproterenol or 1.0 m*M* epinephrine reduced the effects of the beta agonists, isoproterenol and epinephrine. However, a concentration of 0.25 m*M* isoproterenol or 1.0 m*M* epinephrine followed by the same combination did not reduce the effects of isoproterenol or epinephrine alone. These results suggested the existence of beta receptor sites in addition to non-specific sites.

The authors thank Ms. Linda Childers for her technical assistance and Mr. Craig Collins for his brief but important collaboration on a Research Medical Student Scholarship. We thank Ayerst Laboratories, Winthrop Laboratories and Parke, Davis and Co. for their kind supply of Inderal, Isuprel and Adrenalin respectively.

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Received June 27, 1977. P.S.E.B.M. 1978, Vol. 157.