ROLE OF ADRENERGIC AGONISTS ON GASTRIC SECRETION IN THE RAT

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Following previous demonstration that isoproterenol stimulated and norepinephrine inhibited gastric acid secretion induced by secretagogues, role of adrenergic agonists was studied by measuring acidity and peptic activity of the effluent of the perfused rat stomach. Response of gastric secretion to isoproterenol was increased by theophylline treatment but was not affected by metiamide treatment. N\textsuperscript{6},O\textsuperscript{2*}-Dibutyrlyadenosine 3', 5'-cyclic monophosphoric acid sodium salt monohydrate (dibutryl-c-AMP) stimulated gastric secretion in a dose-dependent manner. These results suggest the possibility that the action of isoproterenol in gastric acid secretion is mediated by c-AMP. However, gastric secretion induced by pentagastrin, histamine, or carbamylcholine was not affected by theophylline treatment. N\textsuperscript{2},O\textsuperscript{2*}-Dibutyrlyguanosine 3', 5'-cyclic monophosphoric acid sodium salt (dibutryl-c-GMP) did not exert any effect on gastric secretion. Depression of pentagastrin-induced gastric secretion by norepinephrine was reversed by EGTA infusion. Moreover, Ca\textsuperscript{2+} depressed pentagastrin-induced gastric secretion. These results suggest that the action of norepinephrine is closely related to the concentration of Ca\textsuperscript{2+}.

Keywords—gastric secretion; isoproterenol; norepinephrine; theophylline; Ca\textsuperscript{2+}; EGTA

INTRODUCTION

In the previous paper,\textsuperscript{1) we reported that isoproterenol stimulates gastric secretion without cholinergic rebound and norepinephrine inhibits that induced by tetragastrin, histamine, or carbamylcholine. We stated that the stimulatory and inhibitory effect of β- and α-adrenergic agonists on gastric secretion might be mediated by cyclic nucleotides. In terms of second mediators in gastric secretion,\textsuperscript{2-4) there are many reports which indicate that the second mediator of pentagastrin\textsuperscript{5,6) and histamine\textsuperscript{7-9) is cyclic adenosine monophosphate (c-AMP). On the contrary, Lippman\textsuperscript{10) and Thompson et al.\textsuperscript{11,12) reported that gastric secretion inhibitory substances activated adenylate cyclase. Moreover there are papers which showed that gastric secretion was not associated with cyclic nucleotides.\textsuperscript{13-15) These discrepancies are due mainly to the differences in methods, species of animals, materials, and preparations used. As is known, β-adrenergic agonists are closely related to cyclic nucleotides. On the other hand, mechanism of α-adrenergic agonists is much less well characterized. In this paper, we demonstrate the presence of possible second mediators of isoproterenol and norepinephrine, paying particular attention to the action of norepinephrine.

METHODS AND MATERIALS

Male Wistar rats weighing about 200 g were fasted for 1 day and used for the perfused rat stomach preparation under urethane anesthesia, according to the method of Ghosh and Schild.\textsuperscript{16) Details of experimental procedure were described in our previous paper.\textsuperscript{1) For the comparison of the effect of a secretagogue, acidities of 10-min effluents above basal secretion were summed for 1 hr after injection of a secretagogue and expressed as µmol hr.\textsuperscript{-1)

Pentagastrin (t-butyloxycarbonyl-β-Ala-Trp-Asp-Phe-NH\textsubscript{2}) was purchased from Sumitomo
Chem. Co. Metiamide was a gift from Smith, Kline and Fujisawa Co. Other materials used were L-norepinephrine bitartrate, d,l-isoproterenol, ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), and carbamylcholine chloride (Sigma Chemical Co.), histamine dihydrochloride, theophylline, and calcium chloride dihydrate (Wako Pure Chemical Industries), $N^6, O^{2'-} $-dibutyryladenosine 3',5'-cyclic monophosphoric acid sodium salt monohydrate (dibutyryl-c-AMP) (Boehringer Mannheim GmbH), $N^2, O^{2'-} $-dibutyrylguanosine 3',5'-cyclic monophosphoric acid sodium salt (dibutyryl-c-GMP) (Kyowa Hakko Co.).

RESULTS
Effect of Metiamide and Theophylline on Gastric Secretion induced by Isoproterenol

Response of gastric secretion to histamine or isoproterenol before and after infusion of metiamide, a histamine H$_2$-blocker, was compared (Fig. 1). After intravenous injection of histamine (500 μg kg$^{-1}$) or isoproterenol (100 μg kg$^{-1}$), same dose of histamine or isoproterenol

FIG. 1. Effect of Metiamide on Isoproterenol-induced Gastric Secretion
Histamine (Hist) (500 μg kg$^{-1}$) and isoproterenol (Iso) (100 μg kg$^{-1}$) were injected into femoral vein at arrows. Metiamide (2 mg kg$^{-1}$ hr$^{-1}$) was infused through the vein of the other femur during the time indicated by the hatched block. Metiamide depressed basal secretion and histamine-stimulated acid secretion. The values represent the mean± s.e. of four rats.

FIG. 2. Effect of Theophylline on Isoproterenol-induced Gastric Secretion
Isoproterenol (Iso) (100 μg kg$^{-1}$) was injected before and after infusion of theophylline (10 mg kg$^{-1}$ hr$^{-1}$). Theophylline slightly enhanced basal acid secretion, and increased the response of acid secretion to isoproterenol. The values represent the mean± s.e. of four rats.

FIG. 3. Stimulation of Gastric Secretion by Dibutyryl-c-AMP
Doses of 5, 10, and 20 mg kg$^{-1}$ of dibutyryl-c-AMP (db-c-AMP) were injected at 90-min intervals. Gastric secretion to cumulative doses of db-c-AMP responded in a dose-dependent manner. The values represent the mean± s.e. of three rats.
Fig. 4a—c. Effect of Theophylline on Gastric Secretion induced by Pentagastrin, Histamine, or Carbamylcholine

Theophylline (10 mg kg\(^{-1}\) hr\(^{-1}\)) was infused after a single injection of (a) pentagastrin (P.G.) (5 \(\mu\)g kg\(^{-1}\)), (b) histamine (Hist) (500 \(\mu\)g kg\(^{-1}\)), or (c) carbamylcholine (Carb) (2 \(\mu\)g kg\(^{-1}\)). Same doses of chemicals were injected at 90 min after starting the infusion. Responses to each secretagogue before and after infusion of theophylline were not significantly different statistically; theophylline did not affect responses to these secretagogues. The values represent the mean ± s.e. of (a) three and (b) (c) four rats.

Fig. 5. Effect of Dibutryl-c-GMP on Gastric Secretion

After infusion of pentagastrin (P.G.), saline (control) or dibutryl-c-GMP (db-c-GMP) was injected. The response to saline or db-c-GMP is expressed respectively by solid and broken lines, and the mean ± s.e. of three and four rats.

was injected under metiamide infusion (2 mg kg\(^{-1}\) hr\(^{-1}\)). Mean ± s.e. of acid secretion to histamine before and after infusion of metiamide were 3.81 ± 0.90 and 0.38 ± 0.30 (\(\mu\)mol hr\(^{-1}\)), and those to isoproterenol were 3.81 ± 1.03 and 2.43 ± 1.19 (\(\mu\)mol hr\(^{-1}\)), respectively. Metiamide inhibited histamine-induced and isoproterenol-induced acid secretion by 93 ± 5 (\(p < 0.01\)) and 39 ± 16 percent (N.S.), respectively.

Responses of gastric secretion to isoproterenol before and after infusion of theophylline were also examined (Fig. 2). Isoproterenol was injected alone, then under theophylline infusion (10 mg kg\(^{-1}\) hr\(^{-1}\)) the same dose of isoproterenol was injected. Mean ± s.e. of acid secretion in response to isoproterenol before and after infusion of theophylline were 2.19 ± 0.51 and 5.25 ± 0.91 (\(\mu\)mol hr\(^{-1}\)). Percentage increase of acid secretion to isoproterenol after theophylline infusion was 188 ± 96 (\(p < 0.05\)). Theophylline caused a slight increase in basal secretion. Isoproterenol-induced pepsin output paralleled that of acid under
FIG. 6a-b. Effect of EGTA on Gastric Secretion reduced by Norepinephrine Under Continuous Pentagastrin-stimulation

Norepinephrine (100 µg kg⁻¹ hr⁻¹) was infused for 2 hr beginning 90 min after starting infusion of pentagastrin (P.G.), and then EGTA (a) (38 mg kg⁻¹ hr⁻¹) or (b) (190 mg kg⁻¹ hr⁻¹) was infused for 1 hr. A dose of 190 mg kg⁻¹ hr⁻¹ of EGTA reversed norepinephrine-depression above P.G.-induced acid secretion control, when norepinephrine depressed P.G.-induced gastric acid and pepsin secretion. The values represent the mean ± s.e. of three rats in both (a) and (b).

FIG. 7a-b. Effect of Ca²⁺ on Pentagastrin-induced Gastric Secretion

CaCl₂ (a) (11.1 mg kg⁻¹ hr⁻¹) or (b) (55.5 mg kg⁻¹ hr⁻¹) was infused for 1 hr at 90 min after starting infusion of pentagastrin (P.G.). A dose of 55.5 mg kg⁻¹ hr⁻¹ of CaCl₂ markedly depressed P.G.-induced acid secretion, but not P.G.-induced pepsin secretion. Stopping of Ca²⁺ infusion caused rapid restoration of acid secretion. The values represent the mean ± s.e. of (a) four and (b) three rats.
metiamide and theophylline infusion.

The response of gastric secretion to single intravenous injection of dibutyryl-c-AMP was examined (Fig. 3). Mean ± s.e. of acid secretion to 5, 10, and 20 mg kg⁻¹ of dibutyryl-c-AMP were 2.75 ± 0.80, 3.75 ± 1.25, and 7.08 ± 1.69 μmol hr⁻¹, respectively. Enhancement of the basal acid secretion was observed after injection of dibutyryl-c-AMP.

Effect of Theophylline on Gastric Secretion induced by Other Secretagogues

Responses of gastrin, histamine (500 μg kg⁻¹), and carbachol (2 μg kg⁻¹) were compared before and after infusion of theophylline (10 mg kg⁻¹ hr⁻¹) (Fig. 4-a, b, c). Percentages of acid secretion induced by pentagastrin, histamine, or carbachol before and after infusion of theophylline were 68 ± 13, 98 ± 41, and 135 ± 20, respectively. Theophylline rather depressed the response of acid secretion to pentagastrin, while it increased that to carbachol. However these values were not statistically significant. Discharge of pepsin from gastric mucosa by pentagastrin, histamine, or carbachol under theophylline infusion seemed to be similar to acid secretion induced by these secretagogues.

Effect of Dibutyryl-c-GMP and Ca²⁺ Concentration on Gastric Secretion reduced by Norepinephrine

Effect of dibutyryl-c-GMP on pentagastrin-induced gastric secretion was examined (Fig. 5). Dibutyryl-c-GMP (expressed by broken lines) did not inhibit acid secretion, if the response to dibutyryl-c-GMP was calculated in relative value to the response to pentagastrin before injection of dibutyryl-c-GMP.

The effect of Ca²⁺ deprivation on inhibitory effect of norepinephrine was examined by using EGTA, a Ca²⁺ chelating compound. Norepinephrine (100 μg kg⁻¹ hr⁻¹) was infused beginning 90 min after starting infusion of pentagastrin (2 μg kg⁻¹ hr⁻¹). When norepinephrine started to depress gastric secretion, EGTA was infused at the rate of 38 (Fig. 6-a) or 190 mg kg⁻¹ hr⁻¹ (Fig. 6b) for 1 hr. EGTA, in a dose of 38 mg kg⁻¹ hr⁻¹, could not antagonize inhibitory activity of norepinephrine, but a dose of 190 mg kg⁻¹ hr⁻¹ rather stimulated acid secretion. Doses of 38 and 190 mg kg⁻¹ hr⁻¹ of EGTA correspond to 1 and 5 × 10⁻⁴ mol kg⁻¹ hr⁻¹ respectively. Profile of pepsin secretion resembled that of acid except for the amount of pepsin output.

Ca²⁺, a form of CaCl₂, did not affect gastric secretion induced by pentagastrin, when 11.1 mg kg⁻¹ hr⁻¹ of CaCl₂ was infused for 1 hr from 90 min after starting infusion of pentagastrin (2 μg kg⁻¹ hr⁻¹) (Fig. 7-a). However a dose of 55.5 mg kg⁻¹ hr⁻¹ of CaCl₂ markedly depressed gastric secretion. When infusion of CaCl₂ was stopped, stimulatory activity of pentagastrin was restored rapidly (Fig. 7-b). The doses of 11.1 and 55.5 mg kg⁻¹ hr⁻¹ of CaCl₂ correspond to 1 and 5 × 10⁻⁴ mol kg⁻¹ hr⁻¹. Ca²⁺ did not depress pepsin output.

DISCUSSION

It is a well known fact that the second mediator of β-adrenergic agonists is c-AMP. We also demonstrated in the present work that the second mediator of gastric acid stimulatory activity of isoproterenol might be c-AMP; stimulation of acid secretion by isoproterenol was enhanced by theophylline treatment; and dibutyryl-c-AMP stimulated gastric acid secretion. However, in our experiments, gastric acid secretion induced by pentagastrin, histamine, or carbachol was not increased by theophylline; that suggested the possibility that these secretagogues might not be associated with c-AMP. Ohkura et al. showed that dibutyryl-c-AMP was one of secretagogues based on an evidence that c-AMP was a second mediator of secretagogues except β-adrenergic agonists. From these results it is thought that there would be at least two mechanisms for stimulation of acid secretion. One is mediated by c-AMP; we think this stimulation is caused by β-adrenergic agonists, and the other is not mediated by c-AMP; we think this stimulation is caused by pentagastrin, histamine, and carbachol.

Concerning the second mediator of norepinephrine, c-GMP was examined at first. In
our preliminary experiments, single injection of dibutyryl-c-GMP, even in a dose of 50 mg kg\(^{-1}\), did not affect gastric secretion. Dibutyryl-c-GMP also did not inhibit pentagastrin-induced gastric secretion. However, infusion of a high dose of Ca\(^{2+}\) caused a marked depression of pentagastrin-induced gastric acid secretion. It seems likely that the depression of pentagastrin-induced gastric acid secretion by norepinephrine resembles that caused by Ca\(^{2+}\). Usually Ca\(^{2+}\) is known to be an inhibitor of gastric acid secretion in the rat.\(^{19,20}\) On the contrary, there are also papers which reported that Ca\(^{2+}\) enhanced the basal gastric acid secretion in the rat,\(^{21}\) the basal and stimulated gastric secretion in the ferret,\(^{22}\) and plasma gastrin level in patients.\(^{23}\) Changes in Ca\(^{2+}\) concentration affect parathyroid hormone and thyrocalcitonin level in the circulation, which cause the change of plasma gastrin level.\(^{24,25}\) In other tissues, Ca\(^{2+}\) was reported as a modulator of hormonal response\(^{26}\) and was closely related to norepinephrine.\(^{27}\) Norepinephrine increased the rate of uptake of Ca\(^{2+}\) in rat aorta.\(^{28}\) However in isolated rat hepatocytes, norepinephrine induced efflux of Ca\(^{2+}\).\(^{29,30}\) Therefore, in these reports Ca\(^{2+}\) is characterized as a second mediator. In our experiments, depression of pentagastrin-induced gastric acid secretion by norepinephrine was reversed by Ca\(^{2+}\) deprivation, using EGTA infusion. These results suggest that depression of acid secretion is closely related to the concentration of intracellular Ca\(^{2+}\); norepinephrine might increase the influx of Ca\(^{2+}\) to parietal cells in the rat. In terms of the effect of Ca\(^{2+}\) concentration, our results rather agree with those of the experiments using isolated gastric mucosa conducted by Main and Pearce.\(^{31}\) Although we did not demonstrate direct evidence that norepinephrine cause influx of Ca\(^{2+}\) to parietal cells, norepinephrine might inhibit gastric secretion by changing the Ca\(^{2+}\) concentration. This has also been documented by Selinger et al.\(^{32}\) that \(\alpha\)-adrenergic agonists introduces Ca\(^{2+}\) into the cell to mediate K\(^{+}\) release from rat parotid slices. Further studies will be required to determine the interaction between norepinephrine and Ca\(^{2+}\) in isolated cell suspen

sion obtained from gastric mucosa.

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