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Possible Mechanism of Stimulation of Gastrin Secretion by Exogenous Serotonin in Rats

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The effect of 6-hydroxydopamine, propranolol, phentolamine, α-methyl-tyrosine and α-methyl-tyrosine plus propranolol on serotonin-stimulated gastrin secretion in rats has been examined. Gastrin secretion in response to administration of serotonin alone (10 mg/kg i.p.) was significantly reduced in rats pretreated with 6-hydroxydopamine or with propranolol. These results suggest that the effect of exogenous serotonin on gastrin secretion can be described as sympathomimetic and indirect. The serotonin-stimulated gastrin secretion was significantly enhanced by previous administration of phentolamine. Pretreatment with α-methyl-tyrosine also elevated serotonin-stimulated gastrin secretion, indicating that in the presence of diminished concentrations of the catecholamines, the influence of exogenous serotonin on secretion by G cells is increased. This enhancement in the serum gastrin levels was also reduced to a significant extent by simultaneous administration of propranolol, which suggested the activation of G-cell β-adrenergic receptors after serotonin administration.

Serotonin has been shown to be widely distributed in various parts of the gastrointestinal tract (9), and it has been described to produce an increase in the serum gastrin levels (15, 16); however, the mechanism responsible for this action is unknown. It appears definitive that the serotonin-induced gastrin secretion is not related to the alkalinization of the antral pH observed after serotonin administration (16), nor to a direct effect on G cells, as it has been shown that the serotonin antagonist cyproheptadine stimulates gastrin secretion as well (17). On the other hand, there are several pieces of evidence indicating that the stimulant action of serotonin on several adrenergically-innervated organs is sympathomimetic and indirect (11, 18, 19, 27, 35), and that stimulation of β-adrenergic receptors increases gastrin secretion, while stimulation of α-receptors inhibits it (4, 22, 31). The question then arises whether this

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effect is the result of a sympathomimetic response by exogenous serotonin.

The present study was done to shed light on the mechanism of exogenous serotonin-stimulated gastrin secretion. We particularly examined the possible interaction between adrenergic and serotoninergic mechanism in gastrin secretion. Subsequently, the serum gastrin levels after administration of exogenous serotonin were investigated in rats treated and untreated with substances blocking endogenous noradrenaline metabolism.

Materials and Methods

Male Charles River rats, 200-250 g, were caged (5 per cage) under controlled temperature conditions (22 ± 2° C) on a standard light dark cycle (14 h light and 10 h dark). The rats were starved for 24 h before use but allowed free access to water until 2 h before experiments. Serotonin creatinine sulfate; 6-hydroxydopamine hydrobromide; DL-propranolol HCL; DL-α-methyl-p-tyrosine methylester; DL-p-Chlorophenylalanine methylester hydrochloride and carboxymethyl cellulose were purchased from the Sigma Chemical Company, and phentolamine mesylate (Regitine®) was kindly supplied by Ciba Geigi AG. All drugs were calculated and expressed in terms of the salt forms and were dissolved in a 2% aqueous suspension of carboxymethyl cellulose to the desired concentration and injected intraperitoneally. The volumes used were always 2 ml.

The animals were divided into 7 groups. Serum gastrin was evaluated after administration of serotonin (10 mg/kg) in: *Group A:* untreated rats. *Group B:* after pretreatment with 6-hydroxydopamine (200 mg/kg, one week before the test). *Group C:* after pretreatment with repeated dosages of α-methyl-tyrosine (50, 100 and 50 mg/kg, 25, 18 and 2 hours, respectively, before the test). *Group D:* after pretreatment with repeated dosages of propranolol (4, 4 mg/kg, 150 and 30 min, respectively, before the test). *Group E:* after pretreatment with phentolamine (5 mg/kg, 120 min before the test). *Group F:* after pretreatment with α-methyltyrosine plus propranolol at doses and times described above. *The control rats* received the drug solvent.

After pretreatment and at 30, 60, 90 and 120 min following serotonin administration in the experimental groups (A, B, C, D, E and F) and carboxymethyl cellulose in the control group, the rats were anesthetized with diethyl ether (Merck). The abdominal cavity was opened, and each animal was exsanguinated by withdrawing blood from the abdominal aorta. The sera were divided into aliquots and stored at —20° C until assayed. Serum gastrin concentration was measured in duplicate by sensitive and specific radioimmunoassay, as described by YALOW and BERSON (37). The gastrin radioimmunoassay kit employed for this measurement was purchased from *Cea Ire Sorin*, using synthetic human gastrin I (G-1-17, ICI; Macclesfield, England). Antibodies were raised in rabbits by immunization with synthetic human gastrin I (G-1-17, ICI), covalently coupled to bovine serum albumin according to MCGUIGAN (25), and used in a final dilution of 1:53 000. Monoiodinated synthetic human gastrin (G-17) was used as a tracer and G-17 as standard. The separation of antibody-bound from free hormone was carried out by dextran-coated charcoal and the labelled free and bound hormone was counted in an automatic gamma scintillation counter (Wallac, LKB, Sweden). The within-assay variation determined by repeated measurements of two pools of sera ranged from 3.8 to 6.2% and between-assay variations were calculated to be from 6.1 to 10.1%. The immunoassay system was sufficiently sensitive to detect 10 pg/ml of serum gastrin.

Results are expressed at the mean ±

one standard error. The Mann-Whitney test for unpaired values was used to analyze the data for statistical significance of differences with a P value of less than 0.05 considered significant.

Results

The administration of exogenous serotonin (10 mg/kg) induced a progressive increase in immunoreactive serum gastrin concentration from 53.41 ± 2.23 pg/ml in the fasting state to 103.28 ± 3.32 pg/ml after 120 min of serotonin administration (table I). This increase is highly significant.

Pretreatment with 6-hydroxydopamine resulted in a rise of basal serum gastrin from 55.01 ± 1.17 pg/ml to 69.23 ± 3.06 pg/ml, which represents a significant increase (table II). In contrast, chemical sympathectomy lessened the secretory properties of serotonin. As shown in figure 1, pretreatment with 6-hydroxydopamine reduced the serum gastrin in response to administration of serotonin from 103.28 ± 3.32 pg/ml to 62.36 ± 2.84 pg/ml. This reduction is highly significant ($p < 0.001$).

Propranolol alone did not significantly alter the basal serum gastrin (table II), whereas pretreatment with propranolol resulted in a significant reduction ($p < 0.01$) of serotonin-stimulated gastrin secretion, attaining a value of 78.13 ± 3.50 pg/ml (figure 1).

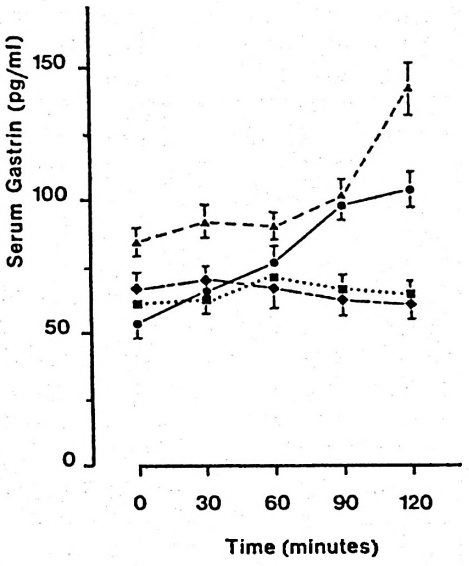

Fig. 1. *Serum gastrin levels after administration of serotonin (●——●); phentolamine + serotonin (▲------▲); propranolol + serotonin (■.....■) and 6-hydroxydopamine + serotonin (◆—·—◆).*
Each value is the mean ± S.E.M. from 10 rats.

Table I. *Serum gastrin levels (pg/ml) after serotonin administration (10 mg/kg i.p.).*
Mean ± S.E.M. N = 10.

Treatment	Basal	30 min	60 min	90 min	120 min
Control	53.41 ± 2.23	59.22 ± 1.84	55.66 ± 1.53	60.86 ± 2.40	57.15 ± 2.53
Serotonin	53.41 ± 2.23	66.26 ± 2.37	76.67 ± 3.10	98.04 ± 3.06	103.28 ± 3.32*

* $p < 0.001$.

Table II. *Basal serum gastrin (pg/ml) after administration of 6-hydroxydopamine (6-OHDA), propranolol, phentolamine, α-methyl-tyrosine (α-MT) or α-methyl-tyrosine plus propranolol (α-MT + Prop.).*
Mean ± S.E.M. N = 10.

Basal	6-OHDA	Propranolol	Phentolamine	α-MT	α-MT+Prop.
55.01 ± 1.17	69.23 ± 3.06*	62.39 ± 2.08	85.86 ± 2.56**	83.03 ± 2.57**	80.25 ± 3.92

* $p < 0.05$; ** $p < 0.01$.

Phentolamine increased significantly the basal serum gastrin (table II). The serotonin-stimulated gastrin secretion was significantly enhanced ($p < 0.01$) by pretreatment with phentolamine from 103.28 ± 3.32 pg/ml to 140.56 ± 4.73 pg/ml (figure 1).

Repeated dosages with α-methyl-tyrosine caused a significant increase in the basal serum gastrin (table II). The serotonin-stimulated gastrin release was significantly enhanced ($p < 0.001$) by pretreatment with α-methyl-tyrosine. In figure 2 it was observed that immunoreactive serum gastrin concentrations were increased from 103.28 ± 3.32 pg/ml to 232.85 ± 6.38 pg/ml. It was also observed that this increase was substantially reduced ($p < 0.001$) by simultaneous administration of propranolol, reaching a value of 25.20 ± 0.95 pg/ml.

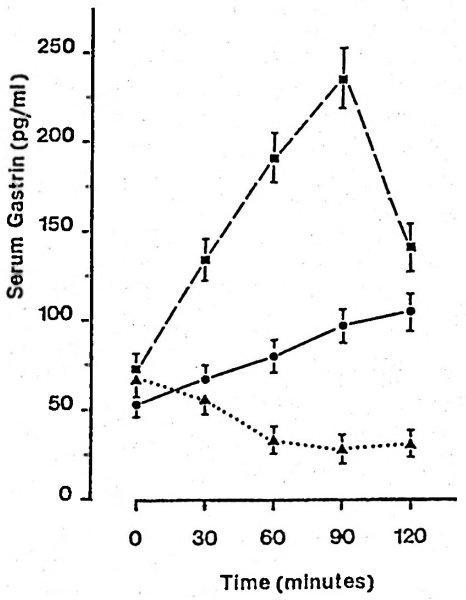

Fig. 2. *Serum gastrin levels after administration of serotonin (●——●); α-methyl-tyrosine +serotonin (■·····■) and propranolol + α-methyl-tyrosine + serotonin (▲·····▲).*

Discussion

In this study the observation was made that administration of serotonin in rats resulted in increased serum gastrin concentration. This finding confirms our previous studies (15, 16). This effect of exogenous serotonin does not seem to be related to its alkalinization of intragastric pH (16) nor to a direct effect on G cells (17). At the same time, there are several pieces of evidence suggesting that the stimulant action of serotonin on rabbit heart and atria (11, 19, 35), isolated cat spleen (18, 27) and cat nictitating membrane (27) is sympathomimetic and indirect, since this effect of serotonin was found to be reduced by pretreatment with 6-hydroxydopamine (11) or with propranolol (11, 35).

During the course of this research it was shown that serotonin-stimulated gastrin secretion is significantly reduced by pretreatment with 6-hydroxydopamine, a drug known to produce long-lasting depletion of peripheral noradrenaline from sympathetically innervated organs as the results of acute and selective degeneration of the sympathetic nerves (21, 34) without altering the serotonin concentration (36). These observations were extended by using phentolamine and progranolol, α and β-adrenoceptor antagonists, respectively. Phentolamine caused a significant increase in serotonin-stimulated gastrin secretion, and propranolol caused a significant reduction. Based on these results, the mechanism of action of exogenous serotonin on gastrin secretion can be described as sympathomimetic and indirect, since gastrin secretion in response to administration of serotonin was significantly reduced by 6-hydroxydopamine and propranolol. In seeking an explanation for the mechanism of the indirect sympathomimetic action of serotonin, one finds two principal possibilities. First, it is well known that serotonin can displace endogenous noradrenaline from storage

sites (1, 13, 18). The other possibility is that serotonin could be accumulated into sympathetic endings in several organs (3, 5, 10, 12, 20, 24, 26, 33). Once transported into the neuron, it could subsequently be released as a «false» transmitter, which, in turn, would act on adrenaline receptors (28). A third possibility could be considered, consistent with previous observations that serotonin is capable of changing the microcirculation in the gastrointestinal tract and kidney where the catabolism and excretion of gastrin occurs (2, 6, 23, 32). In spite of conflicting data, most evidence indicates that serotonin, at same dose of 10 mg/kg, increases gastric blood flow (7) and produces a vasoconstriction in the kidney's afferent arterioles (8). The mechanism responsible for the catabolism of gastrin in the gastrointestinal tract and kidneys is poorly understood, and data concerning the interference of circulatory changes after serotonin administration on excretion and catabolism of endogenous gastrin are not available. However, the supression of hypergastrinaemia by propranolol and 6-hydroxydopamine is not considered likely as a major role of circulatory phenomena in affecting gastrin concentration after serotonin administration.

The use of α-methyl-tyrosine further amplifies the study of the mechanism of action of serotonin on gastrin secretion. Pretreatment with α-methyl-tyrosine, a drug known to selectively reduce central and peripheral noradrenaline levels without lowering serotonin levels (14, 29, 30), resulted in an increase of serotonin-stimulated gastrin secretion. The implication here is that in the presence of diminished concentrations of the catecholamines, the level of gastrin release by exogenous serotonin is increased. This may be due to a rise in the accumulation of serotonin into catecholaminergic endings, since serotonin and catecholamines compete for the same uptake sites in catecholaminergic neurons (28). Based on the results reported in this paper it is proposed that exogenous serotonin could be transported into gastric sympathetic endings. Once accumulated into the neuron, it would be released as a «false» adrenergic transmitter. It could subsequently act on G cell β-adrenergic receptors. To confirm this assumption will require further studies.

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Resumen

Se estudia el efecto de la 6-hidroxidopamina, propranolol, fentolamina, α-metiltirosina, y α-metiltirosina más propranolol, sobre la secreción de gastrina estimulada por la serotonina. La secreción de gastrina, en respuesta a la inyección de serotonina (10 mg/kg de peso, i.p.), se reduce significativamente en los animales tratados con 6-hidroxidopamina o propranolol, se potencia significativamente por el tratamiento previo con fentolamina y más marcadamente con pretratamiento con α-metiltirosina. Este aumento de la secreción de gastrina es abolido por el tratamiento simultáneo con propranolol, lo que sugiere que la serotonina exógena ejerce su acción estimuladora de la secreción de gastrina a través de una activación de los receptores β-adrenérgicos de las células G del estómago.

References

1. ANDEN, N. E.: *Acta Pharmac. Tox.*, 21, 59-75, 1964.
2. BECKER, H. D., REEDER, D. D. and THOMPSON, J. C.: *Gastroenterology*, 65, 903-906, 1973.
3. BERTLER, A., FALCK, B. and OWMAN, C.: *Acta Physiol. Scand.*, 63, suppl., 239, 1-18, 1964.
4. BRANDSBORG, O., BRANDSBORG, M. and CHRISTENSEN, N. J.: *Eur. J. Clin. Invest.*, 6, 395-401, 1976.

5. BURGEN, A. S. V. and IVERSEN, L. L.: *Br. J. Pharmac. Chemother.*, **25**, 34-39, 1965.
6. CLENDINNEN, B. G., REEDER, D. D., BRANDT, E. N. and THOMPSON, J. C.: *Gut*, **14**, 462-467, 1973.
7. DOLCINI, H. A., ZAIDMAN, I. and GRAY, S. J.: *Amer. J. Physiol.*, **199**, 1157-1160, 1960.
8. ERSPAMER, V.: *Ricerca scient.*, **22**, 694-702, 1952.
9. ERSPAMER, V.: In «Handbook of Experimental Pharmacology», Vol. XIX (Erspamer, V., ed.). Springer-Verlag, New York, 1966, pp. 132-181.
10. FILLION, G. M. B., LLUCH, S. and UVNAS, B.: *Acta Physiol. Scand.*, **83**, 115-123, 1971.
11. FOZARD, J. H. and MWALUKO, G. P. M.: *Br. J. Pharmac.*, **57**, 115-125, 1976.
12. FUXE, K. and UNGERSTEDT, U.: *J. Pharm. Pharmacol.*, **19**, 335-337, 1967.
13. GILLIS, C. N.: *J. Pharm. exp. Ther.*, **146**, 54-60, 1964.
14. HERMA, Z. S.: *Psychopharmacology*, **17**, 234-241, 1970.
15. HIERRO, J. H., SÁNCHEZ, M.ª J. and PEÑA, J.: *Rev. esp. Fisiol.*, **34**, 187-190, 1978.
16. HIERRO, J. H., SOLANA, R., REQUENA, F. and PEÑA, J.: *Rev. esp. Fisiol.*, **35**, 465-468, 1979.
17. HIERRO, J. H., DE LA FUENTE, M., REQUENA, F., MONTENEGRO, E. and PEÑA, J.: *Arch. Farmacol. Toxicol.* Madrid, 1980. (In press).
18. INNES, I. R.: *J. Pharmacol.*, **19**, 427-441, 1962.
19. JACOB, J. and POITE-BEVIERRE, M.: *Arch. Intern. Pharmacodyn.*, **127**, 11-26, 1960.
20. JESTER, J. and HORST, W. D.: *Biochem. Pharmac.*, **21**, 333-338, 1972.
21. JONSSON, G. and SACHS, C.: *Eur. J. Pharmacol.*, **9**, 141-155, 1970.
22. KAES, H., UTZ, G., TECKENTRUP, U., HAUK, A. M. and DORNER, M.: *Eur. J. Clin. Invest.*, **5**, 401-408, 1975.
23. KORMAN, M. G., LAVER, M. C. and HANSKY, J.: *Br. Med. J.*, **1**, 209-210, 1972.
24. LICHTENSTELGER, W., MUTZNER, U. and LANGEMANN, H.: *J. Neurochem.*, **14**, 489-497, 1967.
25. MCGUIGAN, J. E.: *Gastroenterology*, **54**, 1005-1011, 1968.
26. NEFF, N. H., BARRET, R. E. and COSTA, E.: *Eur. J. Pharmacol.*, **5**, 359-384, 1959.
27. PLUCHINO, S.: *Naunyn-Schmiedebergs Arch. Pharmacol.*, **272**, 189-224, 1972.
28. SHASKAN, E. and SNYDER, S. H.: *J. Pharmacol. exp. Ther.*, **175**, 404-418, 1970.
29. SPECTOR, S.: *Pharmacol. Rev.*, **18**, 599-609, 1966.
30. SPECTOR, S., SJOERDSMA, A. and UDENFRIEND, S.: *J. Pharmacol. exp. Ther.*, **147**, 86-95, 1965.
31. STADIL, F. and REHFELD, J. F.: *Gastroenterology*, **65**, 210-215, 1973.
32. STRAUS, E. and YALOW, R. S.: *Gastroenterology*, **66**, 936-943, 1974.
33. THOA, N. B., ECCLESTON, D. and AXELROD, I.: *J. Pharmacol. exp. Ther.*, **169**, 68-73, 1969.
34. THOENEN, H., TRANZER, J. D. and HAEUSLER, G.: In «New Aspects of Storage and Release Mechanism of Catecholamines» (Kroneberg, G. and Schümann, H. J., eds.). Springer-Verlag, Berlin and Heidelberg, 1970, pp. 130-143.
35. TRENDELENBURG, U.: *J. Pharmacol. exp. Ther.*, **125**, 55-65, 1959.
36. VOTANOVA, M., BOULLIN, D. J. and COSTA, E.: *Life Sci.*, **10**, 87-91, 1971.
37. YALOW, R. S. and BERSON, S. A.: *Gastroenterology*, **58**, 1-14, 1970.