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MODE OF ACTION OF A GASTRIC-SECRETION ANTAGONIST

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We embarked on the present study with the object of throwing light on the mode of action of gastrin. In this study a compound with specific antigastrin properties would seem to be useful. The actions of the pure natural peptides, gastrins I and II, in stimulating and inhibiting gastric acid secretion, as stimulants of pepsin and pancreatic secretion, and of gastric and small intestinal tone and motility, have been recorded by Tracy & Gregory (1964). Little is known of the mechanisms by which the gastrins display these numerous actions. As to the mode of action of gastrin in exciting acid secretion, pertinent facts have recently appeared. It was disclosed that injection of gastrin, as well as feeding, distension, and vagus stimulation, produced a striking and long lasting elevation of the histamine forming capacity (HFC) within the region of the parietal cells (Kahlson, Rosengren, Svahn & Thunberg, 1964 ; Kahlson, Rosengren & Thunberg, 1967). These authors further found that the mucosal histamine formed at high rates was sufficient to stimulate acid secretion, that additional histamine was formed only in the region containing parietal cells, that the elevation of HFC was not merely a concomitant of the process of secretion *per se*, and that the elevation of HFC occurred in all species studied.

The authors believe that gastrin acts by evoking a mobilization of histamine and a concurrent acceleration of HFC, thus providing adequate amounts of histamine available for effective stimulation of acid secretion, so a specific antigastrin was expected to hinder the elevation of mucosal HFC without lowering the sensitivity of the parietal cells to histamine. The present experiments reveal that the newly synthesized compound antigastrin (Cook & Bianchi, 1967), 2-phenyl-2-(2-pyridyl)-thioacetamide (SC-15396) inhibits acid secretion not by interfering with the elevation of mucosal HFC induced by gastrin but by rendering the parietal cells less sensitive to histamine. The lack of specificity is shown further by the present finding that antigastrin reduces the secretory response of the peptic cells to stimulation by gastrin and histamine.

METHODS

Animals

The rats used weighed about 200 g, and belonged to two strains ; a strain bred at this Institute for gastric secretory studies, and Sprague-Dawley rats for determination of histamine forming capacity (HFC) of the glandular part of the gastric mucosa. Urinary histamine was estimated in the latter strain.

Stomach preparations

Heidenhain pouches were prepared as described by Alphin & Lin (1959). Total stomach fistulae were established according to Lane, Ivy & Ivy (1957). Before killing the Heidenhain pouch rats, the completeness of vagal denervation was ascertained by stimulating the vagal nerves in the neck; presence of motor response in the main stomach but absence in the pouch was taken to indicate effective denervation. Gastric juice was collected at 30 min intervals from the total stomach fistula and Heidenhain-type pouch as described by Lilja & Svensson (1967).

Drugs

Gastrin II was obtained by courtesy of Professor R. A. Gregory, Liverpool. Antigastrin was donated by G. D. Searle & Co., Chicago. For infusion, gastrin was dissolved in 0.9% NaCl. Antigastrin was dissolved in 50% polyethylene glycol (molecular weight 380–420) in water, kindly supplied by Ferring, Ltd. Histamine was used in the form of the diphosphate and doses are expressed in terms of the base.

Drugs were given by continuous infusion through plastic tubes inserted into a tail vein.

Determination of HCl, pepsin and histamine

Hydrochloric acid was determined by titration against 0.1 N NaOH with phenol red as an indicator. The peptic activity was determined by a modification of the original method of Hunt (1948) using a preparation of γ-globulin free fraction of human plasma (donated by Kabi, Ltd.), and expressed as μg, referring to the corresponding effect of a commercial crystalline preparation of pepsin (Sigma, lot 95B-1270), as employed by Bitsch (1966).

The amount of histamine excreted in the urine was determined by bioassay on the isolated guinea-pig gut, and fluorometrically by a modification of the procedure described by Shore, Burkhalter & Cohn (1959). Urine was collected in serial 2 hr samples while the rats were kept in the restraining cage. In order to compensate for the loss of fluid and to obtain a sufficiently high rate of urine flow, solutions of 0.9% NaCl or 5.5% glucose were infused intravenously at suitable rates.

Determination of HFC

The rate of histamine formation in the gastric mucosa—that is, the activity of histidine decarboxylase—was determined by a procedure originally devised by Schayer and fully described by Kahlson, Rosengren & Thunberg (1963). These estimations were made on the region of the mucosa containing parietal cells. Enzymic activity, the histamine forming capacity (HFC), is expressed in terms of μg of histamine formed in 3 hr per g of excised mucosa.

Blood pressure and bronchial tone

Arterial blood pressure was recorded in rats and cats anaesthetized with chloralose. Bronchoconstriction was recorded in anaesthetized guinea-pigs and rats by adapting a principle originally introduced by Konzett & Rössler (1940).

RESULTS

Inhibition of gastrin-induced gastric secretion

Gastrin was infused in amounts (2 μg/hr to each rat) which, in the individual rat, produced a sustained secretion substantially higher than the rate of interdigestive secretion. The innervated total fistulated stomach appeared slightly more sensitive in the secretory response to gastrin than was the denervated pouch. This slight difference in sensitivity has no bearing on the present report and will be fully analysed in a separate investigation. In thirteen experiments in eight different Heidenhain pouch rats, varying

doses of antigastrin, 1–5 mg per rat, were injected. As seen in Fig. 1, following a transient peak, a constant rate of secretion was obtained about 2 hr after the onset of gastrin infusion. The plateau was maintained for several hours. Antigastrin was injected during this plateau phase of secretion. Within 30 min of antigastrin injection, the HCl secretion was significantly reduced. The average inhibition of HCl secretion was 70% with 1 mg of antigastrin (six experiments). The same degree of inhibition was obtained with 5 mg (five experiments). The duration of the inhibition resulting from one injection was about 3 hr. No inhibition of acid or pepsin secretion was seen with 0.1 mg of antigastrin per rat. Increasing the dose of antigastrin to 10 mg per rat produced nearly maximal inhibition (90%) of acid secretion in three rats bearing total stomach fistulae.

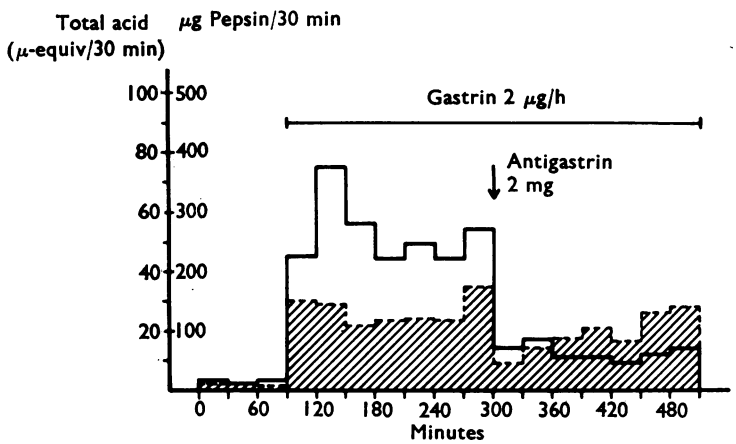

Fig. 1. Heidenhain-type pouch in an unanaesthetized rat. Ordinates indicate secretion of HCl (continuous line) and pepsin (cross hatched columns). Gastrin infused and antigastrin injected as indicated. Note low rate of spontaneous secretion, strong stimulation of both types of secretion by gastrin, and inhibition of both secretions by antigastrin.

Pepsin secretion was investigated only in the Heidenhain-pouch preparation. The peptic activity (in the following referred to as pepsin) of the secreted juice was augmented on continuous infusion of gastrin 2 μg/hr. There was an immediate steep increase, presumably resulting from a washing out of stored pepsin, followed by a gradual decline down to a maintained plateau which persisted for the duration of the gastrin infusion. The inhibition of pepsin secretion after injection of antigastrin 1–5 mg/rat was not so consistent as the inhibition of acid secretion. In five experiments the reduction was less than 10%, and in the remaining eight experiments it varied between 35 and 80%.

Inhibition of histamine-induced gastric secretion

Histamine was infused intravenously at constant rates and the resulting secretory response of the Heidenhain pouch recorded. In this group, five experiments were performed in four rats. In order to obviate the systemic circulatory effects of histamine, which might otherwise interfere with blood supply to the pouch, the rats were given the histamine antagonist mepyramine, 1–2 mg per rat, intravenously. The sensitivity of various rat tissues to histamine, including the stomach glands, varies widely among individual rats. This is in contrast to the secretory response to gastrin. For this reason it was essential to establish the effective secretory histamine dosage in each rat. In the actual experiment an effective infusion rate was established by successively increasing

the dose, and a level of secretion was achieved corresponding to approximately the plateau maintained by gastrin infusion. Injection of antigastrin, 1–2 mg per rat intravenously, reduced the rate of histamine induced secretion of hydrochloric acid by about 75% (Fig. 2).

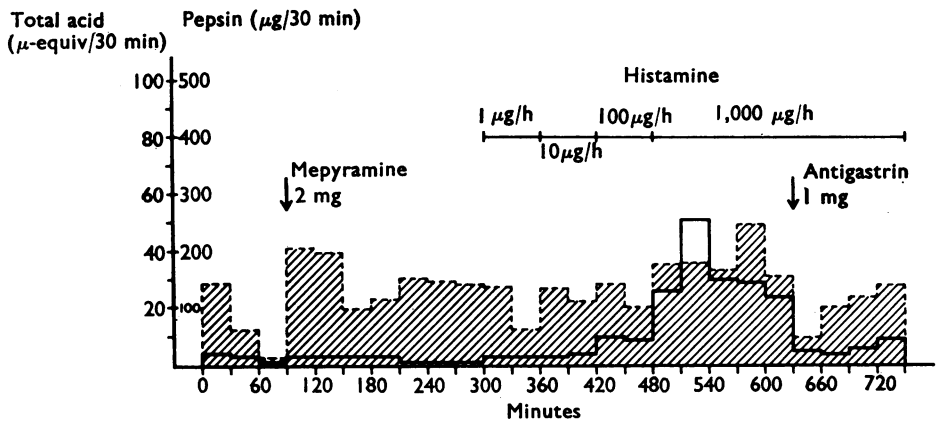

Fig. 2. Heidenhain-type pouch in an unanaesthetized rat. Ordinates and columns represent secretions as in Fig. 1. Note transient enhancement of spontaneous pepsin secretion by mepyramine. Histamine infusion and antigastrin injection as indicated. Strong and prolonged inhibition of histamine-induced HCl-secretion by antigastrin, strong but transient inhibition of pepsin secretion.

Infusion of histamine at the rates employed significantly increased the secretion of pepsin in every rat. Injection of antigastrin reduced the pepsin secretion to about 40% of the rate prevailing before antigastrin. In two experiments the pepsin secretion was reduced for only half an hour.

Rate of histamine formation in the gastric mucosa

As previously noted, injection of gastrin has been shown to induce a substantial elevation of gastric mucosal HFC. This was confirmed in the present study. After infusion of gastrin, the excised glandular part of the mucosa exhibited a striking elevation of HFC. Injection of various doses of antigastrin to rats during infusion of gastrin did not significantly influence the elevation of HFC. To investigate further the effect of antigastrin on HFC, the secretory antagonist was administered before the injection of gastrin. In Table 1, determinations on twenty-four rats are arranged in three groups. After fasting for 16 hr, group 1 was injected with antigastrin 5 mg per rat intravenously and 10 min later with 1 μg gastrin subcutaneously; group 2 was injected with the solvent used for antigastrin, followed by gastrin and group 3 with solvent and saline only. The animals were killed 2 hr after the injection of gastrin. The figures in Table 1 show that gastrin strongly raised mucosal HFC, and that in animals treated with antigastrin and gastrin the HFC was significantly higher than in group 2.

TABLE 1

HFC (μg/g) OF GASTRIC MUCOSA OF RATS UNDER THE INFLUENCE OF ANTIGASTRIN AND GASTRIN

Significant difference ($P<0.01$) between each group.

Group 1 Antigastrin+gastrin	Group 2 Solvent+gastrin	Group 3 Solvent+saline
15·2	8·2	3·7
15·2	9·7	6·1
20·0	17·8	5·8
20·2	13·0	5·3
17·5	7·7	3·7
17·3	8·1	6·3
15·0	10·4	3·7
11·3	15·4	2·8
Mean ± S.D. 16·5±2·68	11·3±3·70	4·7±1·24

Urinary histamine

Serial samples of urine were collected during infusion of gastrin and after injection of antigastrin, and examined for their content of free histamine. During the course of gastrin infusion, the histamine excretion was about trebled. This confirmed earlier results from this laboratory which showed that injection of gastrin greatly raised the gastric mucosal HFC resulting in increased histamine excretion (Kahlson *et al.*, 1964). Following injection of antigastrin, the increase in histamine excretion induced by gastrin disappeared, and in some instances the excretion was reduced to values below those seen before gastrin infusion. The paradoxical effect of antigastrin in elevating HFC without a concomitant augmented excretion of histamine remains unexplained. One reason could be the unfavourable reduction of urine flow caused by antigastrin (see below).

Miscellaneous effects of antigastrin

Gastric tone. The stomach of rats given gastrin appeared normal. On the other hand if, in addition to gastrin, antigastrin was injected, the stomach contained neutral gastric juice, and its wall was dilated. In rats under the influence of antigastrin and allowed to drink water, the stomach was found enormously dilated.

Blood pressure. In anaesthetized rats and cats intravenous injection of antigastrin was followed by a transient fall in blood pressure, the magnitude of which depended on the dose of antigastrin. This effect was not abolished by the antihistaminic drug, mepyramine, injected in doses which effectively abolished the fall produced by histamine injected intravenously.

Broncho-constriction. In anaesthetized rats and guinea-pigs intravenous injections of antigastrin evoked a transient broncho-constriction which was not prevented by pre-treatment with mepyramine.

Urine flow. In the experiments in which urine was collected for determination of histamine content, injection of antigastrin reduced urine flow by about 80–90%. Besides recording arterial blood pressure, no attempts were made further to investigate this unfavourable side-effect.

DISCUSSION

Previous workers report incompatible findings regarding the actions of antigastrin. Cook & Bianchi (1967), in experiments on rats and dogs, noted strong inhibition of gastrin-induced acid secretion, whereas stimulation by histamine was not significantly affected. They concluded that the compound is a specific inhibitor of gastrin-induced acid secretion. Working on vagally innervated and denervated Heidenhain-type pouches in dogs, Bedi, Gillespie & Gillespie (1967a) found about 50% inhibition of pentagastrin-induced and about 70% inhibition of gastrin II-induced acid secretion. By contrast, the single intravenous injection of 10 mg of the antigastrin had no effect on the plateau responses of the vagally innervated pouches to the continuous intravenous infusion of histamine ; accordingly, these authors designate the inhibition as specific. In a study by workers in Belfast and Edinburgh (Connell, Sircus, Hill, Macleod & Thomson, 1967) observations were made which in the main agree with ours. These workers report that antigastrin effectively depresses the acid secretory response to gastrin II and pentagastrin in conscious dogs and anaesthetized rats. They found that the drug also inhibits histamine-stimulated secretion, 10 mg/kg given intravenously to a fistula dog producing 75% inhibition of secretion. These authors suggest that the drug is not a specific gastrin antagonist. Bedi, Gillespie & Gillespie (1967b) extended their previous study and administered 10–20 times the original dose of antigastrin. This dose suppressed the acid response to histamine in dogs. To these authors there now seems to be no difference between their findings and those of the Belfast and Edinburgh workers.

The discrepancies in observations reported so far seem to have arisen chiefly from differences in dosage and form of administration of antigastrin and histamine. There is no evidence for an essential species difference in the secretory responses under study between dogs and rats. And there can be no doubt that antigastrin effectively inhibits histamine-stimulated acid secretion. The unanaesthetized rat, fitted with the stomach preparations employed, is particularly well suited for studies of acid secretion. Proper dose-response relations for gastrin and histamine, and a persistent plateau response, can be established, and secretory responses are consistent and reproducible.

If accelerated rate of mucosal histamine formation constitutes an essential part of the mechanism by which gastrin acts, and antigastrin leaves the histamine formation unaffected, why then does antigastrin inhibit the acid secretory response to gastrin? The obvious answer is that antigastrin reduces the responsiveness of the parietal cells to histamine. Moreover, the inhibition by antigastrin of gastrin-induced acid secretion parallels approximately the degree of depressed sensitivity to histamine produced by antigastrin. Further work is required to see whether interference with the blood supply plays a part in lowering the responsiveness. The greatly reduced urine flow under the influence of antigastrin points towards derangement of the circulation.

The search for a specific inhibitor of the stimulatory effect of gastrin on acid secretion is likely to continue. The final discovery of an agent of this kind will make fresh experiments rewarding on the lines of the present study.

In the course of the present experiments noteworthy findings were made which are not directly related to the object of the study. The innervated stomach was found to be only slightly more sensitive to stimulation by gastrin than the denervated Heidenhain-

type pouch ; and histamine infusion was found to stimulate pepsin secretion. These and other results which are at variance with conventional views on the subject will be reported in detail elsewhere.

SUMMARY

1. In unanaesthetized rats carrying a total stomach fistula or a denervated Heidenhain-type pouch, the effect of antigastrin, 2-phenyl-2-(2-pyridyl) thioacetamide (SC-15396) on hydrochloric acid and pepsin secretion evoked by infusion of gastrin II or histamine was investigated.

2. Antigastrin inhibited the gastrin and histamine induced secretion of hydrochloric acid by approximately 80%.

3. The secretion of pepsin provoked by gastrin or histamine was also inhibited although to a lesser and varied extent.

4. Infusion of gastrin strikingly enhanced the rate of histamine formation in the glandular region of the gastric mucosa, and also increased the urinary excretion of free histamine. Antigastrin did not restrain the gastrin-induced elevation of mucosal histamine forming capacity.

5. Antigastrin acts by greatly reducing the sensitivity of the parietal and peptic cells to otherwise effective secretory stimulation, and not by directly interfering with the action of gastrin.

6. Antigastrin causes side effects on muscular tone of the stomach and bronchi and kidney function, the precise analysis of which is outside the scope of the present study.

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REFERENCES

ALPHIN, R. S. & LIN, T. M. (1959). Preparation of chronic denervated gastric pouches in the rat. *Am. J. Physiol.*, **197**, 257–259.

BEDI, B. S., GILLESPIE, G. & GILLESPIE, I. E. (1967a). Effects of a specific gastrin antagonist on gastric acid secretion in pouch dogs. *Lancet*, **1**, 1240–1243.

BEDI, B. S., GILLESPIE, G. & GILLESPIE, I. E. (1967b). A gastric-secretion antagonist. *Lancet*, **2**, 841–842.

BITSCH, V. (1966). A modification of the Hunt method for estimating peptic activity in gastric juice. *Scand. J. clin. Lab. Invest.*, **18**, 357–358.

CONNELL, A. M., SIRCUS, W., HILL, R. A., MACLEOD, I. B. & THOMSON, C. G. (1967). A gastric secretion antagonist's effect. *Lancet*, **2**, 720.

COOK, D. L. & BIANCHI, R. G. (1967). SC-15396: A new antiulcer compound possessing anti-gastrin activity. *Life Sci.*, **6**, 1381–1387.

HUNT, J. N. (1948). A method for estimating peptic activity in gastric contents. *Biochem. J.*, **42**, 104–109.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1963). Observations on the inhibition of histamine formation. *J. Physiol., Lond.*, **169**, 467–486.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol., Lond.*, **174**, 400–416.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1967). Accelerated mobilization and formation of histamine in the gastric mucosa evoked by vagal excitation. *J. Physiol., Lond.*, **190**, 455–463.

KONZETT, H. & ROSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.*, **195**, 71–74.

LANE, A., IVY, A. C. & IVY, E. K. (1957). Response of the chronic gastric fistula rat to histamine. *Am. J. Physiol.*, **190**, 221–228.

LILJA, B. & SVENSSON, S. E. (1967). Gastric secretion during pregnancy and lactation in the rat. *J. Physiol., Lond.*, **190**, 261–272.

SHORE, P. A., BURKHALTER, A. & COHN, V. H. (1959). A method for the fluormetric assay of histamine in tissues. *J. Pharmac.*, **127**, 182–186.

TRACY, H. J. & GREGORY, R. A. (1964). Physiological properties of a series of synthetic peptides structurally related to gastrin I. *Nature, Lond.*, **204**, 935–938.