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The effect of SC-15396, atropine and mepyramine on gastrin-, bethanechol- and histamine-stimulated gastric acid secretion in rats and guinea-pigs

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The effect of SC-15396 ("antigastrin"; 2-phenyl-2-(2-pyridyl)thioacetamide), atropine and mepyramine on gastrin-, bethanechol- and histamine-stimulated gastric acid secretion was studied in rats and guinea-pigs. For all three stimulants parallel dose response curves were obtained except in guinea-pigs where bethanechol even in very high doses displays a poor activity in stimulating gastric acid secretion. The maximal secretory response was found to be $12{\cdot}7 \pm 5{\cdot}0$ μ-equiv HCl/10 min in rats and $53{\cdot}2 \pm 27{\cdot}9$ μ-equiv HCl/10 min in guinea-pigs. All stimulating effects on gastric acid secretion were reduced by SC-15396; atropine abolished the secretory responses to bethanechol. Mepyramine was ineffective. In accordance with these findings the mechanism of action of gastrin and a receptor model on the oxyntic cell are discussed.

Several chemically different compounds are potent gastric acid secretagogues, among those the antral hormone gastrin can be regarded as the physiological stimulus for gastric secretion. It has been postulated by several authors that histamine may be the common final chemostimulator (Code, 1965; Lorenz & Pfleger, 1968). This hypothesis up to now has not been established unequivocally. The present investigation was designed to bring some light to the question as to whether gastrin, bethanechol and histamine act on the oxyntic cell by the same common mechanism. The effects of the alleged specific gastrin antagonist SC-15396 ["antigastrin"; 2-phenyl-2-(2-pyridyl)thioacetamide], the anticholinergic atropine and the antihistamine mepyramine on gastrin-, bethanechol- and histamine-stimulated gastric acid secretion have been examined in anaesthetized rats and guinea-pigs.

EXPERIMENTAL

Methods

The experiments were made on 95 male Wistar rats (180–400 g) and 95 male mongrel guinea-pigs (200–530 g) from which food was withheld for 24 h; there was free access to drinking water. The animals were prepared under urethane anaesthesia (rats 1·25 g/kg, i.p., guinea-pigs 1·5 g/kg i.p.) according to Lai (1964). Gastric acid secretion was collected in 10 min periods and the acid output was estimated titrimetrically with 0·01 N NaOH using bromothymol blue as indicator. The dose response curves were obtained from six different doses of each stimulus. The lowest dose was numbered as 1 and the highest as 6. For convenience, in a single animal three doses (1–3–5, 5–3–1, 2–4–6, 6–4–2) were infused intravenously for 15 min at intervals of 70 min. The infusion volume was 0·935 ml/15 min for rats and 2·35 ml/

15 min for guinea-pigs. Each dose of the agonists was tested in the presence of only one dose of the antagonists. SC-15396 has a rather short duration of action and was therefore injected intravenously 5 min before each infusion. Atropine and mepyramine were administered intravenously as a single dose 20 min before the first infusion. The combination of drugs and groups of drugs (see above) were randomized for each species. Each point of the dose-response curves represents the mean value of 4 single experiments (the highest dose of bethanechol in combination with SC-15396 in rats and the highest dose of gastrin in combination with SC-15396 in guinea-pigs are mean values of only 3 experiments). Differences in the dose-response curves were tested by analysis of variance.

Compounds and dosage

Agonists. Synthetic human gastrin I (American Gastroenterological Association): rats and guinea-pigs 0·2, 0·4, 0·8, 1·6, 3·2, 6·4 μg/kg in 15 min. Bethanechol chloride (Schuchardt, Munich): rats and guinea-pigs 20, 40, 80, 160, 320, 640 μg/kg in 15 min. Histamine dihydrochloride (La Roche, Grenzach): rats 50, 100, 200, 400, 800, 1600 μg/kg in 15 min; guinea-pigs 1·25, 2·5, 5, 10, 20, 40 μg/kg in 15 min.

Antagonists. SC-15396 [2-phenyl-2-(2-pyridyl)thioacetamide (G. D. Searle, Chicago and Dr. Karl Thomae, Biberach]: rats and guinea-pigs 3 × 10 mg/animal. Atropine sulphate (Merck, Darmstadt): rats 0·5 mg/kg; guinea-pigs 5·0 mg/kg. Mepyramine maleate (Bayer, Leverkusen): rats 10 mg/kg; guinea-pigs 1·3 mg/kg.

All compounds were given as weights of the base dissolved in 0·9% saline and were injected or infused into the jugular vein. SC-15396 was dissolved in a mixture of 0·5 ml dimethylsulphoxide and 0·5 ml 0·9% saline. After each injection or infusion the venous cannula was rinsed with 0·2 ml saline.

pA_2 values

The pA_2 values of SC-15396 for histamine and acetylcholine were determined by the method of Schild (1947).

RESULTS

Rats

The basal secretion varied between 2–4 μ-equiv HCl/10 min.

Gastrin (Fig. 1A). The infusion of gastrin resulted immediately in a dose-dependent secretion of HCl. When the infusion was stopped gastric secretion returned to the basal level within 30 min. The maximal response of the dose response curve was not obtained with the doses used. The slightly stronger effect of gastrin in the presence of mepyramine and the weaker in the presence of atropine was not statistically significant. The gastrin effect was significantly reduced by SC-15396.

Bethanechol (Fig. 1B). With bethanechol, 160 μg/kg in 15 min, the maximal response of the dose-response curve was obtained. The bethanechol effect was not influenced by mepyramine, diminished by SC-15396 or abolished by atropine.

Table 1. *pA_2 values of atropine, mepyramine and SC-15396 for acetylcholine and histamine*

	Atropine	Mepyramine	SC-15396
Acetylcholine	8·27+	4·71+	3·90
Histamine	5·73+	8·71+	3·50

+ Values from Schild (1947).

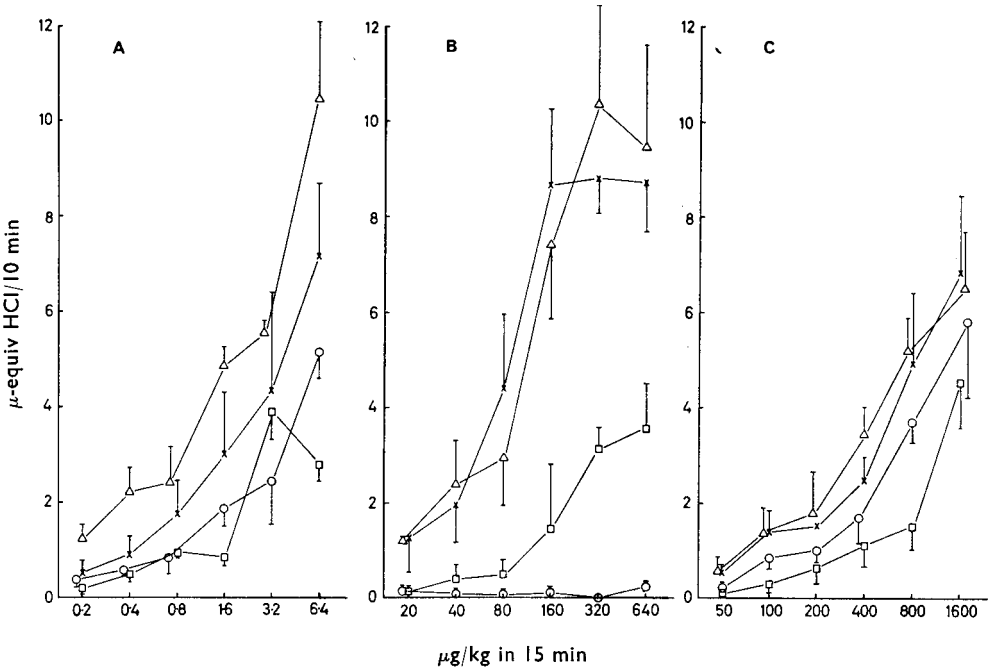


FIG. 1. Dose response curves of gastrin I (A), bethanechol (B) and histamine (C) in rats without pretreatment (×), in the presence of SC-15396 (□), atropine (○) or mepyramine (Δ). Vertical bars = s.e.

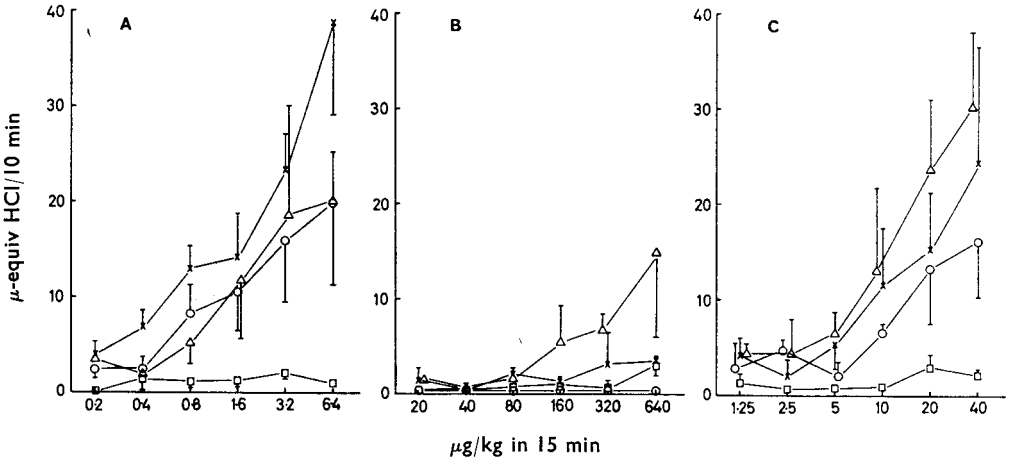


FIG. 2. Dose response curves of gastrin I (A), bethanechol (B) and histamine (C) in guinea-pigs without pretreatment (×), in the presence of SC-15396 (□), atropine (○) or mepyramine (Δ). Vertical bars = s.e.

Histamine (Fig. 1C). The dose-dependent histamine response was not influenced by mepyramine or atropine, but was significantly reduced by SC-15396. The effects of all doses of histamine were submaximal.

In comparing the relative potencies of the three stimulants at a secretory rate of 5 μ-equiv HCl/10 min, it was calculated that on a molar basis gastrin was 4000 times and bethanechol 14 times as potent as histamine.

Guinea-pigs

In guinea-pigs the basal secretion varied over a wide range from 2–15 μ-equiv HCl/10 min.

Gastrin (Fig. 2 A). When the same dose of gastrin used in rats was given to guinea-pigs, much larger responses were obtained. Here also the maximal response was not observed. Atropine and mepyramine had no influence on the gastrin response. A significant inhibition was obtained by SC-15396.

Bethanechol (Fig. 2 B). Doses of bethanechol covering the whole dose-response curve in rats were only weakly potent in guinea-pigs. Even with a dose of 640 μg/kg in 15 min a secretory response of 3·5 μ-equiv HCl/10 min was not exceeded. The augmented secretion rate by mepyramine was insignificant. SC-15396 was ineffective. The bethanechol effect was abolished by atropine. At a constant perfusion rate of the stomach, the outflow from the pyloric cannula was markedly increased by bethanechol. Therefore in separate experiments free (Toepfer's reagent) and total (phenolphthalein) acid was determined. No difference in comparison on titration with bromothymol blue was observed. Furthermore, the protein content of the gastric perfusate was determined (Lowry, Rosebrough & others, 1951). A dose-dependent increase in protein content during the infusion of bethanechol was probably due to the known strong pepsigogue effect of cholinergic stimuli. As a side-effect, a marked salivary secretion was observed.

Histamine (Fig. 2 C). With doses of histamine being about 1/40 of those used in rats a good dose response relation was obtained in guinea-pigs. Doses of 40 μg/kg in 15 min were nearly maximal. The histamine response was almost completely abolished by SC-15396. Neither atropine nor mepyramine had any augmenting or inhibiting effect.

At a secretion level of 10 and 20 μ-equiv HCl/10 min, gastrin was on a molar basis about 270 times as potent as histamine. The relative potency of bethanechol could not be evaluated because of its weak effect.

DISCUSSION

The results demonstrate parallel dose-response curves for all stimulants in rats and for gastrin and histamine in guinea-pigs. The weak bethanechol effect in guinea-pigs may be the consequence of a relative insensitivity of the target organ since salivary and pepsin secretion seemed to be strongly stimulated. In either species gastrin was the most potent secretagogue, underlining the importance of gastrin as the physiological stimulant for gastric acid secretion. The smaller difference in the relative potencies between gastrin and histamine in guinea-pigs than in rats may be due to a less-pronounced sensitivity for histamine in rats.

The maximal secretory response is determined only by the total parietal cell mass and not by the stimulus applied (Makhlouf, McManus & Card, 1966). This would explain the higher secretion rates in guinea-pigs than in rats. Makhlouf's model for describing the dose response relation of gastric acid secretion can be expressed by the following equation:

$$c = \frac{c_{max} \times D}{K + D} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where c = the observed response, c_{max} = maximal response, D = concentration of the stimulus, K = ED50.

By transformation of equation (1) a linear relation between the reciprocals of both dose and response is obtained:

$$\frac{1}{c} = \frac{1}{c_{max}} + \frac{K}{c_{max}} \cdot \frac{1}{D} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

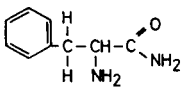
Knowing three parameters of these equations it is possible to determine the unknown. Since in our experiments only the lower ends of the dose response curves were determined (except for bethanechol in rats) it was of interest to get information about the maximal response (c_{max}) in both species and to investigate whether c_{max} was depressed by one or more of the antagonists. Therefore a program was elaborated (Winne, 1969, unpublished) to calculate by a nonlinear regression the parameters by which we could determine c_{max} from the experimental data. From such a calculation c_{max} was found for rats to be $12{\cdot}7 \pm 5{\cdot}0$ μ-equiv HCl/10 min and for guinea-pigs $53{\cdot}2 \pm 27{\cdot}9$ μ-equiv HCl/10 min. Unfortunately we were not able to ascertain whether or not one of the antagonists used depressed c_{max} significantly since in some drug combinations the data available covered too small a range of the total dose response curve to determine c_{max} with a margin of safety.

The inability of antihistamine drugs to antagonize histamine-stimulated gastric acid secretion confirmed earlier observations. In our experiments a stimulating effect of mepyramine on gastric secretion as it has been described for salivary secretion (Lorenz & Pfleger, 1968; Lorenz, Haubensak & others, 1968) was not observed.

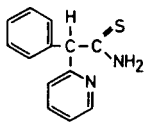
The blockade of cholinergic receptors by atropine was clearly demonstrated in both species when gastric secretion was stimulated with bethanechol. Neither the histamine nor the gastrin response was diminished by atropine.

In both species SC-15396 reduced the effect of all three stimulants. The one exception being the effect of bethanechol in guinea-pigs which was not diminished since the bethanechol effect was already low. The pA_2 values (Table 1) did not give any indication that the effect of SC-15396 was specifically anticholinergic or antihistaminic.

SC-15396 has been reported to be a specific gastrin antagonist in dogs (Bedi, Gillespie & Gillespie, 1967) and rats (Cook & Bianchi, 1967). Connell, Sircus & others (1967) demonstrated an inhibiting effect of SC-15396 on histamine-stimulated dogs. But the opinion that SC-15396 is a specific gastrin antagonist has been changed because it has since been shown to antagonize gastric secretion stimulated by gastrin, histamine and insulin (Connell, Hill & others, 1968).



Phenylalanine amide



SC-15396

SC-15396 chemically resembles the C-terminal phenylalanine amide of the gastrin molecule. This resemblance could be the key to the molecular mechanism of the action of gastrin thus offering the following hypothesis: The active centre of the gastrin molecule covering the entire range of physiological properties is the C-terminal tetrapeptide amide Trp.Met.Asp.Phe-NH_2 (Tracy & Gregory, 1964). The polar end group $-C\langle^{O}_{NH_2}$ of the phenylalanine amide is capable of accepting

H^+ ions supplied by intramolecular electron transfer. These H^+ ions may come from the aspartyl residue. The importance of the aspartyl residue and the C-terminal acid amide group is underlined by several observations. Morley (1968) found that modification of the aspartyl group led to either inactive compounds or to compounds whose activity was so weak as to be questionable. The same happened when the amide group was substituted by two methyl groups. On the other hand, the activity of the C-terminal tetrapeptide amide was increased when substituted at the N-terminal end by a β-alanyl residue or a carbamoyl group. Both groups could serve as additional acceptors for H^+ ions supplied by the aspartyl residue. This would explain the increase in activity. These considerations do not allow the conclusion that the activity of the total gastrin molecule *in vivo* is only the action of its C-terminal tetrapeptide amine.

SC-15396, because of its similarity with the phenylalanine amide, is likely to interfere with the receptor for the $-C\begin{matrix}O\\ \diagdown NH_2\end{matrix}$ group. The large dose necessary to display an antisecretory activity may be due to its poor solubility which limits its use for more detailed molecular studies.

The antagonistic effect of SC-15396 to all three stimulants suggests either a common receptor for gastrin, bethanechol and histamine or one common chemical transmitter for stimulating the secretion of gastric hydrochloric acid. The common chemical transmitter may be gastrin since its active centre shows the closest chemical resemblance to the inhibitor for all three stimulants—SC-15396.

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