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Gastrin – Histamine as a Normal Sequence in Gastric Acid Stimulation in the Rabbit

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ABSTRACT

In a suspension of isolated gastric glands the effect of secretagogues on the oxyntic cells can only be detected by direct stimulation. An indirect stimulus like gastrin inducing one type of cell to liberate histamine which then acts on the oxyntic cells will not be detectable because of the very high dilution of the liberated substance. Thus the isolated gland preparation presents a means by which two steps in a sequential stimulation can be separated.

There is no evidence that gastrin acts directly on the oxyntic cells but it does liberate histamine in a dose-effect relationship, which would in an intact stomach give histamine concentrations sufficient to effectively stimulate the acid secretion. Thus in the rabbit histamine seems to be a normal physiological mediator for gastrin stimulation.

INTRODUCTION

Ever since Code in 1956 (7) suggested histamine as a final common mediator for all stimulants of gastric acid secretion this has been a matter of dispute, and so far unequivocal results allowing a final conclusion have not been reported. Several bits of evidence support the view that histamine constitutes the final step in a stimulatory chain. For instance both cholinergic stimulation and gastrin mobilize histamine from the gastric mucosa and increase the "histamine-forming capacity" of the gastric mucosa (for references see 14); H_2-receptor blockers inhibit secretion not only after histamine but also after gastrin and cholinergic agonists (5); the isolated frog gastric mucosa is exhausted by repeated stimulation with cholinergic agents or gastrin, but still responds to histamine (13); the parietal cells in the rabbit mucosa seem to lack gastrin receptors (3, 4). There is, however, evidence that appears inconsistent with simple sequential hypothesis that gastrin acts by the liberating of histamine (11, 18, 19). For reviews on this topic the reader is referred to elsewhere (8, 12, 14, 15).

One reason for the divergent opinions is that the experimental results

have not allowed conclusive interpretations. It has been shown undoubtedly that gastrin can form and probably release histamine, and it is equally true that histamine stimulates gastric secretion, but it has not been possible to prove that these two events work sequentially.

This paper deals with a technique that has made it possible to analyze the two steps separately. We conclude that it is probable that histamine is in fact the final stimulatory mediator for gastrin in the rabbit gastric mucosa.

METHODS

Isolated gastric glands. The experiments were performed on isolated glands from the rabbit mucosa prepared as to Berglindh & Öbrink (3). Briefly, saline is perfused at high pressure through the mucosal blood vessels producing a slight mechanical separation of the glands. Collagenase treatment of the exised mucosa completes the separation (see Fig. 1). The yield is normally about 750 mg (wet weight) per stomach. The glands were subsequently suspended in a nutrient solution in a concentration of about 15 mg/ml corresponding to 3 mg dry weight per ml.

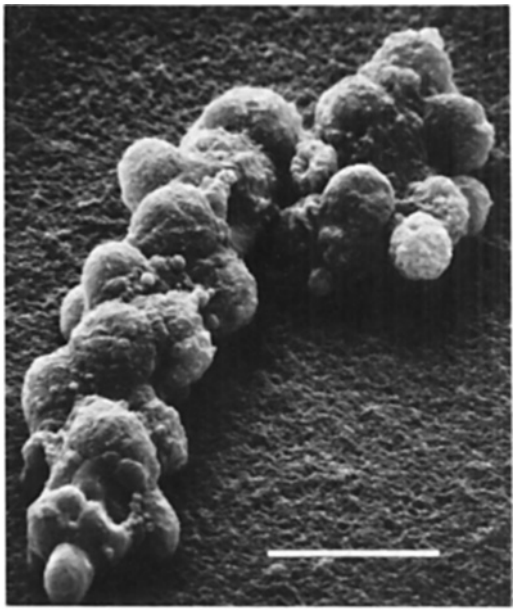

Fig. 1. A scanning electron microscopic view of an isolated gastric gland from the rabbit gastric mucosa. The bulging cells are the parietal cells. The white line represents 50 μm.

Experimental procedure.

Determination of glandular activity was made by measuring the oxygen consumption (3) and/or the accumulation of the weak base Aminopyrine (AP) (2).

Histamine liberated after pentagastrin treatment estimated as follows: Glandular suspension were prepared in the usual way (3). When pentagastrin was used it was added from the beginning to the suspension which was kept in a 37°C water bath for 15 minutes. The glands were then spun down at 2000 x g

(2 min) and the supernatant kept for subsequent histamine analyses.

Histamine determination. Histamine in the suspending medium was measured by a modification (9, 10) of the original method of Shore et al. (17) as follows : The glandular suspension was centrifuged at 2000 x g for a few minutes. The supernatant (about 5 ml)was then centrifuged for 20 minutes at 28.000 x g. The new supernatant was mixed with 36% (w/v) trichloroacetic acid (TCA) in the proportion 10:1 in order to precipitate the albumin added during the preparation of the glands. The mixture was left over night at $+4^{o}C$.

The precipitate was centrifuged down at 2000 x g (10 min). The supernatant was freed from TCA by shaking with a double volume of ethylether. To 4 ml of the water phase 1.2 g of NaCl, 0.6 ml 3M NaOH and 10 ml n-butanol were added. The mixture was shaken for 5 minutes and centrifuged. 9.5 ml of the butanol-phase were pipetted into 5 ml heptane together with 2.3 ml of 0.12 M HCL and thoroughly mixed. Two ml of the water-phase were used for condensation with o-phthalaldehyde (OPT) as follows (all the reactions were performed at ice-temperature): To the 2 ml sample 400 µl 1M NaOH and 100 µl freshly prepared 0.2% (w/v) OPT dissolved in absolute methanol were added and thoroughly mixed. The resulting pH = 12.5. After 30 minutes the reaction was stopped by addition of 250 µl 0.88 m H_2SO_4 which gave a pH of 2.5.

The sample was now allowed to warm up to room temperature and the fluorescence was determined with a Farrand Ratio Fluorometer-2.

Standards with known amounts of histamine were treated in an identical manner, except for the absence of glands.

Pentagastrin was obtained either as a pure substance from ICI or as Peptavlon^{R}.

Aminopyrine (n-4-dimethyl-^{14}C) with a specific activity of 3.15 GBq (85.0 mCi per mmole)was purchased from NEN Chemicals.

RESULTS

The effect of histamine on isolated gastric glands. These results are those reported earlier from this laboratory (3). Fig. 2 shows a dose-effect curve relating the increase in oxygen consumption to the dose of histamine. It can be seen that the ED50 for histamine is about $3 \cdot 10^{-6}$M. The lowest concentration of histamine giving a detectable increase in oxygen consumption was about 10^{-7}M.

The effect of pentagastrin on isolated gastric glands has likewise been reported from our laboratory(2). Pure pentagastrin was completely without effect while Peptavlon^{R} showed a transient increase in oxygen consumption. The discrepancy was shown to depend on the NH_4^+ ions in the Peptavlon-solvent.

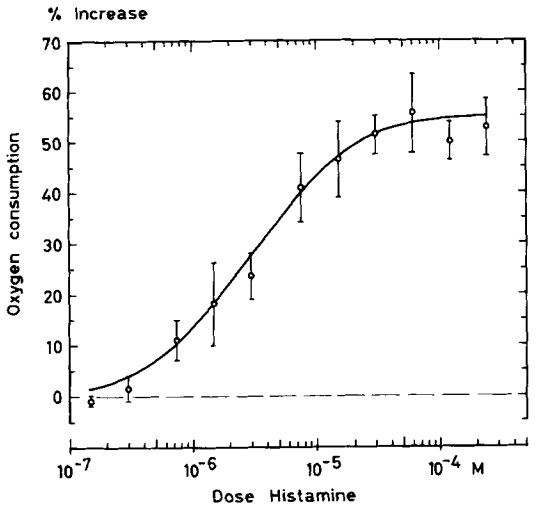

Fig. 2. Dose-effect relationship between histamine stimulation and increase in oxygen consumption in isolated gastric glands. From (3).

It was suggested from these experiments that the rabbit parietal cells in the glandular preparation lacked gastrin receptors. The findings could result either because the parietal cell receptors for gastrin had been lost during preparation, or because the parietal cells do not normally have such receptors. In the latter case, the failure of gastrin to stimulate the isolated glands (in contrast to its stimulatory effect in the intact stomach)could be explained in the following way : gastrin normally liberates histamine from histamine-containing cells in the vicinity of the parietal cells. Due to the very narrow space in which this occurs, there will be a rather high concentration of histamine at the parietal cell surface. In the case of isolated glands, however, the liberated histamine will be highly diluted thus giving only subthreshold concentrations at the parietal cell surface.

Logically the next step would be to try to identify histamine possibly released into the gland suspension after gastrin administration.

The effect of pentagastrin on histamine liberation. In order to facilitate the detection of histamine in the suspension the glands were concentrated about fourfold to about 10 to 12 mg dry weight/ml. A further increase was not possible due to their tendency to clump.

Pentagastrin as Peptavlon^{R} was added in different concentrations to the glandular preparation. Fig. 3 shows the resulting concentrations of histamine in the supernatant recalculated to the concentration of histamine that would have been liberated by 3 mg dry weight of glands/ml. A dose dependent release of histamine was obtained and detectable amounts of histamine appeared after 10^{-10}M pentagastrin. Different populations of glands, however, gave different maximal concentrations of liberated histamine. The highest obtained was 0.85×10^{-7} M per 3 mg dry weight of glands per ml, whereas the lowest maximal release was about 0.36×10^{-7}M.

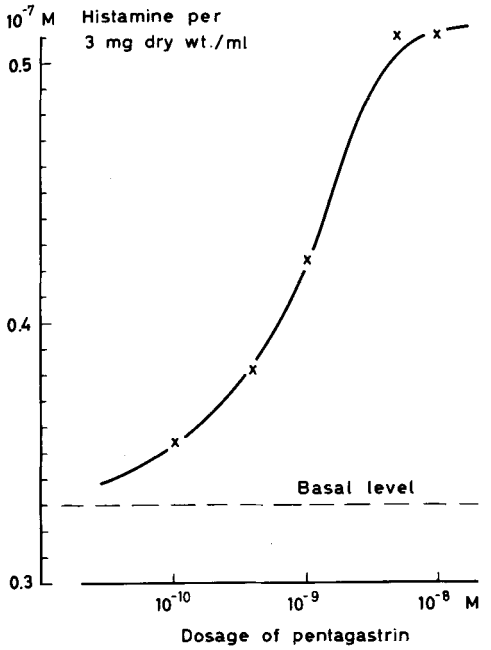

Fig. 3. Histamine liberated in a suspension of isolated gastric glands after treatment with different doses of pentagastrin. The histamine concentration has been calculated for a glandular concentration of 3 mg dry weight/ml of suspension, which was the condition in previous reports (2, 3).

It is therefore of doubtful value to analyse the dose-effect curve statistically but Fig. 3 shows a typical result. Pure pentagastrin, dissolved in a NaCl solution alkalinized with NaOH, also gave similar results.

Is the fluorescent activity really histamine ?

The OPT-condensation method detects not only histamine but also other compounds such as spermidine, arginine, glutathione, histidine and peptides with N-terminal histidine. Most of the substances reacting with OPT do not show the same spectral peaks for activation and fluorescence (17), although there are a few exceptions.Histidine may show similar reactions as histamine, but the butanol extraction mostly eliminates this interference. Similarly spermidine can be distinguished from histamine by formaldehyde-treatment which eliminates the histamine reaction, or cadmium treatment which eliminates the spermidine reaction (10). In this way spermidine was excluded from our results.

Spectral analyses as shown in Fig. 4 as well as results with butanol extraction, formaldehyde and cadmium treatments indicate that the fluorescence in all probability was due to histamine.

Attempts to increase the histamine concentration.

As it seems necessary to obtain a histamine concentration higher than 10^{-7}M in order to detect parietal cell activity (see Fig. 1), an obvious procedure would be to increase the concentration of glands in the suspension. This was not feasible, however, due to the clumping tendency when the gland concentration

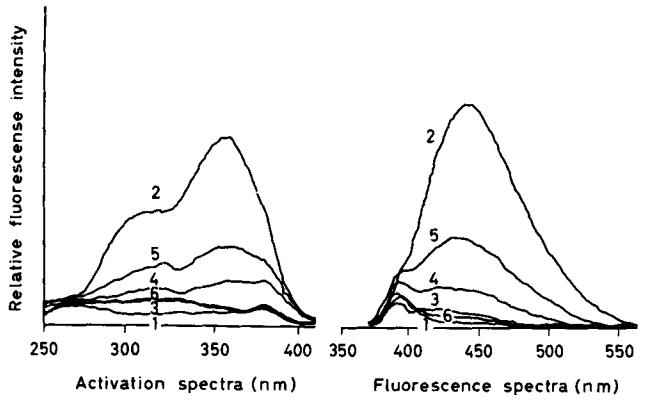

Fig. 4. Activation and fluorescence spectra in the "histamine" condensation with o-phthalaldehyde. 1. Blank; 2. 2.7 x 10^{-7} M histamine; 3. 2.7 x 10^{-7}M histamine + 1 M HCHO; 4. Extraglandular fluid, background; 5. Extraglandular fluid, after 10^{-7} M pentagastrin; 6. Extraglandular fluid, after 10^{-7} M pentagastrin + 1 M HCHO. Note the similarities of the spectra from the extraglandular fluid and from pure histamine.

was higher than the 10 - 12 mg dry weight per ml, used in the present experiments.

Attempts to potentiate the histamine effect on the isolated glands.

It is well documented that histamine acts through the activation of adenyl cyclase. Concomitant treatment with a phosphodiesterase inhibitor like theophylline, necessarily leads to an effect which is greater than the sum of the two individual treatments (4). This fact was utilised in an attempt to unmask an activity of histamine too small to be detected on its own.

In the first type of experiment, subthreshold doses of histamine (similar in size to those liberated by pentagastrin) were combined with theophylline.

In a second type of experiment,pentagastrin was combined with theophylline.

Fig. 5 shows the effect of these combined treatments. In the control experiments the AP accumulation in unstimulated glands was observed for 90 minutes at which time theophylline (5 x 10^{-3}M) was added. A clear increase in the AP accumulation was seen indicating increased parietal cell activity. When histamine (1.6 x 10^{-7}M) was added at 60 minutes no effect was seen until the theophylline was administered (at 90 minutes), when an effect markedly enhanced compared to that with theophylline alone, was obtained revealing a histamine action that could not be detected without potentiation.

A similar experiment with pentagastrin (10^{-7}M) is also shown in the figure. As expected, this did not in itself give any effect on the AP accumulation, but

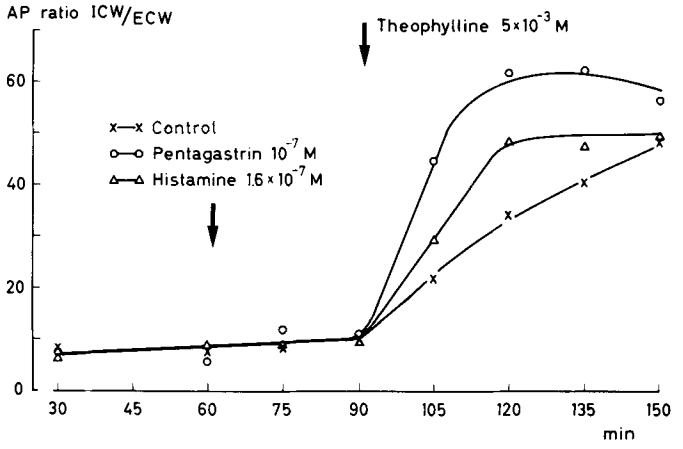

Fig. 5. The ineffectiveness of histamine (1.6×10^{-7}M) or pentagastrin (10^{-7}M) to cause an accumulation of aminopyrine (AP). When combined with a stimulation by theophylline (5×10^{-3}M) both however, potentiated the effect of the phosphodiesterase inhibitor.

again, when theophylline was also present a pronounced potentiation of the effect appeared.

The interpretation of the latter results depends on whether or not pentagastrin stimulates adenyl cyclase. Most workers have failed to demonstrate such a stimulation, but on the other hand an increase in cAMP after gastrin has been found in Necturus (16), frog (6) and rat (14).

If pentagastrin does not stimulate cAMP formation in the rabbit glands, then our results showing the potentiated effect of theophylline favour the hypothesis that the histamine liberated was the responsible co-stimulator just as in the previous experiments with subthreshold doses of histamine alone. If, however, pentagastrin can stimulate adenyl cyclase directly then the potentiating effect would depend on the gastrin itself and the histamine liberated might just be a byproduct without any physiological significance. Even if this possibility seems unlikely, as neither low nor high doses of gastrin by itself will stimulate the glands, the same two types of experiments were repeated after treatment with the H_2 -blocker cimetidine.

As would be expected cimetidine alone did not lower the control AP-accumulation but it did significantly reduce the effect of theophylline (Fig. 6). The results in Fig. 6 are derived from the same type of experiments as shown in Fig. 5 and the first groups of columns are the values from controls untreated by either histamine or pentagastrin. The second and the third groups of columns are from glands treated with histamine (1.6×10^{-7}M) or pentagastrin (10^{-8}M). The potentiating effects of the theophylline given 45 minutes later

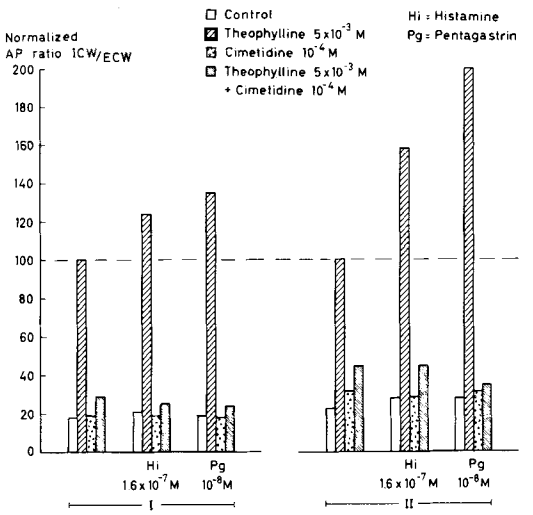

Fig. 6. From the kinetic type of experiments shown in Fig. 5 the situation at time 135 min is depicted in this figure, i.e. histamine or pentagastrin were added at 60 min and theophylline at 90 min. (see Fig. 5). Cimetidine was added at 60 min. I and II denote experiments with two different populations of isolated glands. The left group of columns in each population represent glands untreated with either histamine or pentagastrin. The second and third groups were treated with histamine (1.6×10^{-7}M) or pentagastrin (10^{-8}M).

is clearly demonstrated but also apparent is that cimetidine the H_2-blocker reduces the potentiation. There was no difference between the histamine and the pentagastrin experiments and it seems therefore justified to conclude that the acting substance after the pentagastrin pretreatment was in fact the liberated histamine.

DISCUSSION

A preparation of isolated gastric glands from rabbits responds with increased oxygen consumption and accumulation of the weak base aminopyrine (AP) after histamine, but not after pentagastrin (2, 3). H_2-blockers are able to inhibit competitively the histamine effect on the glands (1). Thus the parietal cells of the rabbit apparently possess intact H_2-receptors but apparently not receptors for gastrin. This might mean that the parietal cell gastrin receptors were destroyed, or it could also be a consequence of a normal absence of gastrin receptors on the parietal cell. If so gastrin might act on some other type of cell liberating histamine, which would then stimulate the H_2-receptors on the oxyntic cells. In the well mixed gland suspension the histamine concentration would, probably, however, be too low to stimulate.

As found in the present experiments, histamine concentrations just too small to be detected by an oxyntic cell activity (10^{-7}M) were liberated

by physiological concentrations of gastrin. Thus gastrin receptors were retained in some cells other than the oxyntic cells.

Both gastrin and histamine do stimulate acid secretion in vivo. Histamine has always been considered to have a direct effect on the oxyntic cells. The action of gastrin, on the other hand, has been explained either as a gastrin → histamine → secretion sequence or a direct action gastrin → secretion. It could also be a combination gastrin → histamine → secretion, or just the last part of this sequence, in which case the often suggested interaction of gastrin and histamine receptors would occur (18).

With the isolated gland preparation it has been possible to distinguish between these possibilities because the stimulating events on the oxyntic cells can be separated from possible events on other cells. In the rabbit gastrin is effective only by liberating histamine from some other cells.

There is no doubt that the histamine liberated by the pentagastrin has a secretagogue effect on the oxyntic cells if only it can reach high enough concentrations. In the intact stomach on the other hand histamine would be liberated into narrow spaces between the closely packed glands, where the histamine concentration can be estimated from the observation that histamine in our preparation reaches concentrations of about 10^{-7}M when about 10 mg dry weight of glands were suspended in 1 ml solution. This corresponds to 50 mg wet weight and a total amount of 10^{-10} moles of histamine. The 50 mg tissue is roughly equal to a glandular volume of 50 µl. Morphologically the glandular volume in relation to the interglandular space volume in the intact mucosa is roughly 10:1. This interglandular space, however, is filled with blood vessels and connective tissue. We assume that the free space left is somewhat between 50 and 10% of the intercellular space or between 5 and 1% of the glandular volume. The histamine liberated in our preparation, 10^{-10} moles, would then be distributed in 1 to 5% of 50 µl, which would result in concentrations between 0.4×10^{-4} and 2.0×10^{-4}M if evenly distributed. This would result in a maximal secretion rate.

Thus we suggest that in the rabbit, gastrin stimulates the oxyntic cells indirectly through the liberation of histamine.

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