Br. J. Pharmacol. (1988), **95**, 1047–1056

Fade and tachyphylaxis of gastric acid secretory response to pentagastrin in rat isolated gastric mucosa

Barry H. Hirst, [1]Joy Holland, *Michael E. Parsons & *[2]Carolyn A. Price

Department of Physiological Sciences, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne NE2 4HH and *Department of G.I. Pharmacology, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR

1 Gastric acid secretory responses to pentagastrin were characterized in the rat isolated gastric mucosa. In particular, the mechanisms underlying fade, declining response upon continued stimulation, and tachyphylaxis, progressively reduced responses upon repeated stimulation, were investigated.

2 Pentagastrin, 10^{-9}–10^{-7} M, resulted in concentration-related increases in acid secretion, with a mean maximum of 2.65 μmol cm^{-2} h^{-1} in response to pentagastrin, 10^{-7} M. Higher concentrations of pentagastrin produced sub-maximal secretory rates; we define this as auto-inhibition. The responses to all concentrations of pentagastrin demonstrated fade. The rate of fade was correlated with the maximum acid secretory rate, declining at about 36% of the peak over the first 16 min.

3 The Po_2, Pco_2, [HCO_3^-], pH, [glucose], [lactate], [Na^+] and [K^+] did not decline during the fade of the acid secretory response to pentagastrin, 10^{-7} M. Addition of a second aliquot of pentagastrin was not able to reverse fade, but these tissues were responsive to histamine. Replacement of the serosal solution, before addition of a second aliquot of pentagastrin, increased the acid response from 3% to 24% of the first response.

4 Serosal solution from donor tissues, allowed to respond to pentagastrin and then the acid secretion to fade, was able to stimulate secretion in fresh recipient tissues, although at lower rates.

5 Acid secretory responses to a second dose of pentagastrin were not significantly different, whether the tissues were previously unstimulated, or stimulated with pentagastrin washed out after attaining its peak secretory response (after 10–20 min). The second response was significantly reduced if the first response was allowed to fade with the pentagastrin in contact for 100 min; i.e. fade significantly influenced the extent of tachyphylaxis.

6 Proglumide, 10^{-2} M, a gastrin receptor antagonist, and omeprazole, 10^{-5} M, an inhibitor of the gastric ($H^+ + K^+$)-ATPase, both inhibited pentagastrin-stimulated acid secretion to similar extents. The second response to pentagastrin after pentagastrin alone, or pentagastrin plus omeprazole were both reduced compared to responses after no stimulation or omeprazole alone, respectively. After pentagastrin plus proglumide, the second response to pentagastrin was not lower than after proglumide alone. Proglumide, but not omeprazole, therefore, prevented pentagastrin tachyphylaxis.

7 It is concluded that gastrin fade and tachyphylaxis are related phenomena. Part of the fade may be due to release of an inhibitor(s). The major proportion of tachyphylaxis is a result of specific interaction of gastrin with its receptors.

Introduction

Gastric acid secretion in response to continuous infusion of gastrin declines with time. This effect has been termed 'fade', and has been observed in the rat (Emås *et al.*, 1981) as well as other mammalian species *in vivo* (e.g. Hirschowitz & Sachs, 1969; Hirst *et al.*, 1978; Hirst, 1988). Fade of the acid secretory response is a particular feature of stimulation with gastrin, and is much less evident with histamine-stimulated secretion (Hirschowitz & Sachs, 1969;

[1] Present address: Norwich-Eaton Ltd, Newcastle upon Tyne.
[2] Present address: Smith Kline & French Research, Philadelphia, U.S.A.

Albinus et al., 1977; Hirst, 1988). The acid secretory response to pentagastrin in the rat *in vitro*, as well as *in vivo*, demonstrates fade (Main & Pearce, 1978; Boughton-Smith & Whittle, 1981). Tachyphylaxis, the phenomenon of progressively reduced responses upon repeated stimulation by the same secretagogue, of pentagastrin-stimulated acid secretion has also been observed in the rat isolated stomach and gastric mucosa (Bunce et al., 1976; Main & Pearce, 1978). Fade and tachyphylaxis are common to all gastrin peptides (Hirst et al., 1978; Hirst, 1988).

Studies in the cat *in vivo* have indicated that fade of the acid secretory response to gastrin may be related to release of an inhibitor (Izzat & Waton, 1987), a limiting gastric mucosal blood flow (Reed & Smy, 1976), and/or inactivation or desensitization of gastrin receptors (Albinus et al., 1977; Hirst et al., 1978; Hirst, 1988). In the present experiments, we have characterized acid secretory responses to pentagastrin *in vitro*, in the absence of vascular and external neural influences, in the rat isolated gastric mucosa. In particular, we have focussed on the mechanisms underlying fade, the relationship between fade and tachyphylaxis, and tested the hypothesis that tachyphylaxis is due to gastrin receptor inactivation or desensitization.

Methods

Wistar (Porton) strain male rats, 10–14 weeks old and weighing between 180 and 220 g, were allowed free access to water, but food was withheld overnight before experiments. Animals were anaesthetized with sodium pentobarbitone ($60\,mg\,kg^{-1}$ i.p.; Sagatal, May & Baker). The dissection technique was modified from the method of Forte et al. (1975) for the piglet, and is essentially as described by Main & Pearce (1978). The isolated mucosa was mounted over a plastic cup, mucosal surface inwards, $1\,cm^2$ area, and placed in an organ bath containing 60 ml of serosal solution at 37°C. The mucosal surface was perfused at $1\,ml\,min^{-1}$ with warmed unbuffered mucosal solution. Acid secretion was recorded continuously by monitoring the pH of the mucosal perfusate, and converting this to H^+ activity by an antilog function generator as described by Hirst et al. (1984). Serosal Na^+ and K^+ concentrations were determined by flame photometry (Instrument Laboratory, IL543), and serosal pH, P_{O_2} and P_{CO_2} using a blood-gas analyser (Radiometer, ABL 3). Serosal glucose was measured by the glucose oxidase method, and serosal and mucosal lactate by the lactate dehydrogenase method, were analysed using an automatic oxidase analyser (Alpha Laboratories, Analox GM6).

Experimental protocols

After mounting in the organ bath, tissues were allowed to stabilize over 30–60 min. In experiments investigating fade, the tissue was incubated in the presence of pentagastrin for 100 min. When a second response to pentagastrin was investigated, a standard washing routine commenced at 100 min, consisting of five changes of serosal solution each separated by 5 min. The second dose of pentagastrin was added at 120 min. In some experiments the washing procedure was omitted. For each given protocol, mucosae were randomly allocated to test and control groups.

Donor-recipient experiments A (donor) mucosa was dissected and mounted, and the acid secretory rate allowed to stabilize. At 0 min, pentagastrin, 10^{-7} M, was added to the serosal solution, and the acid response monitored for 120 min. During this time, a fresh (recipient) mucosa was dissected and mounted at approximately 60 min, and its response allowed to stabilize. The serosal solution from the donor mucosa was then added to the recipient mucosa at 120 min, and the acid response of the recipient mucosa monitored.

Influence of fade on tachyphylaxis Tissues were randomly allocated to one of three groups. All mucosa were allowed to equilibrate. In group A, tissues were left unstimulated until 100 min, followed by the standard washing routine, and the addition of pentagastrin, 10^{-7} M, at 120 min. In group B, pentagastrin, 10^{-7} M, was added at 0 min, and at the peak of the response the pentagastrin was washed out by three changes of the serosal solution (at approximately 10–20 min). This was followed by the standard washing routine and addition of pentagastrin, 10^{-7} M, at 100 and 120 min, respectively. The protocol for group C was similar to group B, except that the first dose of pentagastrin was allowed to remain in contact with the tissue, and the response to fade, until 100 min, when the protocol for groups A and B was followed.

Influence of inhibition of the first response on tachyphylaxis Tissues were randomly allocated to three groups, and in each group tissues were allocated to control or test experiments. In control experiments, tissues were incubated for 100 min in the absence of pentagastrin, whilst in test experiments pentagastrin, 10^{-7} M, was present. At 100 min, all tissues were subjected to the standard washing routine, and at 120 min, pentagastrin, 10^{-7} M, was added. Tissues allocated to group A were either unstimulated or incubated with pentagastrin alone for the first 100 min. In group B, proglumide, 10^{-2} M, was added to all tissues 10 min before the addition of

pentagastrin or at an equivalent time in the unstimulated tissues. In group C, omeprazole, 10^{-5} M, was added to all tissues 20 min before the start of the experiment at 0 min.

Treatment of results

Acid secretory rates were calculated from the H^+ activity, and expressed as μmol H^+ cm^{-2} h^{-1}. In most cases the acid secretory responses are presented as the increase in secretory rate above pre-stimulation unstimulated rates, Δ acid secretory rate, μmol cm^{-2} h^{-1}.

Results are expressed as mean ± 1 s.e.mean (*n*). Significance of difference between means was analysed by Mann–Whitney U-test, or where appropriate Kruskall–Wallis analysis of variance followed by Mann–Whitney U-test. Correlation coefficients (*r*) between two variables were calculated by the method of least squares. Significance was set at $P < 0.05$.

Materials

The serosal solution, a modified Krebs–Henseleit solution (in mM: NaCl 113, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.0, glucose 11.1; pH 7.4) was gassed vigorously with a 95% O_2, 5% CO_2 gas mixture. The mucosal solution was of similar composition except that it was unbuffered, made isotonic with NaCl (in mM: NaCl 141.2, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 5.6) and was continuously gassed with 100% O_2.

Pentagastrin was supplied as a solution (Peptavlon; ICI Pharmaceuticals). Histamine acid phosphate (BDH) was dissolved in serosal solution immediately before use. Proglumide (sodium salt; Rotta Research Laboratorium) was prepared immediately before use by dissolving in saline. Omeprazole (AB Hassle) was dissolved in polyethylene glycol 400, and diluted in $NaHCO_3$, 6.7 mM, immediately before use. Compounds were added to the serosal solution in volumes of less than 0.5 ml.

Results

Time course and pattern of acid secretory responses to pentagastrin

Examples of traces of acid secretory responses to pentagastrin 10^{-9}–10^{-6} M with time are illustrated in Figure 1. The responses to the various doses of pentagastrin displayed different characteristics such as peak secretory rate, initial rate of fade after peak, time for the response to fade back to pre-stimulation levels, and the change in basal acid secretory rate

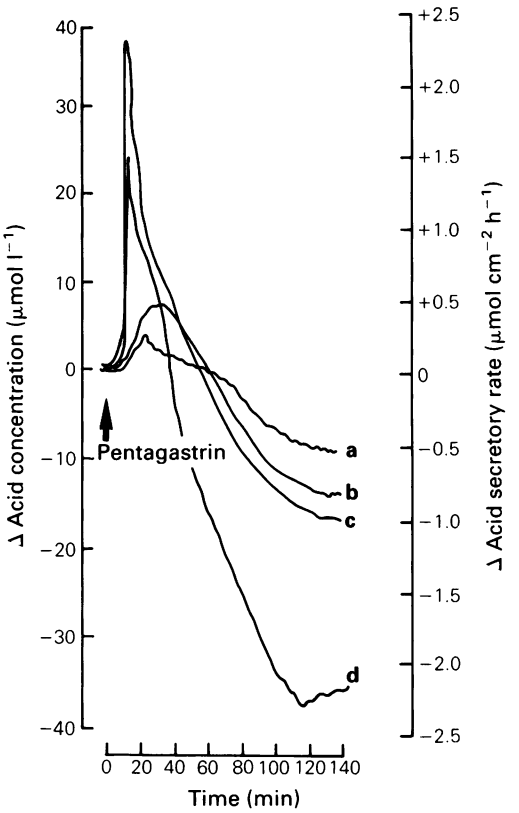

Figure 1 Typical traces of acid secretory responses to pentagastrin added at 0 min. Acid concentration in the mucosal effluent is given on the left axis, and this is converted to acid secretory rate on the right axis. Pre-stimulation values are plotted as zero. The four traces illustrate the responses to different concentrations of pentagastrin; (a) 10^{-9} M, (b) 10^{-8} M, (c) 10^{-7} M and (d) 10^{-6} M.

following fade. These variables were quantified (Table 1), and their relationship to either the dose of pentagastrin, or the magnitude of the increase in acid secretory rate investigated.

The dose-response relationship between $\log_{10}$ molar concentration of pentagastrin and the absolute maximum acid secretory rate is illustrated in Figure 2. Maximum acid secretion was observed with pentagastrin, 10^{-7} M, and higher concentrations produced successively lower responses. The absolute acid secretory rates are paralleled by the increase in acid secretion above basal (Δ acid secretory rate; Table 1).

After obtaining a peak, the acid secretory response to all concentrations of pentagastrin demonstrated fade. The rate of fade of secretion over the first 16 min following attainment of the peak was signifi-

Table 1 Calculated time course and pattern of acid secretory responses by rat isolated gastric mucosa to a range of serosal concentrations of pentagastrin.

Serosal concentration of pentagastrin (M)	*Acid secretory rate* (μmol cm^{-2} h^{-1})	*Fade* (%)	*Fade time* (min)	*Post-stimulation secretory rate* (μmol cm^{-2} h^{-1})
10^{-9}	0.24 ± 0.06 (6)	45.0 ± 13.7 (5)**	30.8 ± 8.7 (5)**	−0.13 ± 0.23 (6)
10^{-8}	0.47 ± 0.06 (6)	43.5 ± 10.0 (6)	35.0 ± 9.8 (6)	−0.32 ± 0.29 (6)
3×10^{-8}	1.52 ± 0.45 (6)	35.7 ± 14.7 (6)	56.8 ± 11.2 (6)	−0.26 ± 0.22 (6)
10^{-7}	2.65 ± 0.35 (7)	26.6 ± 5.0 (7)	73.3 ± 4.5 (7)	−0.38 ± 0.15 (7)
3×10^{-7}	2.13 ± 0.42 (6)	36.2 ± 9.1 (6)	67.7 ± 17.5 (6)	−0.92 ± 0.24 (6)*
10^{-6}	1.33 ± 0.12 (6)	32.8 ± 5.2 (6)	48.8 ± 14.2 (5)	−0.84 ± 0.43 (5)*

Each value is the mean ± s.e.mean of 5, 6 and 7 observations in 5, 6 or 7 gastric mucosae. Only first acid responses in freshly mounted tissues were considered. Acid secretory rate is expressed as the peak increase above pre-stimulation rates. Fade is the percentage decrease in acid secretory rate over the 16 min after peak secretory rate. Fade time is the time taken for acid secretory rate to decline to unstimulated rates, measured from peak acid secretory rate. Post-stimulation secretory rate is the difference between the pre-stimulation unstimulated rate, and the plateau value achieved after pentagastrin stimulation.

* Significantly different from the change in basal acid secretory rate over the same time period in unstimulated controls (+0.16 ± 0.13 (16) μmol cm^{-2} h^{-1}).

** One tissue failed to produce an acid response.

cantly correlated with the peak acid secretory rate (Figure 3). Expressed as a percentage of the peak acid response, fade did not significantly differ with the varying concentrations of pentagastrin, declining by approximately 36% of the peak response over the first 16 min (Table 1). The time for the response to fade to prestimulation basal levels was also signifi-

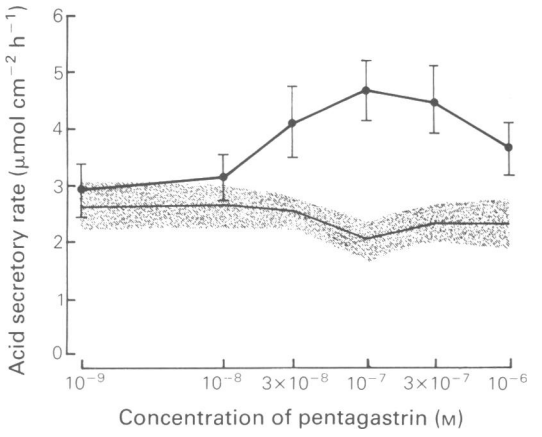

Figure 2 Relation between the log molar concentration of pentagastrin and the absolute acid secretory responses (uncorrected for pre-stimulation acid secretory rates). The peak acid response in tissues not previously exposed to pentagastrin is illustrated. The shaded area includes ±1 s.e. about the mean pre-stimulation secretory rate for each group of tissues. Results illustrated as mean with vertical lines indicating 1 s.e.mean (n = 6 or 7).

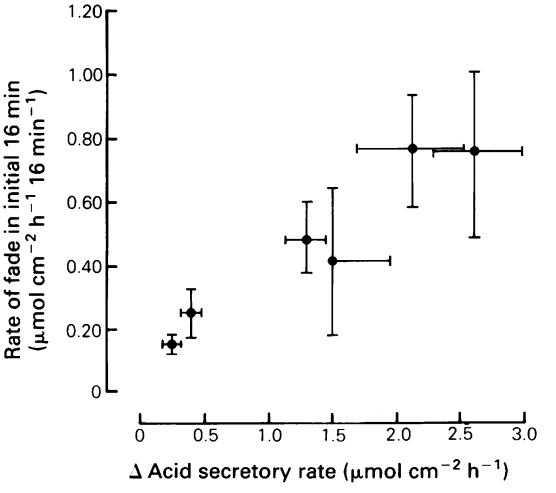

Figure 3 Relation between the increase in acid secretory rate in response to pentagastrin, 10^{-9}–10^{-6} M, and the initial rate of fade of the response over the first 16 min after attaining peak acid secretory rate, calculated as the difference between rates at the peak and after 16 min. Results are illustrated as mean and the vertical and horizontal lines represent ±1 s.e.mean (n = 6 or 7). The two variables were significantly correlated (r = 0.717).

cantly correlated with the magnitude of the acid secretory response (Figure 4), the maximum time being observed with pentagastrin, 10^{-7} M (Table 1).

After an initial stabilization period of 30–60 min subsequent to mounting the tissue in the organ bath, mean unstimulated acid secretory rates were stable over a 2 h period. The difference in basal acid secretory rate at 120 min compared with 0 min was $+0.16 \pm 0.13$ μmol cm^{-2} h^{-1} ($n = 16$). Acid secretory responses to pentagastrin, 10^{-9}–10^{-7} M, were followed by a return to values not significantly different from those before addition of pentagastrin. Responses to pentagastrin, 3×10^{-7} and 10^{-6} M, were followed by a significantly depressed rate of secretion (Table 1 and Figure 1). The post-stimulation acid secretory rate was not related to the dose of pentagastrin nor the magnitude of the acid secretory response to pentagastrin.

In all subsequent experiments, the dose of pentagastrin was standardized at 10^{-7} M, the concentration that produced maximum acid secretory rates (Figure 2 and Table 1).

Composition of the serosal solution during pentagastrin fade

The composition of the serosal solution was monitored throughout the stimulation of acid secretion with pentagastrin, to investigate whether any changes could account for a component of fade. Pentagastrin, 10^{-7} M, was added to the serosal solution, and the acid secretory response allowed to reach a maximum and then fade to a plateau. A second dose of pentagastrin, 10^{-7} M, was then added without changing the serosal solution, and the acid response again followed until it faded to a plateau. Finally, in order to investigate the ability of the tissue to secrete acid, histamine, 10^{-4} M, was added to the same serosal solution. The peak acid secretory response to the first dose of pentagastrin was 1.3 ± 0.29 μmol cm^{-2} h^{-1} ($n = 7$), and after the response had faded, the response to the second dose of pentagastrin was significantly lower (0.06 ± 0.04 μmol cm^{-2} h^{-1} ($n = 7$). However, the tissues were still able to secrete acid, as exemplified by the response to histamine (1.11 ± 0.33 μmol cm^{-2} h^{-1}; $n = 7$) which was not significantly different from the first response to pentagastrin. The composition of the serosal solution remained relatively stable throughout these experiments. Neither mean P_{O_2} (540 mmHg) nor glucose (14.1 mM) declined during these experiments. Mean P_{CO_2} fluctuated between 28 and 40 mmHg, but HCO_3^- (22.5 mM) and pH (7.4) remained stable. Mean Na^+ and K^+ concentrations tended to rise throughout the experiment ([Na^+], 140 to 144 mM; [K^+], 5.76 to 5.88 mM) which might be explained by losses due to evaporation over the

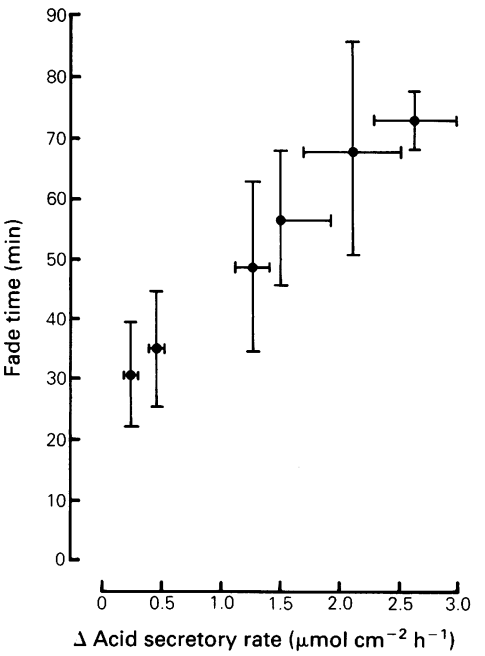

Figure 4 Relation between the increase in acid secretory rate in response to pentagastrin, 10^{-9}–10^{-6} M, and the time taken for the response to fade to pre-stimulation values from the peak acid response. Results are illustrated as mean and the vertical and horizontal lines represent ±1 s.e.mean ($n = 6$ or 7). The two variables were significantly correlated ($r = 0.554$).

> 135 min time course of the experiments.

In a separate series of experiments, mucosal and serosal lactate concentrations were measured. Serosal lactate concentrations were low, even 60 min after stimulation with pentagastrin, and did not increase during stimulation with pentagastrin (0.07 ± 0.03 mg 100 ml^{-1} ($n = 6$) at 30 min of stimulation). Mucosal lactate concentrations tended to be greater than serosal (0.18 ± 0.14 mg 100 ml^{-1} ($n = 6$) at 30 min), but did not vary from pre-stimulated values during stimulation by pentagastrin and subsequent fade.

Acid stimulative capacity of pentagastrin-containing serosal solution before and after fade

Fade of the acid response to pentagastrin cannot be explained by the progressive inactivation of pentagastrin, since addition of a second quantity of pentagastrin did not reverse fade (see above and next section). However, fade may be associated with the release of acid secretory inhibitors by the tissue. In addition, some inactivation of pentagastrin in the

serosal solution may have occurred. The contribution of these latter two factors was investigated by donor-recipient experimental protocols (see Methods). The serosal solution, to which pentagastrin, 10^{-7} M, had been added to stimulate acid secretion and its subsequent fade in donor tissue, was removed and added to a freshly dissected recipient tissue, and the acid response recorded. The peak response of the donor tissue (2.54 ± 0.30 μmol $cm^{-2} h^{-1}$; $n = 14$) was significantly greater than that of the recipient tissue (0.98 ± 0.25 μmol $cm^{-2} h^{-1}$; $n = 14$).

Influence of replacement of serosal solution on second response to pentagastrin

The extent of tachyphylaxis following fade of the acid secretory response to pentagastrin was investigated with and without replacement of the serosal solution. Tissues were incubated with pentagastrin, 10^{-7} M, until fade was complete. A second dose of pentagastrin, 10^{-7} M, was then added to the tissues, either to the original serosal solution, or to fresh serosal solution after a washing regime of five changes of solution separated by 5 min intervals. Without replacement, the second response was only $3.3 \pm 1.7\%$ ($n = 7$) of the first response. With replacement, the second response was increased to $23.9 \pm 8.2\%$ ($n = 7$) of the first (Figure 5). These observations are consistent with the possibility that a serosal factor may be released during fade, which subsequently depresses the second response. However, this could not fully account for the tachyphylaxis, since even after extensive washing, the second response is markedly depressed.

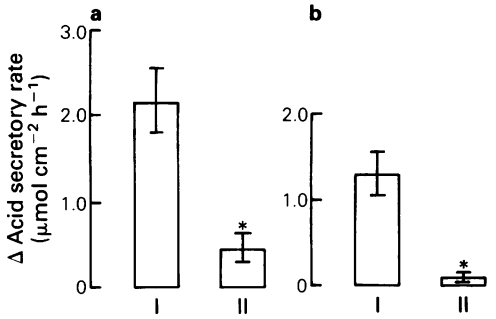

Figure 5 Effect of replacement of the serosal solution on a second response to pentagastrin (tachyphylaxis). Tissues were stimulated with pentagastrin, 10^{-7} M, and the response (I) allowed to fade. In (a), the serosal solution was replaced at 100 min followed by four further changes of solution, and the addition of a second dose of pentagastrin, 10^{-7} M, (II). In (b), the second dose of pentagastrin, 10^{-7} M, was added at 120 min without changing the serosal solution. Results are illustrated as mean peak increases in acid secretory rate and vertical lines indicate ±1 s.e.mean ($n = 7$). *Significantly different from first acid secretory response.

Influence of fade on tachyphylaxis

The acid secretory response to a dose of pentagastrin, 10^{-7} M, given at 120 min was not significantly different whether the tissue had previously been unstimulated (Figure 6a), or been stimulated by pentagastrin, which had subsequently been washed out after a peak secretory response had been attained (Figure 6b). If, however, the first dose of pentagastrin was allowed to remain in contact with the mucosa for 100 min, the first response faded, and the response to a second dose of pentagastrin was significantly reduced (Figure 6c) compared with that of the control tissue (Figure 6a). Thus the process of fade significantly influences the extent of tachyphylaxis.

Influence of inhibition of the first response on tachyphylaxis

An attempt was made to determine whether tachyphylaxis was a result of activation of gastrin receptors, or due to the process of acid secretion. The first acid secretory response to pentagastrin was inhibited by proglumide or omeprazole. Proglumide is believed to be a gastrin receptor antagonist. In contrast omeprazole exerts its acid-inhibitor effect by directly inhibiting the gastric $(H^+ + K^+)$-ATPase, and therefore pentagastrin should still have access to its receptors.

Six experimental protocols were investigated, and the results are illustrated in Figure 7. Compared with the first response to pentagastrin (2.35 ± 0.38 μmol cm^{-2} h^{-1}; $n = 12$), proglumide (0.47 ± 0.18 μmol cm^{-2} h^{-1}; $n = 8$) and omeprazole (0.44 ± 0.20 μmol cm^{-2} h^{-1}; $n = 8$) significantly reduced the response by 80%. In tissues which had been exposed to proglumide or omeprazole, and then stimulated by pentagastrin after 120 min (control experiments) the acid responses (1.17 ± 0.39 ($n = 8$) and 1.59 ± 0.50 μmol $cm^{-2} h^{-1}$ ($n = 8$)) were significantly reduced compared with tissues not exposed to the inhibitors (2.25 ± 0.52 μmol $cm^{-2} h^{-1}$; $n = 12$). Thus there was some degree of carry-over of the inhibitors, but this was similar for the two agents (i.e. the responses after proglumide and omeprazole were not significantly different).

The second response to pentagastrin at 120 min (0.47 ± 0.11 μmol $cm^{-2} h^{-1}$; $n = 12$), after an initial response had been allowed to fade, was significantly reduced compared to the control response to pentagastrin at 120 min (Figure 7a). Similarly, the second response to pentagastrin after pentagastrin plus omeprazole (0.83 ± 0.40 μmol $cm^{-2} h^{-1}$; $n = 8$), was

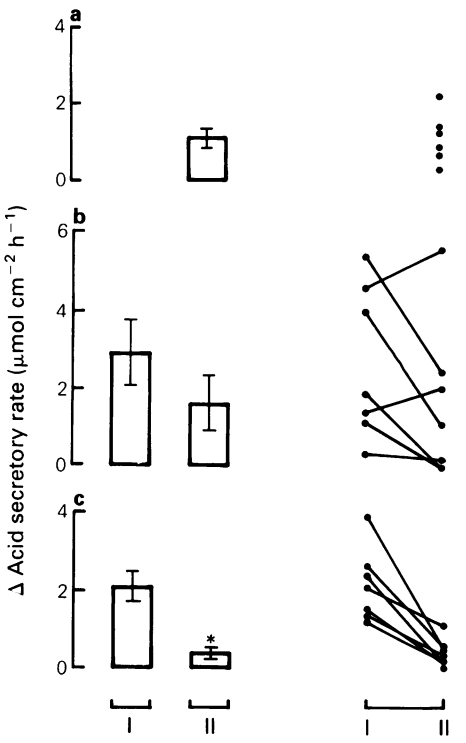

Figure 6 Influence of previous stimulation by pentagastrin on a second response to pentagastrin (tachyphylaxis). The mean (with bars indicating ±1 s.e.mean) ($n = 6$ or 7) (left) and individual (right) acid secretory responses to the first (I) and second (II) doses of pentagastrin, 10^{-7} M, are illustrated. In all tissues the second dose of pentagastrin was added at 120 min after the standard five changes of serosal solution between 100–120 min. The initial stimulation differed for the three groups: (a) unstimulated; (b) pentagastrin, 10^{-7} M, washed out after attaining peak; (c) pentagastrin, 10^{-7} M, remained in contact with mucosa until 100 min. *Significantly different from response II in (a).

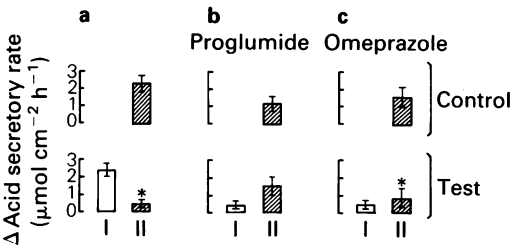

Figure 7 Influence of inhibition of the first response (I) by proglumide and omeprazole upon the extent of tachyphylaxis to a second dose of pentagastrin, 10^{-7} M, (II). The control tissues remained unstimulated until 120 min when pentagastrin was added after the standard serosal solution changes between 100–120 min. The test tissues received pentagastrin, 10^{-7} M, at 0 min (open columns) followed by standard changes of serosal solution at 100–120 min, and a second dose of pentagastrin at 120 min (hatched columns). The tissues were allocated to three groups: (a) no inhibitor; (b) proglumide, 10^{-2} M, added at −10 min; (c) omeprazole, 10^{-5} M added at −20 min. Results are illustrated as mean and bars indicate ±1 s.e.mean ($n = 12$ (group a) or 8 (groups b and c)). *Significantly different from response in control experiments in same group.

significantly reduced compared to the control response to pentagastrin after incubation with omeprazole alone (Figure 7c). In contrast, the response to pentagastrin after pentagastrin and proglumide (1.56 ± 0.50 μmol cm^{-2} h^{-1}; $n = 8$) was not significantly different from the response after proglumide alone (Figure 7b). Thus proglumide, but not omeprazole, prevented the tachyphylaxis of the pentagastrin response.

Discussion

The present study has quantified and characterized the gastric acid secretory response to pentagastrin in the rat isolated gastric mucosa. This *in vitro* preparation demonstrates the phenomena of fade and tachyphylaxis. Evidence is presented that fade may be in part related to release of acid secretory inhibitors into the serosal medium, whilst tachyphylaxis is a result of gastrin interaction with its receptors.

Acid secretory response to pentagastrin

Pentagastrin caused a concentration-related increase in the acid secretory rate over the range 10^{-9}–10^{-7} M (Figures 1 and 2). The mean maximal acid secretory rate to pentagastrin 10^{-7} M (administered as the first stimulant to fresh tissues) was around 2 μmol cm^{-2} h^{-1}. This value is similar to values previously found in this preparation (Main & Pearce, 1978; 1982). The response to concentrations of pentagastrin greater than 10^{-7} M evoked lower acid responses (Figures 1 and 2). The biphasic nature of the dose-response curves to pentagastrin and gastrin has been observed in other preparations *in vitro*, including the mouse, rat, cat and dog (Bunce *et al.*, 1978; Kuo & Shanbour, 1978; Davison & Najafi-Farashah, 1982; Spencer, 1982; Hirst *et al.*, 1984; Lotti *et al.*, 1986) and rabbit isolated gastric parietal cells (Magous & Bali, 1983). In addition, supramaximal concentrations of other stimulants (bethanechol, histamine and theophylline) resulted in reductions of acid secretion in the rat stomach *in vitro* (Spencer, 1982). Sub-maximal acid secretory

rates in response to large doses of gastrin have also been obtained in the dog and cat *in vivo* (Emås & Grossman, 1967; Hirst *et al.*, 1978). It has been proposed that the auto-inhibition observed with high concentrations of gastrin is the result of an interaction with low-affinity inhibitory receptors (Ariens, 1964; Prugh *et al.*, 1975; Davison & Najafi-Farashah, 1982). This postulated second receptor may not be localised to the parietal cell (Soll *et al.*, 1984a). In the piglet isolated gastric mucosa, concentrations of pentagastrin above 4×10^{-6} M reversibly inhibited the acid secretory response to histamine (Forte *et al.*, 1975), consistent with the second inhibitory receptor hypothesis, and similar effects have been demonstrated in the dog *in vivo* (Gillespie & Grossman, 1963). However, only a single class of gastrin receptors have been found in rat gastric mucosa (Takeuchi *et al.*, 1979). An alternative explanation is that the auto-inhibition is related to desensitization of gastrin receptors (Hirst *et al.*, 1978; Magous & Bali, 1983).

The auto-inhibition exhibited by high concentrations of gastrin may be related to the phenomena of fade and tachyphylaxis. As well as exhibiting submaximal secretory rates, the concentrations of pentagastrin greater than 10^{-7} M were also associated with a significantly depressed acid secretory rate, compared to pre-stimulation values, after fade (Table 1). This might be taken as evidence in favour of the release of inhibitory substances.

Mechanisms of fade and tachyphylaxis

The secretory responses to all concentrations of pentagastrin were transient (Figure 1). Both the initial rate of decline of the response (Figure 3), and the time taken for the response to decline to pre-stimulation values (Figure 4) were related to the magnitude of the initial acid response. The rate of fade of the pentagastrin response expressed as a percentage of the peak response was not related to the dose of pentagastrin, declining at about 36% $16\,min^{-1}$. This value is considerably greater than values, 4.6–7.8% $15\,min^{-1}$, obtained for the decline of acid secretion in the anaesthetized and conscious cat and conscious rat (Reed & Smy, 1976; Albinus *et al.*, 1977; Hirst *et al.*, 1978; Emås *et al.*, 1981; Hirst, 1988). The fade of the response *in vitro* is unlikely to be explained by depletion of an essential nutrient. The glucose, oxygen and K^+ concentrations were stable, whilst there was no evidence for progressive loss of tissue viability from the stable lactate concentrations. Moreover, after fade of the pentagastrin response was complete, histamine could still stimulate acid secretion at rates not different from those initially observed to pentagastrin, as has been previously demonstrated for frog isolated gastric mucosa (Kasbekar *et al.*, 1969; Kasbekar, 1972) and cat *in vivo* (Hirst, 1988). The very transient nature of the response to pentagastrin has been noted in other studies in isolated stomach and gastric mucosa of the rat (Main & Pearce, 1978; Boughton-Smith & Whittle, 1981), but it appears less marked in other species, e.g. the mouse, ferret, cat and dog (Kuo & Shanbour, 1978; Yates *et al.*, 1978; Black & Shankley, 1985). However, a more systematic study would be required to determine if these differences are specific or methodological.

Inactivation of gastrin cannot explain fade *in vitro*, or *in vivo* (Hirst *et al.*, 1980; Hirst, 1988). Addition of a second aliquot of pentagastrin to the same serosal solution did not elicit a secretory response similar to the first addition (Figure 5). That the serosal solution still contained acid-stimulating activity, albeit reduced, was demonstrated by the donor-recipient experiments.

The rapid fade of the acid secretory responses *in vitro*, as compared with *in vivo*, might suggest the progressive accumulation of inhibitory factors. In the anaesthetized cat evidence has been presented that pentagastrin releases 5-hydroxytryptamine, and that this may be responsible for fade of the acid secretion in this preparation (Izzat & Waton, 1987). This suggestion may find support in the observations of a reduced acid stimulating activity of donor serosal solution, and that changing the serosal solution significantly increased the acid secretory response to a fresh addition of pentagastrin (Figure 5); i.e. changing the serosal solution reduced tachyphylaxis. Both these experiments, however, also indicate that release of inhibitory factors into the serosal solution cannot entirely explain fade and the subsequent tachyphylaxis. In addition, inactivation of pentagastrin in the donor serosal solution, and recovery of gastrin responsiveness during the 20 min required to change the serosal solution are alternative explanations for these results. In the conscious cat, fade cannot be satisfactorily ascribed to release of 5-hydroxytryptamine (Hirst, 1988), and in rat isolated gastric mucosa 5-hydroxytryptamine reduced the fade of pentagastrin-stimulated acid secretion (Holland, 1983).

That fade influences tachyphylaxis is demonstrated by marked tachyphylaxis if the response to pentagastrin is allowed to fade (Figure 6). However, this tachyphylaxis is markedly alleviated if, after the initial peak response, the pentagastrin is washed out. Thus tachyphylaxis is increased by the continued presence of pentagastrin, even after an extensive washing routine.

Stimulation of acid secretion by pentagastrin in rat isolated gastric mucosa is associated with mobilization of histamine (Main & Pearce, 1982). Thus the decline in acid secretion may be related to deple-

tion of endogenous histamine stores. We did not investigate this possibility in the present studies. However, histamine is able to reverse gastrin tachyphylaxis in frog isolated gastric mucosae (Kasbekar, 1972), and in the anaesthetized cat (Pearson, 1966). In contrast, histamine was not able to prevent fade or tachyphylaxis of pentagastrin-stimulated acid secretion in the conscious cat (Hirst, 1988).

Based on experiments in the conscious cat, we have argued that fade of the gastrin response may be explained by receptor inactivation or desensitization (Albinus *et al.*, 1977; Hirst *et al.*, 1978; Hirst, 1988). Proglumide is a specific gastrin-receptor antagonist, which prevents gastrin binding to its receptors, and selectively inhibits gastrin stimulation of parietal cell function (Magous & Bali, 1983; Soll *et al.*, 1984b; Lotti *et al.*, 1986). Addition of proglumide to pentagastrin inhibited the acid secretory response, and also prevented the tachyphylaxis to a second dose of pentagastrin (Figure 7). Omeprazole, is a specific inhibitor of the $(H^+ + K^+)$-ATPase (Wallmark *et al.*, 1985). Although omeprazole was equally effective with proglumide in inhibiting the initial response to pentagastrin, the subsequent response showed marked tachyphylaxis (Figure 7). These data are consistent with a general model accounting for receptor desensitization (Swillens & Dumont, 1977), and are supported by observations on autoregulation of gastrin receptors (Takeuchi *et al.*, 1980).

In conclusion, we have shown that fade and tachyphylaxis of the responses to gastrin are related. Part of the fade may be due to the local mucosal release of inhibitor agents. These inhibitors may include 5-hydroxytryptamine (Pilot, 1979; Izzat & Waton, 1987), somatostatin (Soll *et al.*, 1984c) or prostaglandins (Pilot, 1979; Reeves & Stables, 1985). In this respect, indomethacin 10^{-4} M significantly reduced, while methysergide 10^{-6} M significantly increased, the rate of fade of pentagastrin-stimulated acid secretion in rat isolated gastric mucosae (Holland, 1983). This would suggest that local prostaglandin, but not 5-hydroxytryptamine, release may contribute towards the fade mechanism. However, the major proportion of the tachyphylaxis is a result of the specific interaction of gastrin with its receptors. The tachyphylaxis may occur at the level of the gastrin receptor, as argued before, or at the level of a gastrin-specific coupling mechanism.

Omeprazole and proglumide were gifts from Dr M.J. Daly, Astra Clinical Research, Edinburgh, and Professor I. Setnikar, Rotta Research Laboratorium, Milan, respectively. J.H. was a SERC-CASE scholar.

References

ALBINUS, M., BLAIR, E.L., HIRST, B.H., REED, J.D., SCHALLY, A.V. & SHAW, B. (1977). The effects of somatostatin and metiamide on tachyphylaxis of pentagastrin stimulated gastric acid and pepsin secretion in the conscious cat. *J. Physiol.*, **266**, 801–817.

ARIËNS, E.J. ed. (1964). *Molecular Pharmacology*, vol. 1. New York: Academic Press.

BLACK, J.W. & SHANKLEY, N.P. (1985). The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmacol.*, **86**, 571–579.

BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1981). The gastric antisecretory actions of prostaglandin E_2 and stable prostacyclin analogues against different secretagogues in perfused whole-stomachs of rat or mouse *in vitro*. *Br. J. Pharmacol.*, **72**, 291–298.

BUNCE, K.T., PARSONS, M.E. & ROLLINGS, N.A. (1976). The effect of metiamide on acid secretion stimulated by gastrin, acetylcholine and dibutyryl cyclic adenose 3',5'-monophosphate in the isolated whole stomach of the rat. *Br. J. Pharmacol.*, **58**, 149–156.

DAVISON, J.S. & NAJAFI-FARASHAH, A. (1982). The effect of dibutyryl cyclic guanosine monophosphate, a competitive antagonist to cholecystokinin-pancreozymin, on gastric acid secretion in the isolated mouse stomach. *Life Sci.*, **31**, 355–361.

EMÅS, S. & GROSSMAN, M.I. (1967). Comparison of gastric secretion in conscious dogs and cats. *Gastroenterology*, **52**, 29–34.

EMÅS, S., NYLANDER, G. & WALLIN, B. (1981). Comparison of the dose-response curves for acid output to pentagastrin determined by two techniques in chronic gastric fistula rats. *Digestion*, **22**, 94–100.

FORTE, J.G., FORTE, T.M. & MACHEN, T.E. (1975). Histamine-stimulated hydrogen ion secretion by *in vitro* piglet gastric mucosa. *J. Physiol.*, **244**, 15–31.

GILLESPIE, I.E. & GROSSMAN, M.I. (1963). Inhibition of gastric secretion by extracts containing gastrin. *Gastroenterology*, **44**, 301–310.

HIRSCHOWITZ, B.I. & SACHS, G. (1969). Pentagastrin in the gastric fistula dog. *Gastroenterology*, **56**, 458–467.

HIRST, B.H. (1988). Investigation of mechanism of fade of gastrin-stimulated gastric acid secretion in the cat. *J. Physiol.*, **395**, 319–331.

HIRST, B.H., HOLLAND, J., MARR, A.P., PARSONS, M.E. & SANDERS, D.J. (1984). Comparison of effect of peptide length and sulphation on acid secretory potency of gastrin in the cat *in vivo* and *in vitro*. *J. Physiol.*, **357**, 441–452.

HIRST, B.H., LABIB, L.A. & REED, J.D. (1978). Characteristics and tachyphylaxis of gastrin-stimulated gastric acid secretion in the cat. *J. Physiol.*, **276**, 1–11.

HIRST, B.H., REED, J.D., LUND, P.K. & SANDERS, D.J. (1980). Sensitivity of parietal cells to gastrin in the cat: comparison with man and dog. *Digestion*, **20**, 300–306.

HOLLAND, J. (1983). Gastric secretory responses to gastrin peptides *in vivo* and *in vitro*. *Ph.D. thesis, University of Newcastle upon Tyne.*

IZZAT, A. & WATON, N.G. (1987). Release of 5-hydroxytryptamine by pentagastrin and its role in the 'fade' of stimulated gastric secretion in cats. *J. Physiol.*, **383**, 499–507.

KASBEKAR, D.K. (1972). Secretagogue-induced tachyphylaxis of gastric H^+ secretion and its reversal. *Am. J. Physiol.*, **223**, 294–299.

KASBEKAR, D.K., RIDLEY, H.A. & FORTE, J.G. (1969). Pentagastrin and acetylcholine relation to histamine in H^+ secretion by gastric mucosa. *Am. J. Physiol.*, **216**, 961–967.

KUO, Y-J. & SHANBOUR, L.L. (1978). Acid secretion by isolated canine gastric mucosa. *J. Physiol.*, **285**, 325–340.

LOTTI, V.J., CHANG, R.S.L., KLING, P.J. & CERINO, D.J. (1986). Evidence that cholecystokinin octapeptide (CCK-8) acts as a potent, full agonist on gastrin receptors for acid secretion in the isolated mouse stomach: lack of antagonism by the specific CCK antagonist asperlicin. *Digestion*, **35**, 170–174.

MAGOUS, R. & BALI, J.P. (1983). Evidence that proglumide and benzotript antagonize secretagogue stimulation of isolated gastric parietal cells. *Regul. Peptides*, **7**, 233–241.

MAIN, I.H.M. & PEARCE, J.B. (1978). A rat isolated gastric mucosal preparation for studying the pharmacology of gastric secretion and the synthesis or release of endogenous substances. *J. Pharmacol. Methods*, **1**, 27–38.

MAIN, I.H.M. & PEARCE, J.B. (1982). Increased histamine-output from the isolated gastric mucosa of the rat in response to pentagastrin and methacholine. *Br. J. Pharmacol.*, **76**, 51–59.

PEARSON, J.A. (1966). The actions of extracts of gastrointestinal mucosa on the stomach and pancreas. *Ph.D. thesis, University of Newcastle upon Tyne.*

PILOT, M-A. (1979). Acid secretion from the completely isolated blood perfused canine stomach. *J. Physiol.*, **299**, 113–123.

PRUGH, M.F., SCHORR, B.A., VLAHCEVIC, Z.R. & MAKHLOUF, G.M. (1975). Intravenous pentagastrin as a partial agonist of gastric secretion in man: evidence in favor of the existence of hormonal inhibitory sites. *Gastroenterology*, **68**, 45–49.

REED, J.D. & SMY, J.R. (1976). Effects of isopropylnoradrenaline on tachyphylaxis of gastrin stimulated gastric acid secretion and mucosal blood flow in the anaesthetized cat. *J. Physiol.*, **254**, 153–168.

REEVES, J.J. & STABLES, R. (1985). Effects of indomethacin, piroxicam and selected prostanoids on gastric acid secretion by the rat isolated gastric mucosa. *Br. J. Pharmacol.*, **86**, 677–684.

SOLL, A.H., AMIRIAN, D.A., THOMAS, L.P., PARK, J., ELASHOFF, J.D., BEAVEN, M.A. & YAMADA, T. (1984a). Gastrin receptors on nonparietal cells isolated from canine fundic mucosa. *Am. J. Physiol.*, **247**, G715–G723.

SOLL, A.H., AMIRIAN, D.A., THOMAS, L.P., REEDY, T.J. & ELASHOFF, J.D. (1984b). Gastrin receptors on isolated canine parietal cells, *J. Clin. Invest.*, **73**, 1434–1447.

SOLL, A.H., YAMADA, T., PARK, J. & THOMAS, L.P. (1984c). Release of somatostatin-like immunoreactivity from canine fundic mucosal cells in primary culture. *Am. J. Physiol.*, **247**, G558–G566.

SPENCER, J. (1982). The rat isolated stomach; an *in vitro* model for the study of the physiology and pharmacology of gastric acid secretion. *J. Pharmacol. Methods*, **8**, 197–212.

SWILLENS, S. & DUMONT, J.E. (1977). The mobile receptor hypothesis in hormone action: a general model accounting for desensitization. *J. Cyclic Nucleotide Res.*, **3**, 1–10.

TAKEUCHI, K., SPEIR, G.R. & JOHNSON, L.R. (1979). Mucosal gastrin receptor. II. Physical characteristics. *Am. J. Physiol.*, **237**, E295–E300.

TAKEUCHI, K., SPEIR, G.R. & JOHNSON, L.R. (1980). Mucosal gastrin receptor. III. Regulation by gastrin. *Am. J. Physiol.*, **238**, G135–G140.

WALLMARK, B., LORENTZON, P. & LARSSON, H. (1985). The mechanism of action of omeprazole—a survey of its inhibitory actions *in vitro*. *Scand. J. Gastroenterol.*, **20**, 37–51.

YATES, J.C., SCHOFIELD, B. & ROTH, S.H. (1978). Acid secretion and motility of isolated mammalian gastric mucosa and attached muscularis externa. *Am. J. Physiol.*, **243**, E319–E326.

(Received January 18, 1988
Revised July 14, 1988
Accepted August 9, 1988)