Br. J. Pharmac. (1983), **80**, 27–32

Inhibition of gastric acid secretion in the conscious dog by the mast-cell stabilizing agent, FPL 52694

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1 The effects of the mast-cell stabilizing agent, FPL 52694, on gastric acid secretion in conscious dogs with gastric fistulae have been studied.

2 FPL 52694 (5 or 10 mg $kg^{-1} h^{-1}$) given intravenously during a plateau response to pentagastrin stimulation (2 $\mu g kg^{-1} h^{-1}$) caused a maximum inhibition of acid output of about 50% but had no significant effect on volume output so that the $[H^+]$ in the juice was markedly reduced. The ratio of mucosal blood flow/acid output (Ra) was increased in the presence of FPL 52694. There was no maintained reduction of $[H^+]$ when inhibition was due to cimetidine (4 μmol kg^{-1}, i.v.).

3 Instillation of FPL 52694 (4.35 mg ml^{-1}) directly into the stomach via the fistula for 30 min also resulted in an inhibition of acid output and reduction of $[H^+]$ during both pentagastrin- (2 $\mu g kg^{-1} h^{-1}$) and histamine-stimulated (30 $\mu g kg^{-1} h^{-1}$) secretion. Inhibition of pentagastrin-stimulated acid output by intragastric administration of FPL 52694 was much greater than the maximum effect seen following intravenous infusion.

4 The results are discussed in relation to the possible mode of action of FPL 52694. It is concluded that FPL 52694 is active orally and has a novel action on acid secretion which may include stimulation of gastric bicarbonate secretion.

Introduction

The therapeutic value of histamine H_2-receptor antagonists in the treatment of duodenal ulcer has underlined the importance of histamine in the physiological regulation of gastric acid secretion. It is therefore of interest to investigate the effects on acid secretion of other groups of compounds known to influence the role of histamine in the body. One such group is the mast-cell stabilizing agents which prevent the release of histamine during the anaphylactic response. Members of this group have previously been shown to inhibit gastric acid secretion in the anaesthetized rat and dog (Nicol, Thomas & Wilson, 1981) and in man (Davies, Rhodes & Thomas, 1981). Studies are described in this paper on the effects of a monochromone mast-cell stabilizing agent, FPL 52694 (5 - (2 - hydroxypropoxy) - 4 - oxo - 8 - propyl - 4H - 1 - benzopyran - 2 - carboxylic acid sodium salt), on acid secretion in the conscious dog equipped with a gastric fistula. A preliminary account of some of this work has been published elsewhere (Canfield & Curwain, 1982).

Methods

Experiments were carried out on male beagles (11–14 kg) equipped with established gastric fistulae and trained to laboratory conditions. Dogs were deprived of food overnight before the experiment but allowed access to water. No food was in evidence when the fistulae were opened before the experiment and basal acid output was very low. Gastric juice was collected by gravity drainage over 15 min periods, its volume measured, and an aliquot titrated to pH 7.4 using a Radiometer auto-titration system. Submaximal infusions of secretagogues were given in sterile isotonic saline throughout the experiment by continuous intravenous infusion (1.5 ml min^{-1}) via a catheter placed in a superficial leg vein under local anaesthesia. In experiments where FPL 52694 was given intravenously, gastric mucosal blood flow was estimated by the clearance of neutral red (Knight & McIsaac, 1977).

Intravenous administration of FPL 52694

Pentagastrin (2 $\mu g kg^{-1} h^{-1}$) was infused for 1.5 h. After 30 min neutral red (NR) was added to the infusion (1 mg $kg^{-1} h^{-1}$) and a loading dose of NR given (1 mg kg^{-1} over 10 min). Blood was withdrawn from a second intravenous catheter every 30 min during the remainder of the experiment for estima-

tion of NR and NR was also estimated in each gastric juice sample following the methods of Knight & McIsaac (1977). After 1.5 h FPL 52694 (5 or 10 mg $kg^{-1} h^{-1}$) was also added to the pentagastrin-NR infusion; acid secretion and mucosal blood flow were followed for a further hour. Control experiments where FPL 52694 was omitted were also carried out.

Intragastric administration of FPL 52694

Pentagastrin (2 $\mu g\, kg^{-1} h^{-1}$) or histamine (30 $\mu g\, kg^{-1} h^{-1}$) were given by intravenous infusion as above. After 1.5 h the gastric fistula was closed and FPL 52694 injected through the fistula into the stomach. The drug was dissolved in water (0.87 or 4.35 mg ml^{-1}) and given as four aliquots of 10 ml each over 30 min. The fistulae were then opened and allowed to drain for 2 min before collection of juice recommenced for 1 h. The solution drained from the stomach was collected and its volume recorded. Control experiments were also carried out where water alone (4 × 10 ml) was injected instead of FPL 52694. Blood flow was not measured in these experiments.

Expression of results

Acid has been expressed as $\mu mol\, H^+\, min^{-1}$, volume as ml per 15 min and H^+ concentration of the juice as mM. The ratio of blood flow to acid output (Ra) is given as ml/$\mu mol\, H^+$.

Statistics

Differences between control and experimental mean values were tested using Student's *t* test.

Results

Intravenous administration of FPL 52694

Figure 1 shows the results of infusion of FPL 52694 at two dose levels (5 and 10 mg $kg^{-1} h^{-1}$) on the response to a continuous infusion of pentagastrin (2 $\mu g\, kg^{-1} h^{-1}$) compared with control experiments in the same four animals in which FPL 52694 was omitted. At the higher dose, acid output was significantly reduced to about half its previous value throughout the period following FPL 52694 addition. There was also a smaller reduction in volume of juice during this time but this did not achieve statistical significance. As a consequence of this difference between acid and volume output there was a progressive fall in H^+ concentration of the juice which was significant for the last three samples. There was a small increase in the ratio of mucosal blood flow/acid output (Ra)

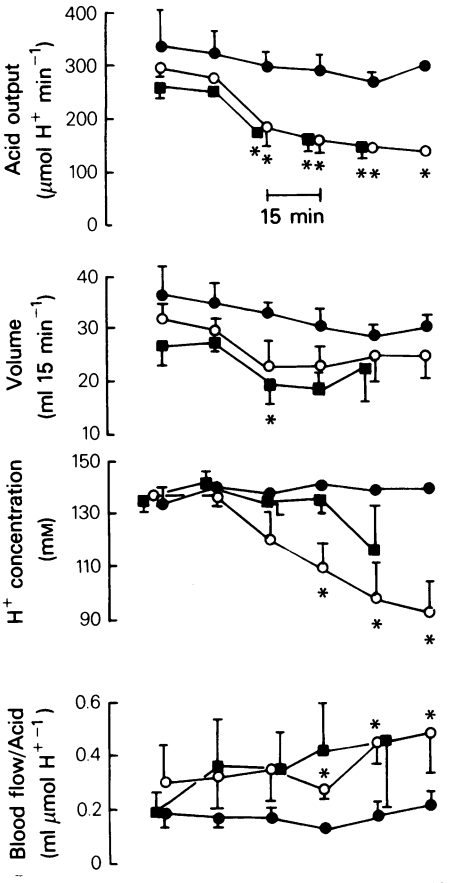

Figure 1 The effect of intravenous infusion FPL 52694 on response to pentagastrin stimulation (2 $\mu g\, kg^{-1} h^{-1}$) in conscious fistula dogs. Values shown are mean of $n = 4$ in each case; vertical lines show s.e.mean. *indicates a significant difference ($P < 0.05$). Filled circles (●) control experiments without FPL 52694, other symbols with FPL 52694 at two dose levels (○) 10 mg $kg^{-1} h^{-1}$, (■) 5 mg $kg^{-1} h^{-1}$. FPL 52694 infusion began after the first two points.

which was significant for the last two periods. The effects of the lower dose of FPL 52694 were only followed for 45 min. The reduction in acid output was not different from that seen with the higher dose and the reduction in volume slightly greater leading to a less marked effect on H^+ concentration. Mean values of Ra were more varied than after the higher dose and the small increase following FPL 52694 was not significant. There were no significant changes of any parameter in the control experiments.

Intravenous cimetidine

The most striking feature of the inhibitory action of FPL 52694 (10 mg $kg^{-1} h^{-1}$) shown in Figure 1 is the

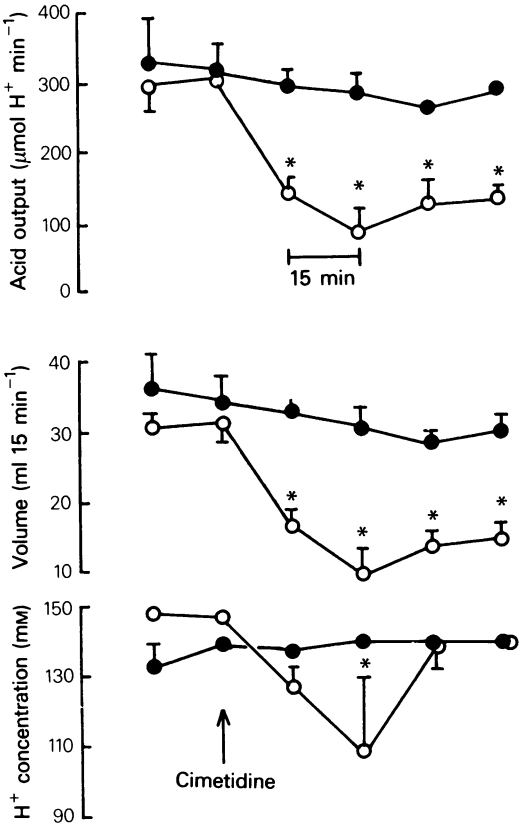

Figure 2 The effect of intravenous injection of cimetidine (4 μmol kg^{-1}) on response to pentagastrin (2 μg kg^{-1} h^{-1}) in conscious dogs with gastric fistulae. Values shown are mean of $n = 5$ in each case; vertical lines show s.e.mean. * indicates a significant difference ($P < 0.05$). Filled circles (●) control and open circles (○) with cimetidine.

reduction in H^+ concentration. This may have been the result of a specific action of this drug or the general consequence of the action of any inhibitor of acid secretion in the fistula animal. To test this, pentagastrin-stimulated secretion (2 μg kg^{-1} h^{-1}) was inhibited by injection of cimetidine (4 μmol kg^{-1} i.v.), a dose that provided a similar average inhibition during the hour following injection to that obtained in the FPL 52694 experiments. The results are shown in Figure 2. Blood flow was not measured in these experiments. There were significant reductions in both acid and volume outputs following cimetidine, both parameters being reduced by the same proportion. There was no sustained fall in H^+ concentration of the kind seen in Figure 1 with FPL 52694 although 30 min after cimetidine there was a transient and statistically significant reduction of the H^+ concentration. During the last two 15 min periods both acid and volume were reduced by half but H^+ concentration was identical with the control values.

Intragastric administration of FPL 52694

The effects of intragastric FPL 52694 at two concentrations (0.89 and 4.35 mg ml^{-1}) during pentagastrin-stimulated secretion (2 μg kg^{-1} h^{-1}) are shown in Figure 3 together with results from control experiments where water but not FPL 52694 was added to the stomach in the same manner as in the test experi-

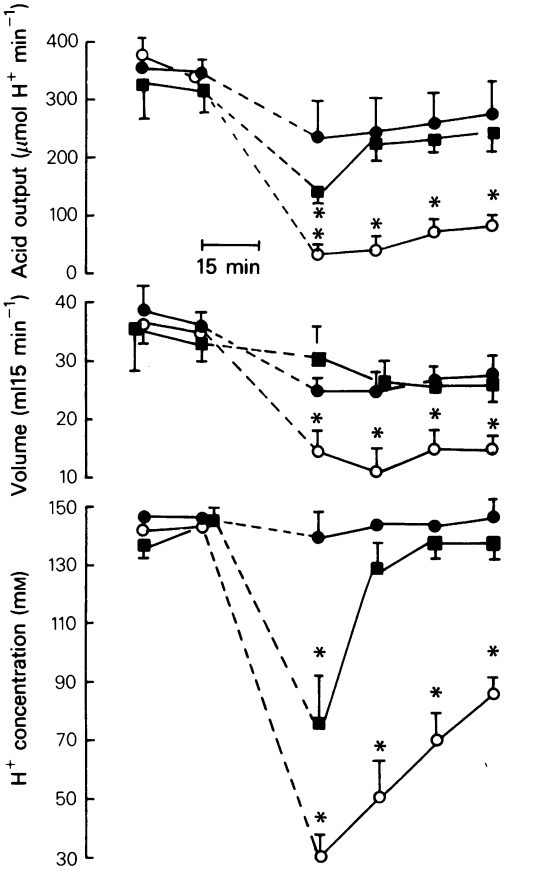

Figure 3 The effect of intragastric instillation of FPL 52694 (4 × 10 ml aliquots over 30 min) on the response to pentagastrin stimulation (2 μg kg^{-1} h^{-1}) in conscious dogs with gastric fistulae. Values shown are means of $n = 5$ for each case; vertical lines show s.e.mean. * indicates a significant difference ($P < 0.05$). The fistula was closed for 30 min during the period covered by the dashed lines. Filled circles (●) control experiments where water alone was instilled into the stomach. Other symbols show results with FPL 52694 instilled into stomach at two dose levels; (○) 4.35 mg ml^{-1} and (■) 0.89 mg ml^{-1}.

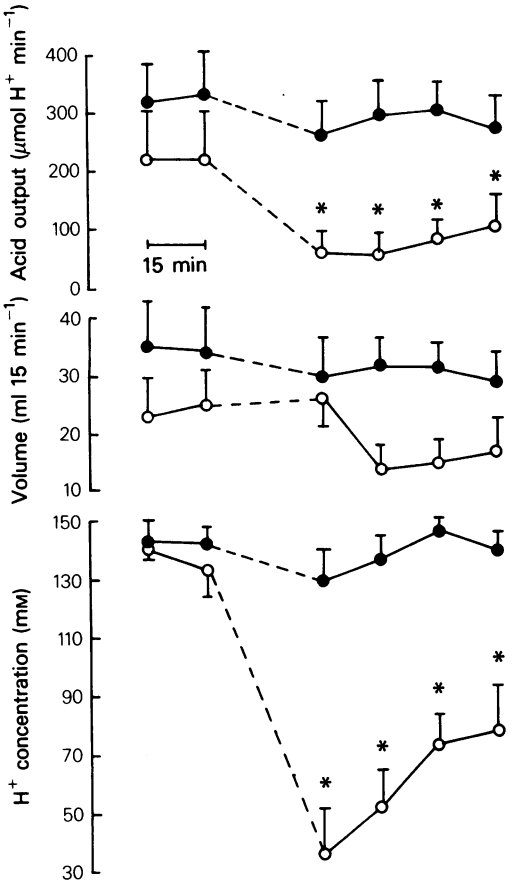

Figure 4 The effect of intragastric instillation of FPL 52694 (4.35 mg ml^{-1} as 4 × 10 ml aliquots over 30 min) on the response to histamine stimulation (30 μg kg^{-1} h^{-1}) in conscious dogs with gastric fistulae. Values shown are means of $n = 5$ for each case; vertical lines show s.e.mean. * indicates a significant difference ($P < 0.05$). Filled circles (●) control experiments where water alone was instilled into the stomach and open circles (○) with FPL 52694 instilled into the stomach.

ments. At the higher concentration of FPL 52694, both acid and volume outputs were significantly reduced and lower than in control experiments throughout the 1 h following the drug. Acid output was reduced to a greater extent than volume, resulting in a significant fall of H^+ concentration. The only significant effects of the lower concentration of FPL 52694 occurred during the first 15 min period following the drug. There were also reductions in acid and volume outputs in the control experiments following closure of the fistula but these were not associated with any change in H^+ concentration in the juice. Figure 4 shows the effect of FPL 52694 (4.35 mg ml^{-1}) on histamine-stimulated secretion (30 μg kg^{-1} h^{-1}) compared with control experiments. The pattern of results is similar to that seen with pentagastrin (Figure 3) but the effects were smaller. Acid output was significantly reduced for the first 3 periods but at no time was the reduction in volume significant. This resulted in the marked reduction in H^+ concentration throughout the 1 h following FPL 52694. There were no significant changes in any of the control experiment parameters in contrast to the control results with pentagastrin (Figure 3).

Volumes recovered from the stomach after fistula closure

When the fistulae were re-opened following the 30 min instillation of drug or water, the stomach contents were drained, collected and the volumes measured. The results are shown in Table 1.

Discussion

Before discussing the results of this study it is necessary to discuss the interpretation of experiments carried out in the gastric fistula dog preparation. There are two main advantages in the use of this preparation which are particularly relevant in studying a novel secretory antagonist. The stomach retains a near normal blood and nervous supply. Gastric secretions are sampled from the whole mucosal surface and so incorporate any regional differences. This latter point is particularly useful with preliminary studies on a compound believed to act topically. There are however, a number of disadvantages. Swallowed saliva may contaminate and dilute the gastric secretions. In the experiments reported here, the H^+ concentrations were approximately 140 mM; comparable with the values of 150 mM reported by Hirschowitz & Sachs (1969) from gastric fistula dogs

Table 1 The volume of fluid recovered (mean ± s.e.mean, $n = 5$) from the stomach of the dog after 30 min closure of the fistula during which time 4 × 10 ml aliquots of water or drug solution were injected

Secretagogue	*Recovered volume* (ml)	
	Control	+FPL 52694 (4.35 mg ml^{-1})
Pentagastrin (2 μg kg^{-1} h^{-1})	16 ± 5	57 ± 10
Histamine (30 μg kg^{-1} h^{-1})	36 ± 6	74 ± 20

where saliva was prevented from entering the stomach by an oesophageal fistula. Thus, salivary contamination would seem not to be a significant factor in the present experiments. Reflux of duodenal contents into the stomach might also complicate the results with fistula preparations and might explain a reduction of H^+ concentration if the reflux was rich in bicarbonate. It is difficult definitely to exclude this possiblity. However, a reduction in H^+ concentration following administration of FPL 52694 has been reported by Nicol *et al.* (1981) using anaesthetized dogs where the pylorus had been ligated. Duodenal reflux cannot therefore, be the sole explanation of the present results. A final difficulty is possible loss of gastric juice from the stomach to the duodenum. This is unlikely to happen with a properly placed fistula which is open but may be a problem when the fistula is closed during administration of FPL 52694 in the intragastric studies. This is discussed below under control experiments.

Intragastric control experiments

In the control experiments the fistulae were closed and water (4 × 10 ml) injected over a 30 min period in the same way as the FPL 52694 studies. If these procedures were without effect upon acid secretion then there would be no statistically significant change in acid output, volume or H^+ concentration during the control experiments. This was the case when histamine was the stimulant (closed symbols, Figure 4) but not with pentagastrin (closed symbols, Figure 3). In the pentagastrin controls there were similar, small reductions of both acid and volume outputs but no significant change in H^+ concentration. Thus, the inhibition seen in pentagastrin control experiments showed quite different characteristics from that seen following administration of FPL 52694 intragastrically. If there were no loss of juice from the stomach during the period that the fistula was closed, one would expect to recover the 40 ml injected plus the volume secreted during the 30 min; a greater volume being obtained from the control experiments than from those where acid output had been reduced by FPL 52694. Contrary to expectation, the recovered volume was greater from the FPL 52694-treated stomachs than from the controls for both stimulants (Table 1). Clearly more juice was lost from the control stomachs than from those receiving FPL 52694. Acidification of the duodenum is well known to cause inhibition of pentagastrin-stimulated acid secretion but results during histamine stimulation are more variable (Andersson, 1967). Thus, it seems likely that acid is lost from the stomach in control experiments leading to a degree of secretory inhibition which was greater with pentagastrin than with histamine. Did this loss occur in the FPL 52694 experiments? It is not possible to give a definite answer to this as the volume of acid secreted during the period of the fistula closure is unknown. An approximation may be made by taking twice the volume secreted in the first 15 min period after FPL 52694 plus 40 ml yielding expected volumes of 70 ml for pentagastrin and 100 ml for histamine where volume was inhibited to a lesser extent. The actual volumes recovered are comparable with this (Table 1). These findings may also suggest that FPL 52694 has an additional action on gastric emptying. No firm conclusions on this can be drawn from these present experiments which were not designed to investigate this. It is worth noting that such an additional effect of FPL 52694 would not have been observed had these experiments been carried out in gastric pouches.

Intravenous studies

FPL 52694 caused a significant reduction in pentagastrin-stimulated acid secretion in the conscious dog. The maximum inhibition by FPL 52694 was about 50% with both 5 and 10 mg kg^{-1} h^{-1}. This agrees with quantitatively similar effects found in the anaesthetized dog with 10 mg kg^{-1} h^{-1} (Nicol *et al.*, 1981) and is comparable with the maximum effect in the anaesthetized rat from dose-response studies reported in that paper. In the present study the inhibition of acid was associated with relatively little reduction of secreted volume leading to a marked fall of H^+ concentration in the juice. This effect was not seen when acid output was reduced to a similar extent with cimetidine which caused a parallel reduction of both acid output and volume and no sustained reduction of H^+ concentration. The results of neutral red clearance suggest that the inhibition was not secondary to a reduced gastric mucosal blood flow and in fact the ratio Ra was significantly raised in the latter half of the FPL 52694 period.

Intragastric studies

Application of FPL 52694 to the gastric mucosa resulted in an almost complete inhibition of pentagastrin-stimulated acid output. The effect was less marked with histamine as a stimulant but was still highly significant. The inhibition of pentagastrin-induced secretion was much greater than the maximum effect seen with intravenous administration of FPL 52694. As with the intravenous study, intragastric application produced an effect where acid output was inhibited to a greater extent than volume output, which was reflected by a very marked reduction in H^+ concentration.

Mechanism of action of FPL 52694

Nicol *et al.* (1981) found that intravenous FPL 52694 inhibited pentagastrin- but not histamine-stimulated acid secretion in the anaesthetized rat. They concluded that it could not be acting as an H_2-receptor antagonist and that the effect might be due to an inhibition of histamine release in the mucosa. The results in this paper clearly indicate that FPL 52694 given intragastrically in the dog inhibits histamine-stimulated acid secretion. This difference from the rat may reflect the different route of administration or a species difference. However, an action against exogenous histamine makes it unlikely that the inhibition is solely the consequence of a prevention of endogenous histamine release although it does not entirely preclude such an effect. It also re-opens the possibility that FPL 52694 may have an antagonistic action at H_2-receptors but there appears to be no evidence to support this hypothesis.

The most striking feature of acid inhibition with FPL 52694 is the very marked reduction in H^+ concentration in the juice, an effect not seen in this preparation with the H_2-antagonist cimetidine at a dose giving a similar degree of secretory inhibition. This is currently being investigated in more detail. A working hypothesis would be that FPL 52694 has two actions; directly to inhibit acid output and also to remove secreted acid from the stomach lumen either by an ion exchange mechanism or by intra-luminal neutralization resulting from an increase in mucosal bicarbonate output. The blood flow results would support such a dual hypothesis as blood flow was reduced less than acid output (increased Ra). The blood flow would still be required to enable the secretion of acid which was subsequently removed or neutralized.

These experiments do not explain the lower maximum inhibition obtained when FPL 52694 is given by the intravenous route. Explanation of this must await a better understanding of the mode of action of the compound. What is clear from this preliminary study is that FPL 52694 is a potent and novel inhibitor of gastric secretion and is orally active. The most striking feature of the inhibition is the marked reduction of H^+ concentration of gastric juice. A better understanding of the mode of action of FPL 52694 must await further work, possibly including experiments in gastric pouch animals and *in vitro* studies.

We would like to thank Fisons (Pharmaceutical) PLC for supply of FPL 52694 and for financial support.

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(*Received November 16, 1982. Revised March 18, 1983.*)