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Gastric juice electrolyte secretion in conscious dogs with gastric fistulae and its modification by FPL 52694, a mast cell stabilizing agent

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1 Studies have been made of the electrolyte output in the gastric juice of conscious dogs equipped with gastric fistulae during stimulation by intravenous infusion of either pentagastrin (2 $\mu g\,kg^{-1}\,h^{-1}$), histamine (30 $\mu g\,kg^{-1}\,h^{-1}$) or insulin (0.1 $u\,kg^{-1}\,h^{-1}$).

2 The mast cell stabilizing agent, FPL 52694 (4.35 $mg\,ml^{-1}$) was instilled into the stomach for 30 min and caused a marked reduction of H^+ output, H^+ concentration and osmotic strength of the juice during stimulation with pentagastrin, histamine, or insulin. There was also a marked increase in the rate of Na^+ output into the juice.

3 When pentagastrin-stimulated acid secretion was inhibited by cimetidine (4 $\mu mol\,kg^{-1}$ i.v.) acid output was reduced but there were no sustained changes in ion concentrations, osmolarity or Na^+ output of the type seen following inhibition with FPL 52694.

4 It is concluded that FPL 52694 may have a dual mode of action in this preparation; a direct reduction of the output of hydrochloric acid and a smaller effect to increase gastric $NaHCO_3$ output leading to a post-secretory neutralization of the juice.

Introduction

FPL 52694 (5 - (2 - hydroxypropoxy) - 4 - oxo - 8 - propyl - 4H - 1 - benzopyran - 2 - carboxylic acid sodium salt) is a monochrome with mast cell stabilizing properties which has previously been reported to have an inhibitory action on gastric acid secretion in rat, dog and man (Davies, Rhodes & Thomas, 1981; Nicol, Thomas & Wilson, 1981; Canfield & Curwain, 1982). In conscious dogs equipped with gastric fistulae, FPL 52694 was more effective when instilled into the stomach than when given intravenously (Canfield & Curwain, 1983). The inhibition of pentagastrin-stimulated acid secretion showed that H^+ output was reduced to a greater extent than secreted volume so that there was a marked fall in the H^+ concentration of the gastric juice. This paper describes further studies in which measurements of both concentration and output of the H^+, Na^+ and Cl^- in the gastric juice together with juice osmolarity have been made in conscious dogs with gastric fistulae.

Methods

Fistula dogs

Experiments were carried out using male beagles (11–14 kg) as previously described (Canfield & Curwain, 1983). Gastric juice was collected by gravity drainage over 15 min periods and its volume measured. Sub-maximal infusions of secretagogues were given in sterile isotonic saline throughout the experiment by intravenous infusion (1.5 $ml\,min^{-1}$) via catheters placed in the superficial leg veins under local anaesthesia. After 1.5 h the gastric fistulae were closed and FPL 52694 injected through the fistulae into the stomach. The drug was dissolved in water (4.35 $mg\,ml^{-1}$) and given as four aliquots each of 10 ml during 28 min. The drug solution had a pH of 6.8. 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 in which water alone was instilled into the stomach were also carried out.

Measurements

Aliquots of juice were titrated to pH 7.4 using a Radiometer autotitration system, Cl^- was measured by electrometric titration (Corning-EEL) and osmolarity by freezing point depression (Osmette A, Precision Instruments Inc.). Sodium content of the juice was measured with a Na^+ selective electrode (E.I.L.)

connected to a millivoltmeter (Phillips PW 9409). Aliquots of juice (5 ml) were treated with 1 M Tris HCl buffer (1.5 ml, pH 10) before measurement giving a final pH of about 8 and compared with sodium chloride standards made up in acid under identical conditions. The electrode response was linear over the range 1–100 mM and stable during the experimental period.

Expression of results

The outputs of H^+ and Cl^- in response to the secretagogues showed between animal variation presumably due to the different sensitivities of the different animals to the stimulants. To avoid superimposing the effects of FPL 52694 on this variation the results have been normalized by expressing the outputs of H^+ and Cl^- during the 1 h following closure of the fistula as a percentage of the average outputs for the two 15 min periods prior to closure of the fistula in each animal. This was not done for Na^+ output which is expressed as μmol Na^+ min^{-1}. There is no evidence that stimulants of acid secretion influence Na^+ output (Makhlouf, McManus & Card, 1966) and in the absence of FPL 52694 Na^+ output was low and the variations between animals small. Ion concentrations have been expressed as mM and osmolarity as mosmol $kg^{-1}H_2O$. The values shown for these parameters are the means for the two 15 min periods before and for the 4 periods following closure of the fistulae.

Statistics

Values given are means with s.e.means throughout. Differences were considered significant if $P < 0.05$ using Students t test for the comparison between mean values before and after instillation of FPL 52694.

Results

Pentagastrin

The mean values of the measured parameters in response to pentagastrin (2 μg kg^{-1} h^{-1}) before closure of the fistulae for both test and control experiments are shown in Table 1 and the effects of FPL 52694 on these parameters are shown in Figure 1. H^+ output fell to 9.5 ± 4.4% and Cl^- output to 30.6 ± 9.8% of preceding values in the first 15 min period following FPL 52694. The H^+ concentration fell to 30 ± 7 mM, the Cl^- concentration to 124 ± 7.3 mM at 30 min after reopening the fistulae. The Na^+ output was significantly increased through-

Table 1 Mean values for measured parameters of gastric juice during the 30 min period before closure of the fistulae

	Control experiments							*Experiments with FPL 52694*							
	Volume	*Ion output* (μmol min^{-1})				*Osmolarity*		*Volume*	*Ion output* (μmol min^{-1})					*Osmolarity*	
Stimulant	(ml min^{-1})	$H+$	Cl^-	$Na+$	H^+ (mM)	Cl^- (mM)	(mosmol $kg^{-1}H_2O$)	n	(ml min^{-1})	H^+	Cl^-	$Na+$	H^+ (mM)	Cl^- (mM)	(mosmol $kg^{-1}H_2O$)	n
Pentagastrin	2.40	363	426	9.6	145	176	290	5	2.47	360	412	13.7	144	160	305	5
(2 μg kg^{-1} h^{-1})	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	0.13	23	24	2.7	2	4	8		0.27	39	54	1.1	2	8	5	
Histamine	2.20	327	393	9.1	138	167	285	5	1.60	209	273	7.5	142	176	292	5
(30 μg kg^{-1} h^{-1})	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	0.47	82	82	2.5	8	5	6		0.47	62	81	2.1	6	7	4	
Insulin	1.35	194	197	7.2	145	170	303	3	1.87	277	315	9.0	145	167	301	4
(0.1 U kg^{-1} h^{-1})	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	0.17	10	21	0.3	3	3	3		0.33	35	44	4.0	4	6	5	

The mean ± s.e.mean values of the parameters measured in the gastric juice from conscious dogs equipped with gastric fistulae during stimulation of secretion with secretagogues given by i.v. infusion. The values are averages for the two 15 min periods prior to closure of the fistulae for both control experiments and experiments with FPL 52694 instillation into the stomach. There were no statistically significant differences between control and test parameters with any of the three stimulants prior to administration of FPL 52694.

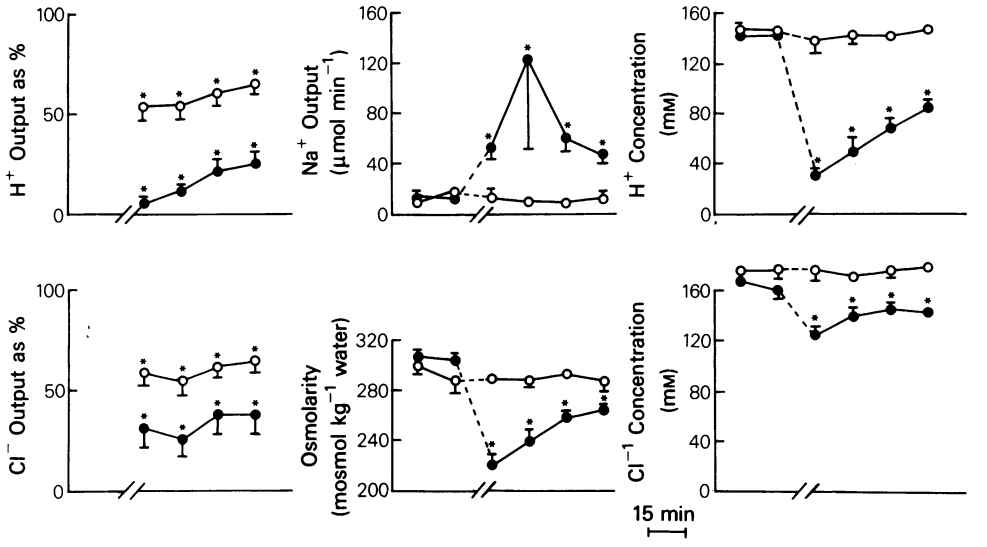

Figure 1 The mean ± s.e.mean values ($n = 5$) of results from conscious dogs with gastric fistulae during continuous i.v. infusion of pentagastrin (2 μg kg^{-1} h^{-1}). The break in the time axis indicates closure of the fistulae for 28 min during which time either 40 ml water (○) or 40 ml of FPL 52694 (4.35 mg ml^{-1}; ●) was instilled into the stomachs as 4 × 10 ml aliquots. The outputs of H^+ and Cl^- after reopening the fistulae are shown as a percentage of the average value obtained during the two 15 min periods prior to closure. Other parameters show these 2 periods plus the four 15 min periods after reopening the fistulae. Where no error bar is shown it lies within the point. *indicates a significant difference ($P < 0.05$).

out the 1 h following treatment with FPL 52694. All parameters showed some recovery during the post-FPL 52694 period but were still all significantly different from pre-FPL 52694 values ($P < 0.05$) at 60 min.

The control experiments in Figure 1 also show a reduction of both H^+ and Cl^- outputs; the asterisks for Figure 1 control values indicate a significant ($P < 0.05$) reduction compared with values prior to closure of the fistula. The fall in output was similar for both ions. There were no significant changes in the other control parameters. The asterisks for FPL 52694-treated data in Figure 1 show significant changes ($P < 0.05$) when compared with the corresponding control data.

Histamine

The results of the experiment with histamine (30 μg kg^{-1} h^{-1}) are shown in Figure 2 and the mean values before the fistula closure in Table 1. Treatment with FPL 52694 produced qualitatively similar results to those seen with pentagastrin; H^+ and Cl^- concentration and total osmolarity were reduced and Na^+ output markedly increased. There was a significant reduction of H^+ output for all but the last 15 min period of the hour following FPL 52694. Cl^- output was reduced to a lesser extent than H^+ and these reductions did not achieve statistical significance. There were no significant changes in any measured parameters in the control experiments.

Insulin

Figure 3 shows the effects of FPL 52694 on gastric juice secretion in response to insulin (0.1 u kg^{-1} h^{-1}). The values before fistula closure are shown in Table 1. The concentrations of H^+ and Cl^-, the osmolarity and the Na^+ output were not significantly different from the values found with pentagastrin or histamine. H^+ output was significantly reduced ($P < 0.05$) during the first two 15 min periods after FPL 52694 but the reduction in Cl^- output was not significant ($P > 0.05$) at any time. There was a large but very variable increase in Na^+ output which was significant only during the last two 15 min periods of the hour following administration of FPL 52694. The reductions in osmolarity and H^+ concentration were significant throughout the 60 min but the reductions in Cl^- concentration were not.

Inhibition with cimetidine

It was desirable to determine if the changes in gastric juice composition following FPL 52694 were due to a specific action of the drug or were a consequence of

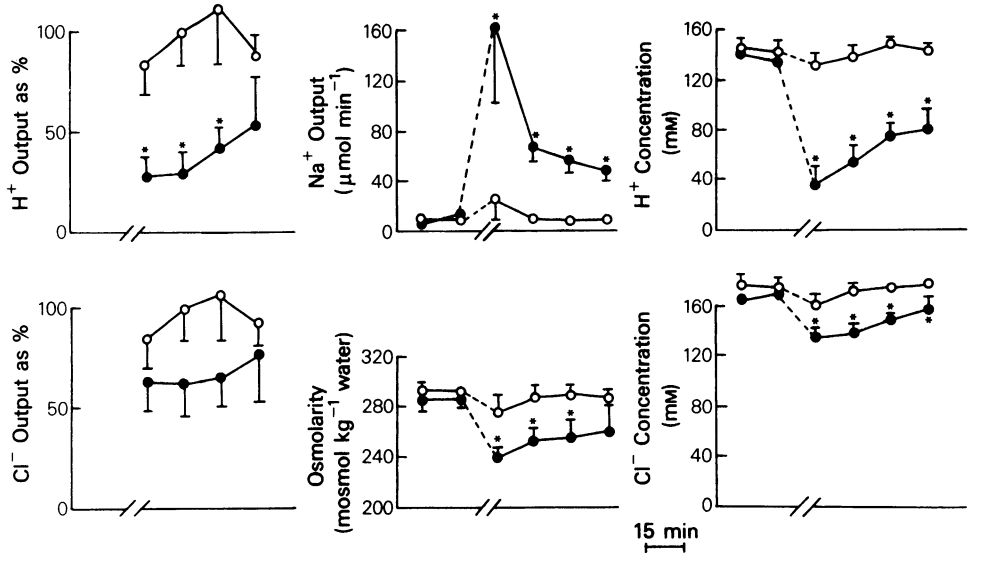

Figure 2 The mean values with s.e.mean ($n = 5$) of results from conscious dogs with gastric fistulae during continuous i.v. infusion of histamine ($30\ \mu g\ kg^{-1}\ h^{-1}$). The break in the time axis indicates closure of the fistulae for 28 min during which time either 40 ml water (○) or 40 ml of FPL 52694 ($4.35\ mg\ ml^{-1}$; ●) was instilled into the stomach as 4 × 10 ml aliquots. The outputs of H^+ and Cl^- after reopening the fistulae are shown as a percentage of the average value obtained during the two 15 min periods prior to closure. Other parameters show these 2 periods plus the four 15 min periods after reopening the fistulae. Where no error bar is shown it lies within the point. *indicates a significant difference ($P < 0.05$).

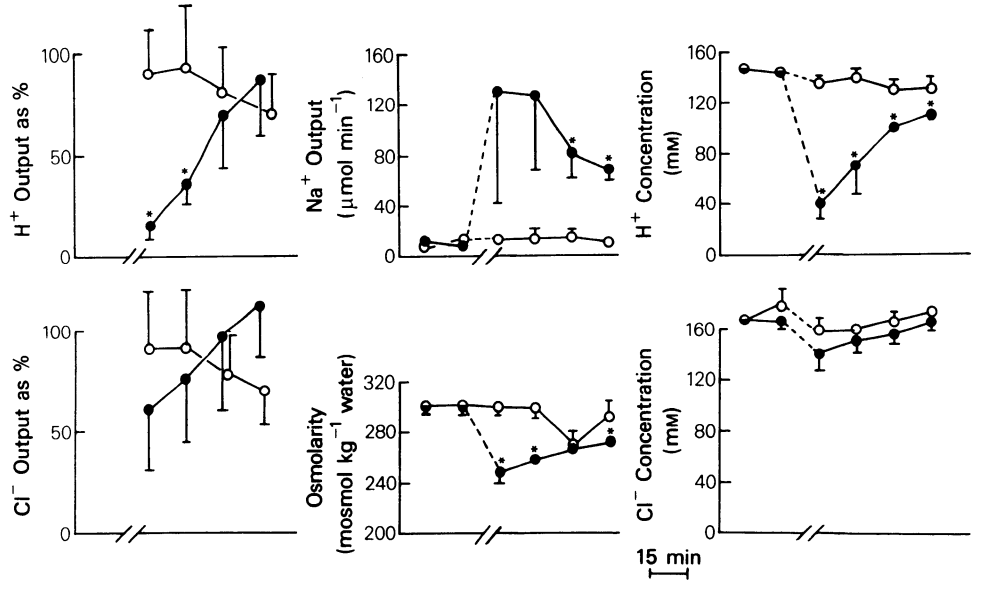

Figure 3 The mean values with s.e.mean of results from conscious dogs with gastric fistulae during continuous i.v. infusion of insulin ($0.1\ u\ kg^{-1}\ h^{-1}$). The break in the time axis indicates closure of the fistulae for 28 min during which time either 40 ml water (○, $n = 3$) or 40 ml FPL 52694 ($4.35\ mg\ ml^{-1}$; $n = 4$; ●) was instilled into the stomach. The output of H^+ and Cl^- after reopening the fistulae are shown as a percentage of the average values obtained during the two 15 min periods prior to closure. Other parameters show these two periods plus the four 15 min periods after reopening the fistulae. Where no error bar is shown it lies within the point. *indicates a significant difference ($P < 0.05$).

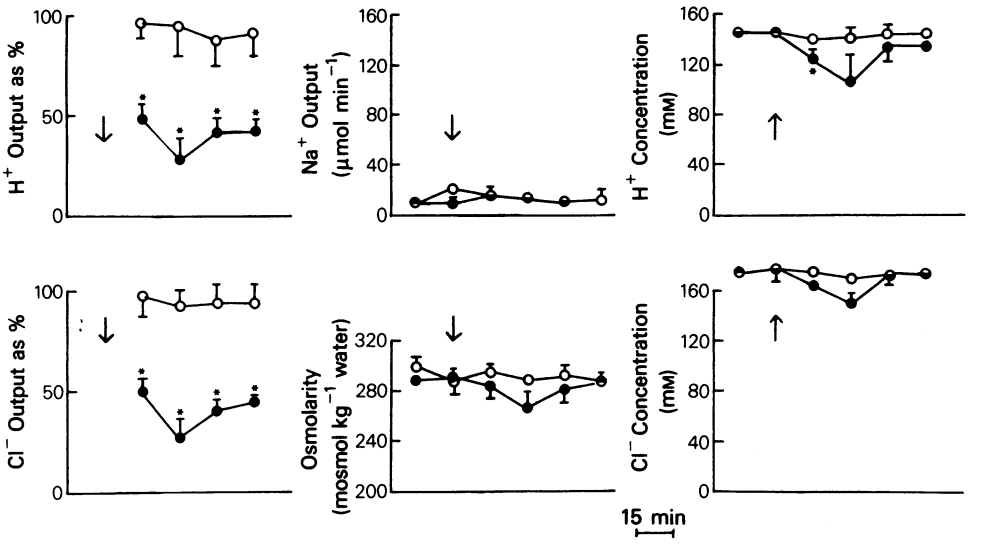

Figure 4 The mean values with s.e.mean ($n = 5$) of results from conscious dogs with gastric fistulae during continuous i.v. infusion of pentagastrin ($2\,\mu g\,kg^{-1}\,h^{-1}$). Open symbols (○) control values and closed symbols (●) following i.v. injection of cimetidine ($4\,\mu mol\,kg^{-1}$) at time indicated by arrow. The outputs of H^+ and Cl^- after cimetidine injection are shown as a percentage of the average value obtained during the two 15 min periods prior to injection. Other parameters show these two periods plus the four 15 min periods after injection. *indicates a significant difference ($P < 0.05$).

non-specific inhibition of acid output in this preparation. Experiments were performed where pentagastrin-stimulated secretion, in the same dogs used in the FPL 52694 study, was inhibited by the histamine H_2-receptor antagonist cimetidine ($4\,\mu mol\,kg^{-1}$ i.v.). The mean values before cimetidine were: H^+ output $311 \pm 12\,\mu mol\,min^{-1}$, Cl^- output $368 \pm 13\,\mu mol\,min^{-1}$ and volume $2.1 \pm 0.1\,ml\,min^{-1}$ (mean ± s.e.mean, $n = 5$ for all). Both H^+ and Cl^- outputs were significantly decreased throughout the 60 min period to a similar extent as shown in Figure 4. There were small reductions of H^+ and Cl^- concentrations and osmolarity and a small increase in Na^+ output following injection of cimetidine. However, only the fall in H^+ concentration at 15 min achieved statistical significance ($P < 0.05$) and this change was not sustained.

Control experiments

In the control experiments 40 ml water was added to the stomach and it might be expected to recover this volume plus a volume similar to that secreted during the 30 min prior to closure of the fistulae. These estimated volumes and the actual volumes recovered on draining the stomachs are shown for pentagastrin and histamine in Table 2. It is more difficult to calculate the expected volumes for the experiments

Table 2 Volumes recovered from the stomachs after closure of the gastric fistulae for the 30 min period

Stimulant	*Control experiments*		*Experiments with FPL 52694*	
	Expected volume (ml)	*Recovered volume* (ml)	*Expected volume* (ml)	*Recovered volume* (ml)
Pentagastrin	112 ± 4	15.6 ± 4.7	68 ± 4.5	57 ± 10.1
Histamine	111 ± 12	36.8 ± 6.0	82 ± 6.6	74 ± 20.5

The results shown are the mean ± s.e.mean ($n = 5$) volumes recovered from the stomachs of conscious dogs equipped with gastric fistulae following closure of the fistulae for 30 min during which time 40 ml of either water or water + FPL 52694 was instilled into the stomach. The expected volumes for control experiments were calculated as the volume secreted by each dog in the 30 min period before closure of the fistulae plus the 40 ml instilled. The expected volumes for the FPL 52694 treatment for each dog were calculated as 40 ml instilled plus twice the volume secreted during the first 15 period after reopening the fistulae.

using FPL 52694 as this depends upon the extent of inhibition of juice output. Volumes have been calculated on the basis of 40 ml plus twice the volume secreted during the first 15 min period following reopening of fistulae. These, together with the actual volumes recovered are also shown in Table 2.

Discussion

Table 1 presents data on the composition of gastric juice from dogs equipped with gastric fistulae during stimulation with three different secretagogues given by intravenous infusion. Similar values for concentrations of H^+ and Cl^- ions, the osmolarity of the juice and the Na^+ output were obtained with each stimulant. The values in Table 1 are comparable with those found by Hirschowitz (1968) and Hirschowitz & Sachs (1969) for gastric fistula dogs which were equipped additionally with oesophageal fistulae to prevent saliva reaching the stomach. This similarity with the values of Hirschowitz (1968) also suggests that there was very little contamination of gastric juice with swallowed saliva in this present study. The results with pentagastrin, histamine and insulin clearly show that FPL 52694 reduced the output of H^+ in the gastric juice. This effect was associated with a very marked fall in the concentration of H^+ ions, a reduction of osmolarity and a marked rise in the rate of Na^+ output into the juice. The reduction of Cl^- output was always less than the reduction of H^+ and in some cases was not statistically significant. This change in the electrolyte composition of the juice following FPL 52694 was qualitatively similar with these three stimulants and characteristically different from that seen when pentagastrin-stimulated secretion was inhibited to a similar average extent with cimetidine (Figure 4). Cimetidine caused a parallel decrease in H^+, Cl^- and volume outputs and was without sustained significant effects on the other parameters. Thus, the observed changes in juice composition following administration of FPL 52694 were not simply due to the inhibition of secretion itself but reflect an action of the drug.

The volumes of fluid recovered from the stomachs when the fistulae were reopened in the control experiments were very much less than might have been expected (Table 2) and in the pentagastrin-stimulated controls some secretory inhibition was apparent. However, this inhibition did not show the characteristic change in juice composition seen with the inhibition following administration of FPL 52694 during pentagastrin-stimulation but was qualitatively similar to the changes seen when inhibition was due to cimetidine. A more general discussion of the problems associated with the use of the gastric fistula preparation for this type of investigation has been previously published (Canfield & Curwain, 1983). The much greater volume of fluid recovered from the stomach after reopening of the fistulae in the FPL 52694 experiments (Table 2) suggest that this compound may also have an effect upon gastric motility leading to delayed gastric emptying under the experimental conditions employed.

The most consistent feature of the action of FPL 52694 was the large increase in Na^+ output with all stimulants. This could be the result of a reflux of alkaline duodenal secretions into the stomach which would cause a reduction of H^+ concentration and increase in Na^+ content. This possibility cannot be entirely excluded from these experiments but a similar reduction of H^+ concentration following FPL 52694 has been reported in anaesthetized dogs where the pylorus had been ligated (Nicol *et al.*, 1981). The appearance of Na^+ in the gastric lumen together with a reduction of H^+ is often indicative of mucosal damage with back diffusion of H^+ ions in exchange for extracellular Na^+. It seems unlikely that this is the correct explanation for the present results as an exchange of Na^+/H^+ would be isosmotic and would not explain the observed reductions in Cl^- concentration and osmolarity. Further, the dogs received FPL 52694 each week for several months and yet showed no signs of gastric bleeding, abdominal tenderness, loss of weight or appetite. An alternative explanation could be that FPL 52694 increases the output of non-parietal cell secretions. Bolton & Cohen (1978) have shown that the non-parietal cell secretion in dogs contain both NaCl and $NaHCO_3$. This would result in a post-secretory neutralization of acid by HCO_3^- which would both reduce the H^+ content and slightly dilute the other constituents of the juice. The Cl^- content of such a secretion might also explain the greater reduction in H^+ output than Cl^- output. A second possibility is that FPL 52694 causes a change in the passive permeability of the gastric mucosa permitting an influx of $NaHCO_3$ from the extracellular fluid into the gastric lumen along the prevailing concentration gradients. In this situation the whole of the increase in Na^+ output would be accompanied by HCO_3^- and this would correspond to a peak rate of about 100 μmol HCO_3^{-1} min^{-1}.

However, it must be stressed that such a HCO_3^- neutralization would only explain a part of the 90% inhibition of pentagastrin-stimulated acid secretion shown in the present results. FPL 52694 must also have a direct inhibitory influence on acid output. It is, therefore, proposed that FPL 52694 applied topically to the mucosa of the conscious dog has two effects, a direct effect inhibiting the output of HCl and a secondary effect which increases the output of $NaHCO_3$. Testing of this hypothesis must await direct measurement of HCO_3^- output under conditions where possible contamination of the juice by

saliva or duodenal contents is excluded. Finally, it is of interest to note that the characteristics of acid secretory inhibition seen with FPL 52694 are not unique. Qualitatively similar results have been obtained in the anaesthetized cat following intragastric sodium fluoride (Bond & Hunt, 1956; Reed & Smy, 1980) and in the conscious fistula dog following inhibitory doses of insulin (Hirschowitz, 1966).

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References

BOLTON, J.P. & COHEN, M.M. (1978). Stimulation of non-parietal cell secretion in canine Heidenhain pouches by 16,16-methyl prostaglandin E_2. *Digestion*, **17**, 291–299.

BOND, A.H. & HUNT, J.N. (1956). The effect of sodium fluoride on the output of some electrolytes from the gastric mucosa of cats. *J. Physiol.*, **133**, 317–329.

CANFIELD, S.P. & CURWAIN, B.P. (1982). Effects of a mast-cell stabilising agent in conscious dogs. *Gut*, **23**, A899.

CANFIELD, S.P. & CURWAIN, B.P. (1983). Inhibition of gastric acid secretion in the conscious dog by the mast cell stabilising agent FPL 52694. *Br. J. Pharmac.*, **80**, 27–32.

DAVIES, H.A., RHODES, J. & THOMAS, M. (1981). Inhibition of gastric acid secretion with a mast cell stabiliser, FPL 52694. *Br. J. clin. Pharmac.*, **1**, 53–56.

HIRSCHOWITZ, B.I. (1966).Characteristics of inhibition of gastric electrolyte secretion by insulin. *Am. J. dig. Dis.*, **11**, 183–198.

HIRSCHOWITZ, B.I. (1968). Histamine dose responses in gastric fistula dogs – decreasing secretion with time. *Gastroenterol.*, **54**, 523–531.

HIRSCHOWITZ, B.I. & SACHS, G. (1969). Pentagastrin in the gastric fistula dog. *Gastroenterol.*, **56**, 456–467.

MAKHLOUF, G.M., McMANUS, J.P.A. & CARD, W.I. (1966). A quantitative statement of the two-component hypothesis of gastric secretion. *Gastroenterol.*, **51**, 149–171.

NICOL, A.K., THOMAS, M. & WILSON, J. (1981). Inhibition of gastric acid secretion by sodium cromoglycate and FPL 52694. *J. Pharm. Pharmac.*, **33**, 554–556.

REED, J.D. & SMY, J.R. (1980). The effects of sodium fluoride on gastric acid and electrolyte output in the anaesthetised cat. *J. Physiol.*, **301**, 39–48.

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