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β-Adrenoceptor agonist stimulation of acid secretion by rat stomach *in vitro* is mediated by 'atypical' β-adrenoceptors

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1 A previous study showed β-adrenoceptor agonists stimulated acid secretion by rat stomach *in vitro*. The receptors could not be classed as either the β_1- or β_2-subtype. This study examines the effect of 2 'atypical' β-agonists on acid secretion.

2 Basal and isoprenaline-stimulated acid secretion were compared in tissues bathed either in HEPES/O_2- or HCO_3^-/CO_2-buffer. Basal secretion was underestimated in HCO_3^- by an amount equal to the rate of base section. Tissues responded well in HEPES buffer and there was no base secretion following acid inhibition with SCH 28080. HEPES was used for the study.

3 SR 58611A stimulated acid in a concentration-related way (0.1–5 μM). Maximum response at 1 μM was equal to the response to a maximal concentration of isoprenaline. BRL 37344 (1 μM) also stimulated to the same extent.

4 Responses to isoprenaline (5 μM) and SR 58611A (1 μM) were reduced by propranolol (10 μM) but not by alprenolol (10 μM) or by practolol (12.5 μM) plus ICI 118551 (1 μM).

5 Exposure to SR 58611A (1 μM) led to desensitization to isoprenaline but not to bethanechol (1 μM) or histamine (50 μM).

6 We conclude that a HEPES/O_2-buffer is advantageous when measuring gastric acid secretion *in vitro* and the stimulatory effect of β-adrenoceptor agonists is mediated by 'atypical' receptors.

Keywords: Gastric acid secretion; 'atypical' β-adrenoceptors; stomach *in vitro*; 'atypical' β-agonists; SR 58611A; BRL 37344; propranolol; alprenolol; SCH 28080

Introduction

Isoprenaline stimulates acid secretion in the rat stomach *in vitro* by an action at β-adrenoceptors. These responses were antagonized by non-selective but not by selective β-antagonists (Canfield & Price, 1981) suggesting that the receptors could not be designated as belonging to either the β_1- or β_2-subtypes. A similar lack of effect of selective β-receptor antagonists has been reported in some adipocytes (Hollenga & Zaagsma, 1989; Hollenga *et al.*, 1991; Carpene *et al.*, 1991) and in various gastrointestinal muscle preparations (Coleman *et al.*, 1987; Bianchetti & Manara, 1990; MacDonald *et al.*, 1990) and ascribed to the presence of 'atypical' β-adrenoceptors. These responded to selective 'atypical' agonists such as BRL 37344 and SR 58611A (Guidice *et al.*, 1989; McLaughlin & MacDonald, 1990). We now report the effects of these two 'atypical' agonists on acid secretion by rat isolated stomach.

Methods

Preparation

Stomachs were taken from male Wistar rats (30–50 g) which had been kept with a lactating female and set up as a flat sheet tied over a plastic tube with the mucosa facing the tube lumen as described previously (Canfield & Price, 1981). The mucosal surface was bathed with 5 ml of solution containing (in mM): NaCl 136, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.4 and glucose 11.7, gassed with 100% O_2. The tube was suspended in a bath containing 30 ml of a similar solution in which either 26 mM $NaHCO_3$ or 10 mM HEPES replaced an equivalent amount of NaCl and maintained at 36°C. The HCO_3^--containing saline was gassed with 95% O_2/5% CO_2 and the HEPES with 100% O_2; both having a pH of 7.4.

Measurement of secretion

The mucosal saline was changed every 15 min throughout the experiment and acid secretion assessed by back-titration with 5 mM NaOH by use of an autotitrator system (ABU 80, Radiometer, Copenhagen). During titration at room temperature the solution was continually gassed with 95% O_2/5% CO_2. The titration end point was determined as that pH obtained when a sample of mucosal saline taken directly from the stock reservoir was similarly gassed and was rechecked several times during the experiment. The method was validated by adding known amounts of HCl to 5 ml of mucosal saline, equivalent to the amounts produced by the tissues. A linear regression of the amount of HCl estimated by titration on the amount added gave a correlation coefficient of 0.9952 ($n = 6$) with a slope of 0.860. This technique is analogous to that used previously to measure bicarbonate secretion (Canfield, 1991) and was adopted because some parts of the study required sequential measurement of both acid and base secretion in the same tissues (with 5 ml HCl titrant).

Expression of results

Acid secretion is expressed either as $\mu mol\ cm^{-2}\ h^{-1}$ or as the secretory ratio = (plateau response to drug/preceeding basal secretion) as used in the earlier study (Canfield & Price, 1981) and values are mean ± s.e.mean with n as the number of tissues. Means were compared by either the paired or unpaired t test as appropriate and values of $P < 0.05$ were taken as indicating a significant difference between means.

Materials

The following were obtained from Sigma (Poole, Dorset); isoprenaline, propranolol and alprenolol. Other compounds were supplied as gifts for which we are grateful: practolol, ICI 118551 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopro-

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pylaminobutan-2-ol; ICI Pharmaceuticals, Cheshire), BRL 37344 (sodium-4[2[2-hydroxy-2(3-chlorphenyl)ethylamino] propyl]phenoxyacetate; SmithKline Beecham, Great Burgh), SR 58611A (N-[(2R)-7-hydroxy-1,2,3,4-tetrahydronapth-2-yl]-(2R)-2-hydroxy-2-(3-chlorphenyl) ethanol hydrochloride; Sanofi Recherche, Milan, Italy), SCH 28080 (2-methyl-8-(phenylmethoxy)imidazol[1,2-a]pyridine-3-acetonitrile; Kirby-Warrick Pharmaceuticals, Bury St Edmunds, Suffolk). Drugs were made up freshly in saline (except SCH 28080 which was dissolved in ethanol) as required and added to the serosal side of the preparation.

Results

Choice of serosal buffer

In previous studies from this laboratory using this preparation the serosal solution was always buffered with HCO_3^-/CO_2. It is likely that acid output was underestimated as a result of being partly neutralised by bicarbonate secreted by surface cells of the stomach (Flemström, 1987). To avoid this possibility we have used a HEPES/O_2 buffered serosal saline in the present study. It was therefore necessary to compare the preparation in this buffer with previous work in HCO_3^-/CO_2. Basal secretion of acid in the HEPES-buffered tissues was well maintained; 1 h into the experiment it was 1.70 ± 0.80 and at 6 h 1.9 ± 0.13 $\mu mol\ cm^{-2}\ h^{-1}$ ($n = 6$). Addition of the proton pump inhibitor SCH 28080 (50 μM) at the peak of response to isoprenaline (5 μM; 3.75 ± 0.25 $\mu mol\ cm^{-2}\ h^{-1}$) abolished acid secretion in all 6 tissues after 75 min. With HEPES as serosal buffer, there was no net alkaline secretion following complete inhibition of acid output which contrasts with the effect of SCH 28080 in HCO_3^--buffered serosal saline shown in Figure 1 described below. We made the following predictions for the HEPES-buffered tissue compared with HCO_3^--buffered; basal secretion would be higher, the increase in secretion in response to isoprenaline would be the same and consequently the response expressed as secretory ratio would be less. The change in basal acid secretion going from HEPES to HCO_3^- buffer should be the same as the measured rate of alkalinisation following total inhibition of acid output with SCH 28080. These predictions were tested experimentally and the results are shown in Figure 1. Basal acid in HEPES was significantly greater than in HCO_3^- (1.98 ± 0.23 and 0.76 ± 0.07 $\mu mol\ cm^{-2}\ h^{-1}$, $n = 6$). Response to isoprenaline (5 μM) in HEPES was not different from HCO_3^- (1.21 ± 0.15 and 1.57 ± 0.16 $\mu mol\ cm^{-2}\ h^{-1}$) but expressed as the secretory ratio, the HEPES value was lower (1.44 ± 0.17 and 1.95 ± 0.1) due to the greater rate of basal secretion in HEPES.

The change in basal secretion going from HEPES to HCO_3^- (1.22 ± 0.28 $\mu mol\ cm^{-2}\ h^{-1}$) was not different from the rate of alkalinisation measured in the presence of SCH 28080 (1.12 ± 0.12 $\mu mol\ cm^{-2}\ h^{-1}$). Responses to repeated stimulation with isoprenaline were well maintained in the HEPES buffer as shown in Figure 2. All subsequent results refer to studies using the HEPES/O_2-buffered saline.

Effect of SR 58611A

Each stomach was first exposed to isoprenaline (5 μM) for 60 min; this was washed out and when acid had returned to a steady rate (usually 60 min), SR 58611A was added at one of the selected concentrations (0.1–5 μM). When the peak response had been obtained (60–75 min) SR 58611A was washed out and isoprenaline (5 μM) was added again. The concentration-response data for SR 58611A is shown in Figure 3. The maximum response was at 1 μM and the secretory ratio was 1.47 ± 0.03 ($n = 4$) whilst the response to the initial isoprenaline for all tissues used in the curve was 1.46 ± 0.07 ($n = 24$).

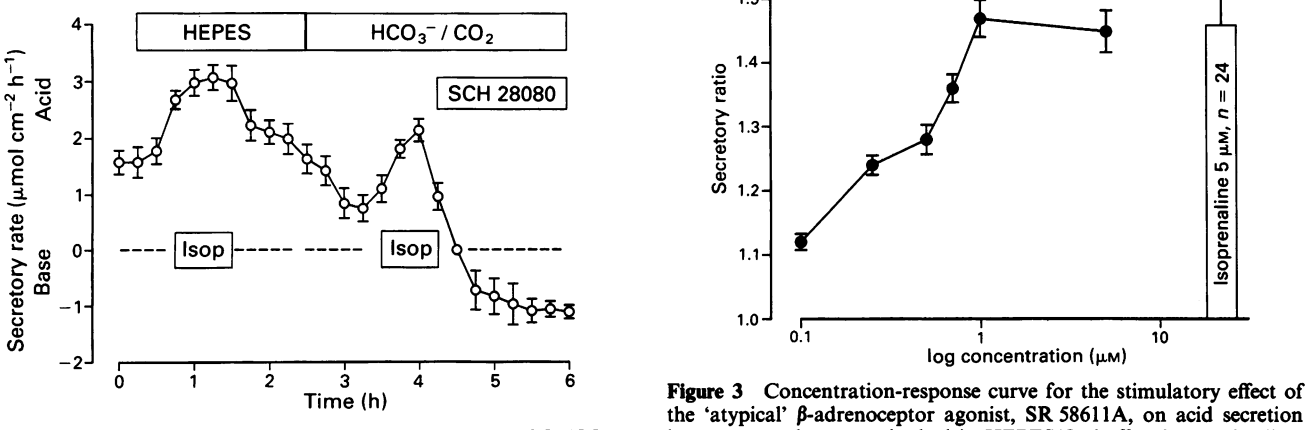

Figure 1 Effect of changing from a HEPES/O_2- to a HCO_3^-/CO_2-buffered (both pH 7.4) serosal saline on basal and isoprenaline (Isop)-stimulated (5 μM) acid secretion by rat stomach *in vitro*. Subsequent addition of the proton pump inhibitor SCH 28080 (50 μM) abolished acid secretion and revealed a net secretion of base (shown as – ve scale). Values are mean with s.e.mean shown by vertical bars, $n = 6$.

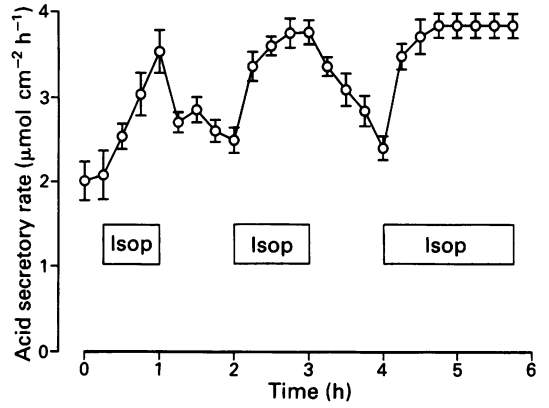

Figure 2 Repeated stimulation of acid secretion with isoprenaline (5 μM) (Isop) in rat stomach bathed with HEPES/O_2 buffered serosal saline *in vitro*. Values are mean with s.e.mean shown by vertical bars, $n = 6$.

Figure 3 Concentration-response curve for the stimulatory effect of the 'atypical' β-adrenoceptor agonist, SR 58611A, on acid secretion by rat stomach *in vitro* bathed in HEPES/O_2 buffered serosal saline. Data shown as secretory ratio (plateau response to drug/preceding basal secretion). Each tissue was first exposed to isoprenaline (5 μM) (Isop) for 60 min and, after washout, to one concentration of SR 58611A (60–75 min). Values are mean with s.e.mean shown by vertical bars, $n = 4$. The mean value for all the initial isoprenaline responses is also shown ($n = 24$) for comparison.

In a separate experiment in HCO_3^-/CO_2-buffered serosal solution containing SCH 28080 (50 μM), the rate of bicarbonate secretion was 1.31 ± 0.09 and, following exposure to SR 58611A (1 μM), was 1.38 ± 0.14 μmol cm^{-2} h^{-1} ($n = 6$) showing that there was no effect of SR 58611A on alkaline secretion. Similar results were obtained with isoprenaline (5 μM, data not shown).

Effect of β-antagonists

The effects of pretreatment of tissues with either propranolol (10 μM), alprenolol (10 μM) or a combination of ICI 118551 (1 μM) plus practolol (12.5 μM) for 60 min was compared with control tissues that had not received antagonist. Tissues were exposed first to isoprenaline (5 μM) and subseqeuntly to SR 58611A (1 μM) as above and the results are shown in Table 1. Only propranolol resulted in a significant inhibition of response to either agonist.

Desensitization following SR 58611A

It was apparent from the initial concentration-response studies that the response to isoprenaline following SR 58611A was much lower than the initial isoprenaline response, a situation not seen with repeated isoprenaline stimulation (Figure 2). We therefore carried out an experiment where all tissues were first exposed to isoprenaline (5 μM), then to SR 58611A (1 μM) as before and finally to either isoprenaline again, bethanechol (1 μM) or histamine (50 μM). The results are shown in Figure 4; SR 58611A significantly reduced the response to the second exposure to isoprenaline but tissues responded well to both histamine and bethanechol.

Discussion

HEPES/O_2-buffered preparations

When the serosal saline was buffered with HCO_3^-/CO_2 in the present study, the maximum response to isoprenaline was the same as in the earlier study (Canfield & Price, 1981). However, the present study differed from the earlier in the introduction of HEPES/O_2 as serosal buffer. HEPES is the preferred buffer as it permits acid secretion to be measured without the complication of serosal bicarbonate being secreted by the surface cells and neutralising part of the acid. With the HCO_3^--buffered system, drug-induced changes in measured acid secretion could be due to either changes in proton pump activity, changes in HCO_3^- flux or a mixture of both. Although an exhaustive comparison of the preparation in the two buffers has not been made, the results suggest that the lack of an exogenous source of CO_2 in the HEPES-buffered tissues does not impair the acid-secretory activity or responsiveness of the tissue. Preparations responded to histamine and bethanechol (Figure 4) as well as the β-adrenoceptor agonists and responses to isoprenaline were well maintained (Figure 1). Additionally, changing from HEPES/O_2 to HCO_3^-/CO_2 buffer containing the proton pump inhibitor SCH 28080 allows easy measurement of both acid and base secretion by the same preparation. The rate of bicarbonate secretion obtained in this way was similar to the value reported with SCH 28080 in guinea-pig stomach (Chiu *et al.*, 1983).

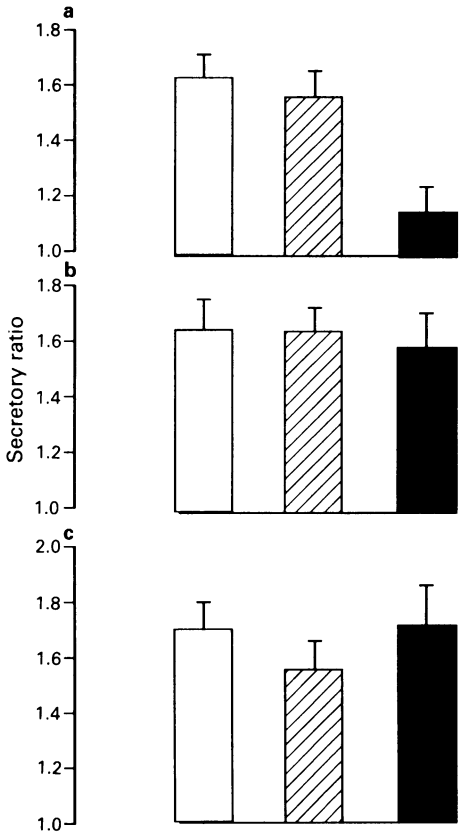

Figure 4 This shows the secretory response as secretory ratio (plateau response to drug/preceding basal secretion) of rat stomach *in vitro* bathed with a HEPES/O_2 buffered serosal saline in response to drug treatments. Three experiments are shown (a, b and c); all tissues were first exposed to isoprenaline for 60 min (5 μM, open columns). This was washed out and all were exposed to the 'atypical' β-adrenoceptor agonist SR 58611A for 60 min (1 μM, hatched columns). Following washout of SR 58611A, tissues were exposed as follows; (a) isoprenaline (5 μM); (b) histamine (50 μM) and (c) bethanechol (1 μM) for 60 min in each case. All values are mean with s.e.mean shown by vertical bars and $n = 6$ in each experiment. The second response to isoprenaline in (a) was significantly lower than the first ($P < 0.05$) in contrast to the situation in Figure 2.

Table 1 Effect of β-adrenoceptor antagonists on acid secretory response to isoprenaline and SR 58611A in rat stomach *in vitro*

Treatment	*Isoprenaline* (5 μM)	*SR 58611A* (1 μM)
Control	1.50 ± 0.02	1.53 ± 0.07
+ Propranolol (10 μM)	1.18 ± 0.03*	1.22 ± 0.06*
+ Alprenolol (10 μM)	1.49 ± 0.08	1.58 ± 0.04
+ Practolol (12.5 μM) + ICI 118551 (1 μM)	1.56 ± 0.05	1.60 ± 0.08

Tissues were preincubated with antagonist (except control) for 60 min before addition of isoprenaline. When response had plateaued, isoprenaline was washed out and when secretion was steady, SR 58611A was added. Values are mean ± s.e.mean of secretory ratio (plateau response/initial basal secretion) and $n = 4$ in each treatment.
*$P < 0.05$ compared with control.

Effects of 'atypical' β-adrenoceptor agonists

The 'atypical' β-adrenoceptor agonists SR 58611A and BRL 37344 were able to stimulate acid secretion to the same maximum extent as isoprenaline. Responses to SR 58611A and isoprenaline were unaffected by the selective β-antagonists but were inhibited by propranolol as expected. The lack of effect of alprenolol was surprising. This has been reported to be particularly effective (but not selective for) 'atypical' β-receptors (Blue *et al.*, 1988; 1990). In addition, in another study in this laboratory in the *in vitro* stimulation of bicarbonate secretion in rat caecum by isoprenaline and SR 58611A, the same alprenolol stock solution inhibited the responses at 10 μM (Canfield & Abdul-Ghaffar, 1992). This may indicate that the 'atypical' β-adrenoceptors in the two tissues are not identical but further studies will be necessary to substantiate this, preferably with a selective antagonist.

The lack of effect of either isoprenaline or SR 58611A on gastric bicarbonate secretion is consistent with the reported lack of effect of β-adrenoceptor agonists on this activity (Flemström, 1987) and contrasts with their stimulation of bicarbonate secretion in rat duodenum (White & Canfield, 1985) and caecum (Abdul-Ghaffar & Canfield, 1990) and bullfrog duodenum (Garner *et al.*, 1984).

Desensitization following SR 58611A

Following the first application of SR 58611A, tissues subsequently showed a very reduced response to either a second application or to isoprenaline but did respond to bethanechol or histamine. It is not possible to say that responses to bethanechol or histamine were totally unaffected by the previous exposure SR 58611A as we do not have repeat control data for these drugs in HEPES/O_2 but the size of responses was comparable with the earlier study (Canfield & Price, 1981). These findings suggest that homologous desensitization of the adrenoceptors may be occurring (Lefkowitz *et al.*, 1990). BRL 37344 also reduced the response to subsequent application of isoprenaline (data not shown). BRL 37344 has been reported to cause desensitization in gastro-intestinal muscle preparations (Coleman *et al.*, 1987; McLaughlin & MacDonald, 1990) and SR 58611A has been used only as a single concentration in each tissue (Guidice *et al.*, 1989). We have also found similar results indicating desensitization following application of these 'atypical' agonists on bicarbonate secretion in rat caecum (Canfield & Abdul-Ghaffar, 1992). Desensitization may thus be a feature of the action of currently available 'atypical' β-agonists.

In summary, the previously reported *in vitro* stimulation of rat gastric acid secretion by β-adrenoceptor agonists (Canfield & Price, 1981) appears to be mediated by so called 'atypical' β-receptors.

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