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Effects of SCH 32651 on resting and stimulated acid secretion in guinea-pig isolated fundic mucosa

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1 Effects of SCH 32651, a novel antisecretory and cytoprotective agent, on resting and stimulated acid secretion by the guinea-pig isolated fundic mucosa were studied.

2 SCH 32651 inhibited resting acid secretion in proportion to concentrations in serosal solution (0.1–10 μM), the IC_{50} being 4.4 μM. Cimetidine and atropine at concentrations up to 100 μM were inactive.

3 Serosal application of SCH 32651 inhibited acid secretory responses to histamine (10 μM), methacholine (1 μM) or dibutyryl cyclic AMP (0.5 mM) plus theophylline (1 mM) in a concentration-dependent manner. The IC_{50}s against histamine, methacholine and db cyclic AMP plus theophylline were 4.2 μM, 0.71 μM and 2.9 μM, respectively. In contrast, atropine and cimetidine each at 100 μM, a concentration that entirely abolished responses to methacholine and histamine, respectively, did not affect acid responses to db cyclic AMP plus theophylline.

4 The inhibitory effects of SCH 32651 on resting and histamine-stimulated acid secretion were readily reversible upon washing.

5 SCH 32651 0.1 mM in the mucosal solution also greatly suppressed the resting and stimulated acid secretion.

6 In the presence of histamine treatment, SCH 32651 concomitantly caused a marked rise in K^+ entry into the mucosal solution in parallel to a decline in the appearance of H^+ in the same solution.

7 The various events demonstrated by SCH 32651 in the present study are shared by omeprazole, a potent antisecretory agent working through inhibition of gastric H^+/K^+-ATPase. We conclude that SCH 32651 as a potent antisecretory agent seems to act directly on the parietal cell, near or at the site of H^+/K^+-ATPase which is a final step in the acid secretory process triggered by various stimuli.

Introduction

SCH 32651 (3-amino-2-methyl-8-phenylmethoxyimidazo [1,2-a] pyrazine HCl 1/3 H_2O; Figure 1) is an orally effective novel anti-ulcer compound with both antisecretory and cytoprotective properties in rats and dogs (Chiu *et al.*, unpublished observations). The pharmacology of a prototype compound, SCH 28080, in animals (Chiu *et al.*, 1983; Long *et al.*, 1983) and in man (Ene *et al.*, 1982) has been reported.

SCH 32651 effectively inhibited gastric acid secretion in rats with pylorus ligation and in dogs receiving stimuli such as histamine, dimaprit, pentagastrin and feeding. The present study was undertaken to elucidate further the mechanism of its antisecretory action in the guinea-pig isolated fundic mucosa. This *in vitro* preparation has frequently been used in physiological and pharmacological studies of acid secretion (Holton & Spencer, 1976; Sjöstrand *et al.*, 1977; Kirkegaard *et al.*, 1982; Chiu *et al.*, 1983; Wallmark *et*

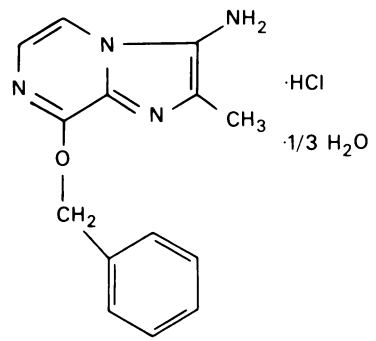

Figure 1 The chemical structure of SCH 32651 (3-amino- 2- methyl- 8- phenylmethoxyimidazo [1,2-a] pyrazine hydrochloride 1/3 hydrate). Empirical formula: $C_{14}H_{14}N_4OHCl$ 1/3 H_2O; mol. wt.: 296.76.

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al., 1983) without complications due to vascular, neuronal and hormonal influences.

The antagonistic effects of SCH 32651 were tested against three inducers of acid secretion, namely histamine (H_1 plus H_2-receptor agonist), methacholine (cholinergic) and dibutyryl (db) cyclic AMP (intracellular messenger).

Methods

The method used was similar to that previously described (Holton & Spencer, 1976; Chiu *et al.*, 1983). Male guinea-pigs (Hartley strain; Charles River Breeding Laboratories, Inc., Wilmington, MA) (250–350 g) were fasted for 24 h with water *ad libitum* before the experiments.

The animals were stunned by a blow to the head and killed by cervical dislocation. The stomachs were quickly excised, opened along the lesser curvature and rinsed with mucosal bathing solution at room temperature (25 °C). The composition of this solution was (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 149, SO_4^{2-} 1.2 and glucose 16. The outer muscle layer was removed by blistering with a slow continuous stream of air through a 24 gauge needle until a section of 2 to 3 cm in diameter could be stripped. The tissue as a sheet of mucosal membrane was then placed over the end of a glass tube (2.0 cm^2 surface area exposed), mucosal side facing inward, and secured with no. 1 surgical silk. The excess tissue was trimmed. In most cases the fundic portion of each stomach yielded two pieces of acid-secreting mucosal preparation, each of which was placed in an organ bath (SGA Scientific Inc., Bloomfield, NJ) containing 50 ml of serosal aqueous buffer solution of the following ionic composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, HCO_3^- 24.9, SO_4^{2-} 1.2, HPO_4^{2-} 1.2 and glucose 16. Temperature of the organ bath was kept at 35 °C and the serosal chamber was aerated with a mixture of 95% O_2 plus 5% CO_2. Five millilitres of mucosal solution, which had been continuously aerated with 100% O_2 to prevent CO_2 accumulation, was immediately added to the mucosal chamber. The latter was adjusted to keep the mucosal and serosal solutions at the same level.

Effects of serosal application

The mucosal solution was collected and replenished with fresh buffer at 30 min intervals. Each experiment consisted of 12 periods of 30 min each.
They were: periods 1–2—stabilization; 3—first measurement for resting acid secretion; 4–6—serosal buffer replaced with buffer with or without agonist (histamine, methacholine, or db cyclic AMP plus theophyline); average acid secretion during periods 5 and 6 taken as the initial response; 7—washed with fresh serosal buffer; 8–9—replenished with plain buffer or buffer containing antagonist (SCH 32651, cimetidine or atropine) in the serosal chamber; 9 taken as the second measurement for resting acid secretion; 10–12—replenished with fresh serosal buffer containing agonist with or without antagonist; average value during periods 11 and 12 taken as the second response.

Acid production was determined from collected samples by titration with 0.01 M NaOH using the Radiometer titration equipment (Copenhagen, Denmark).

Reversibility of the antisecretory action of SCH 32651

The experimental procedure as indicated in Figure 6 differed from that described above in that during periods 4–6 the isolated fundic mucosa was in contact with a serosal buffer containing histamine alone or with a combination of histamine and SCH 32651, that the serosal side was washed with fresh solution at periods 7–9 and that during periods 10–12 the preparation was again challenged with histamine.

Effects of mucosal application

The experimental procedure differed from those depicted above only in that SCH 32651 was added to the mucosal solution instead of the serosal solution. The mucosal solution containing the dissolved SCH 32651 was titrated to pH 7.0 first with 0.1 N NaOH and then with 0.01 N NaOH while being gassed with 100% O_2.

Concomitant measurements of H^+ and K^+ output in mucosal solution of the histamine-stimulated fundic mucosa

The preparation was set-up as described above. The entire experiment was divided into 9 periods of 30 min each, including: periods 1–2—stabilization; 3—resting; 4–9—histamine 10 μM in serosal buffer, superimposed with control or SCH 32651 at 10 μM over periods 6–9. Starting with period 3, a 3.5–4 ml aliquot of the mucosal solution was removed for acid titration, the remainder was measured for K^+ concentration on a flame photometer. The H^+ and K^+ output in the mucosal solution was calculated from the measured volume and respective ion concentration of the individual samples.

Statistical analysis

All values are expressed as mean ± s.e.mean. Dunnett's analysis of variance and Student's *t* test for paired or unpaired comparisons were employed to ascertain significant ($P < 0.05$) differences from con-

trol. A least-square analysis to yield the best-fit straight line of log drug concentration vs. response was performed to obtain IC_{50} (compound concentration giving 50% inhibition of resting or stimulated acid secretion) by using the Statistical Analysis System (SAS) computer program (Helwig & Council, 1979).

Drugs

The following were used: histamine phosphate (Fisher); methacholine (mecholyl chloride, MSD); dibutyryl cyclic AMP (Sigma); theophylline (Sigma); cimetidine (SK & F); atropine sulphate monohydrate (Schwarz/Mann). SCH 32651 HC1 1/3 hydrate has a white solid appearance, with a mealting point of 121.5–122.5°C and water solubility of $>30\ mg\ ml^{-1}$. All concentrations refer to free base weight.

Results

Effects of serosal application of SCH 32651 on resting acid secretion

In six fundic mucosal preparations without any treatment, the mean resting acid production decreased moderately from 3.18 ± 0.40 (period 3, see Methods) to 2.08 ± 0.32 (period 9) $\mu Eq\ H^+\ h^{-1}\ cm^2$ over an interval of 4·5 h ($P < 0.05$, Student's paired t test). Other preparations undergoing various treatments yielded similar resting values during corresponding time periods. Effects of serosal application of SCH 32651, cimetidine and atropine are summarized in Figure 2. When the period 9 acid secretion values are compared between the untreated and antagonist-treated groups, SCH 32651 caused inhibition of resting acid secretion in proportion to the concentration (3–10 μM) present in the serosal buffer. The IC_{50} was 4.4 μM. By contrast, cimetidine (1–100 μM) and atropine (0.001–100 μM) were without effect ($P > 0.05$).

Effects of serosal application of SCH 32651 on stimulated acid secretion

Each fundic mucosal preparation underwent two challenges with individual agonists (see Methods). The first response was used only as an indicator of viability. Acid secretion in response to a second serosal application of histamine, methacholine and db cyclic AMP (plus theophylline), respectively, was used to evaluate effects of individual antagonists. The concentration of each agonist was selected to produce comparable increases in acid secretion and was tested against different concentrations of the individual antagonist (Chiu *et al.*, 1983).

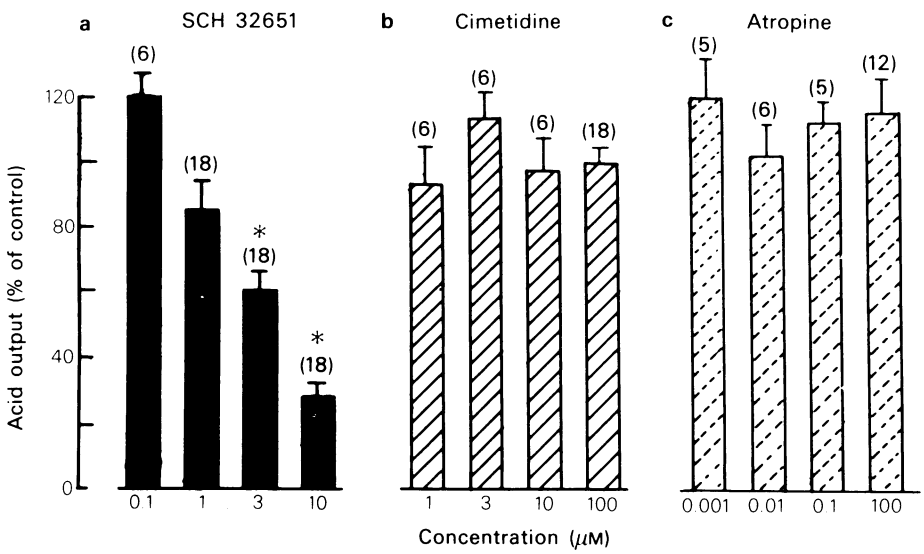

Figure 2 Comparative effects of (a) SCH 32651, (b) cimetidine and (c) atropine on resting acid secretion in guinea-pig isolated fundic mucosa. The period 9 acid secretion (see Methods), in which the preparations were exposed to plain buffer or individual antagonists, was compared between the untreated control and treated groups. Changes are expressed in terms of % of control resting acid secretion, which was $2.15 \pm 0.10\ \mu Eq\ H^+\ h^{-1}\ cm^2$ ($n = 39$). Individual columns represent the mean of (n) experiments as indicated above each column; vertical lines represent s.e.mean; the asterisks indicate significant ($P < 0.05$, Dunnet's Analysis of Variance) difference from control.

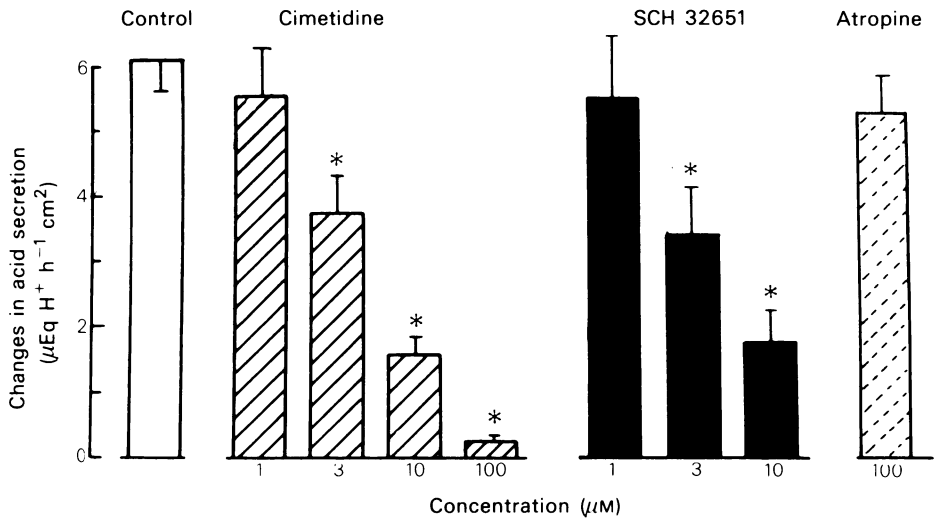

Figure 3 Inhibition of acid secretory responses to histamine by serosal applications of SCH 32651, cimetidine or atropine in guinea-pig isolated fundic mucosa. Acid secretory responses to histamine (10 μM, serosal application) were computed from the difference of average acid secretion (in μEq H^+ h^{-1} cm^2) between periods 11 plus 12 (histamine plus antagonist) and period 9 (antagonist alone) (see Methods). Each column represents the mean of six experiments except the control ($n = 8$); vertical lines are s.e.mean. The asterisks indicate significant difference from control ($P < 0.05$, Dunnett's Analysis of Variance).

Histamine SCH 32651 (1–10 μM) or cimetidine (1–100 μM) inhibited the acid secretory responses to histamine at 10 μM in a concentration-related fashion (Figure 3). The IC_{50}s, estimated from linear regression analysis, were 4.2 μM for SCH 32651 and 5.8 μM for cimetidine. Thus the antisecretory potency of both drugs is similar, as demonstrated previously in the pyloric-ligated rat and the Heidenhain pouch dog preparations stimulated by histamine (Chiu *et al.*, unpublished observations). Atropine was inactive against the effect of histamine in the guinea-pig fundic mucosa.

Methacholine Atropine was ineffective at 1 nM but caused 91 and 99% inhibition, at 0.01 and 0.1 μM of the secretory responses to methacholine at 1 μM, respectively (Figure 4). SCH 32651 significantly suppressed methacholine responses at a concentration

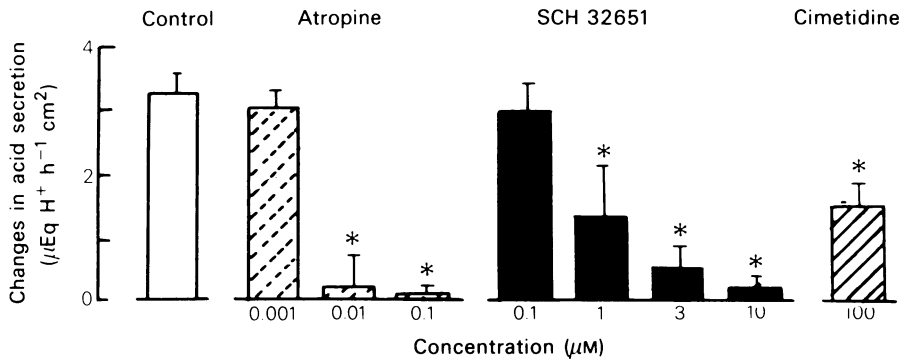

Figure 4 Inhibition of acid secretory response to methacholine by serosal application of SCH 32651, cimetidine or atropine in guinea-pig isolated fundic mucosa. Acid secretory responses to methacholine (1 μM, serosal application) were computed from the difference of average acid secretion (in μEq H^+ h^{-1} cm^2) between periods 11 plus 12 (methacholine plus antagonist) and period 9 (antagonist alone) (see Methods). Each column represents the mean of six experiments except the control ($n = 8$) and two atropine groups (0.001 and 0.1 μM, $n = 5$ each); vertical lines show s.e.mean. The asterisks indicate significant difference from control ($P < 0.05$, Dunnett's Analysis of Variance).

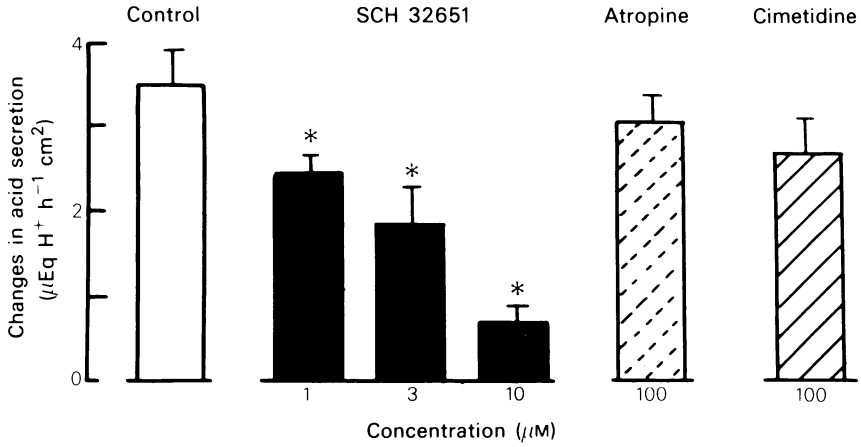

Figure 5 Inhibition of acid secretory responses to dibutyryl cyclic AMP by serosal application of SCH 32651, cimetidine or atropine in guinea-pig isolated fundic mucosa. Acid secretory response to db cyclic AMP (0.5 mM, serosal application) plus theophylline (1 mM) were computed from the difference of average acid secretion (in μEq H^+ h^{-1} cm^2) between periods 11 plus 12 (agonist plus antagonist) and period 9 (antagonist alone) (see Methods). Each column represents the mean of six experiments except the control ($n = 8$); vertical lines are s.e.mean. The asterisks indicate significant difference from control ($P < 0.05$, Dunnett's Analysis of Variance).

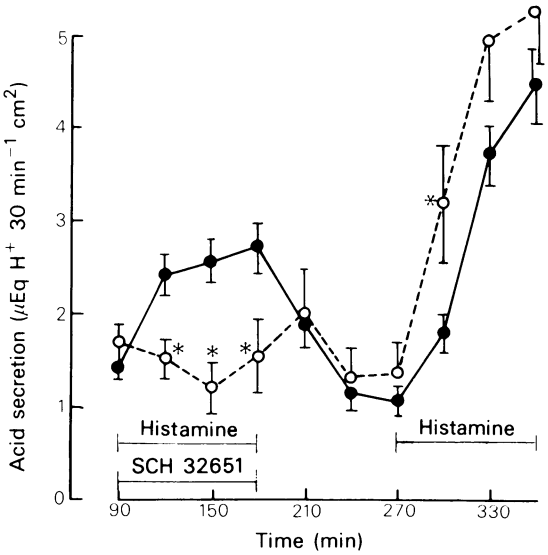

Figure 6 Reversibility of the inhibitory effect of SCH 32651 on histamine-stimulated acid secretion in the guinea-pig isolated fundic mucosa. Histamine (10 μM) and SCH 32651 (10 μM) were added to the serosal solution over the time interval as indicated by the horizontal bars. The time refers to the time interval elapsed after the preparation was set up (time zero). Each point represents the mean of eight values for the control (●) and of six values for the SCH 32651 (○) treated group; vertical lines show s.e.mean. The asterisks indicate significant difference from control ($P < 0.05$, Dunnett's Analysis of Variance).

range between 1 and 10 μM. The IC_{50}s for atropine and SCH 32651 were 4.2 nM and 0.71 μM, respectively, indicating that SCH 32651 is 169 fold less potent than atropine against methacholine. Cimetidine 0.1 μM caused a significant inhibition of the methacholine response, probably due to a non-specific effect (Soll, 1978a). Alternatively, the results are suggestive of interactions between histamine and methacholine on acid secretion (Soll, 1978b).

Dibutyryl (db) cyclic AMP plus theophylline Atropine and cimetidine each at 0.1 mM, a concentration that totally abolished responses to methacholine and histamine, respectively, did not affect secretory responses to the combination of db cyclic AMP (0.5 mM) and theophylline (1 mM) (Figure 5). By contrast, SCH 32651 (1–10 μM) antagonized db cyclic AMP responses in proportion to the concentrations used; $IC_{50} = 2.9$ μM.

Reversibility of SCH 32651 action

The reversibility of the inhibitory effect of SCH 32651 on acid secretion is demonstrated in Figure 6. In the control, the initial secretory response to histamine 10 μM in the serosal buffer was rapidly terminated upon washing. The second application of histamine yielded more acid secretion than the first. In this regard, Main & Pearce (1978) have previously shown, in rat isolated gastric mucosa, that second and subsequent responses to histamine were larger than the first response. With SCH 32651 10 μM in the

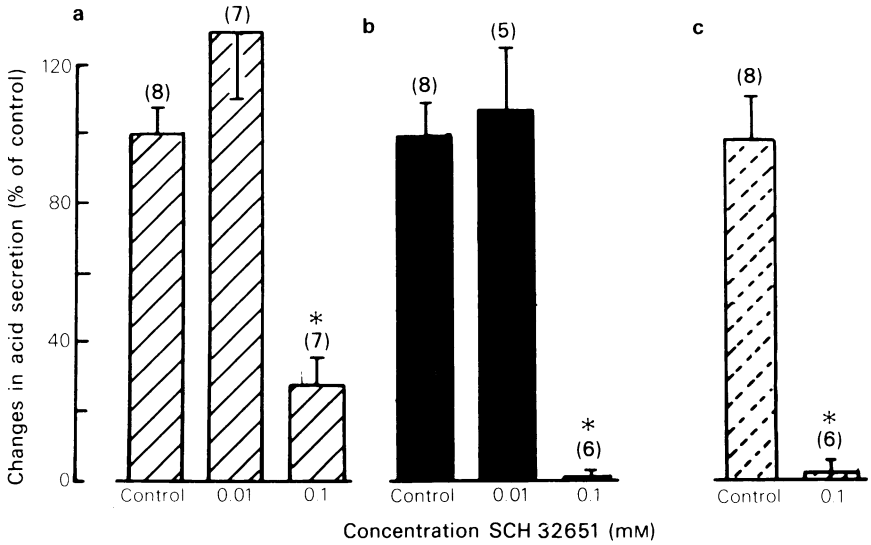

Figure 7 Inhibition of acid secretory responses to (a) histamine (10 μM), (b) methacholine (1 μM) or (c) dibutyryl cyclic AMP (0.5 mM) plus theophylline (1 mM) by SCH 32651 added to the mucosal solution of the guinea pig isolated fundic mucosa. Acid secretory responses to serosal application of agonists were computed from the difference of average acid secretion between periods 11 plus 12 (agonist plus SCH 32651) and period 9 (SCH 32651 alone) (see Methods). Each column represents the mean of (*n*) experiments as indicated, as % of the control; vertical lines are s.e.mean. Control responses (in μEq H^+ h^{-1} cm^2) to histamine (10 μM), methacholine (1 μM) and combination of db cyclic AMP (0.5 mM) and theophylline (1 mM) were 6.12 ± 0.51, 3.21 ± 0.31 and 3.52 ± 0.42, respectively. The asterisks indicate significant difference from control ($P < 0.05$, Dunnett's Analysis of Variance).

serosal buffer, histamine failed to elicit increases in H^+ output. Upon removal of SCH 32651, however, the resting acid secretion was promptly restored to control levels and the subsequent secretory response to histamine was similar to the control.

Effects of the mucosal application of SCH 32651 on resting and stimulated acid secretion

The period 9 acid secretion values (see Methods) were compared between the untreated and drug-treated groups to determine effects of the mucosal application of individual antagonists on resting acid secretion. SCH 32651 had no significant effect on the resting acid secretion at 10 μM (1.91 ± 0.13 μEq H^+ h^{-1} cm^2, $n = 12$, vs. a control value of 2.15 ± 0.15 μEq h^{-1} cm^2, $n = 39$) but caused a 74% decrease at 0.1 mM (0.56 ± 0.06 μEq H^+ h^{-1} cm^2, $n = 19$; $P < 0.05$).

Similarly, SCH 32651 10 μM in the mucosal solution did not affect acid secretion stimulated by serosal histamine (10 μM) or methacholine (1 μM) but at 0.1 mM produced marked inhibition (Figure 7). SCH 32651 0.1 mM in the mucosal solution almost entirely abolished the secretory responses to the combination of db cyclic AMP (0.5 mM) and theophylline (1 mM).

Effect of SCH 32651 on K^+ transport

The ability of SCH 32651 to inhibit acid secretion induced by histamine, methacholine and db cyclic AMP, a property in common with omeprazole, which is a potent inhibitor of the H^+/K^+-ATPase (Wallmark *et al.*, 1983), suggested that SCH 32651 acts beyond the adenylate cyclase-cyclic AMP system and presumably near or at the site of the H^+/K^+-ATPase.Wallmark *et al.* (1983) demonstrated that suppression of histamine-induced H^+ secretion by omeprazole was associated with an elevation of the mucosal K^+ in the guinea-pig fundic mucosa. Therefore, the assumption that SCH 32651 is an inhibitor of H^+/K^+-ATPase was further tested by determining its effect on the mucosal K^+ transport.

As shown in Figure 8, an increase in H^+ secretion occurred promptly upon the introduction of histamine (10 μM) into the serosal solution and was abolished when SCH 32651 (10 μM) was added. Concomitantly SCH 32651 caused a marked rise in the appearance of K^+ in the mucosal solution in parallel to the fall in mucosal H^+. By comparison, the control secretion of H^+ and K^+ in non-stimulated fundic tissues over the same 3 h period was relatively stable. Thus SCH 32651 produced reciprocal

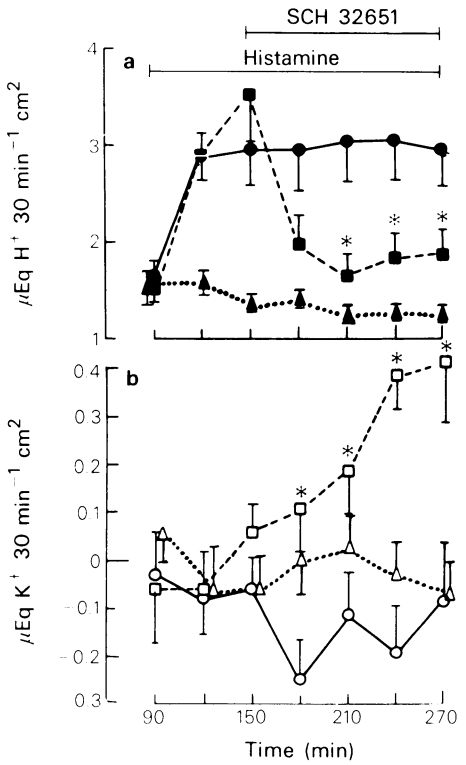

Figure 8 Time course of changes of (a) H^+ and (b) K^+ in mucosal solution of control and the histamine-stimulated fundic mucosa following SCH 32651. Both histamine and SCH 32651 were added to the serosal solution over the time interval indicated by the horizontal bars. The time refers to the time interval elapsed after the preparation was set up (time zero). Each point represents the mean of nine values and vertical lines show s.e.mean. (▲, △) Tissues that did not receive any treatments; (●, ○) histamine stimulation only; (■, □) histamine stimulation superimposed on SCH 32651 treatment. The asterisks indicate significant difference from histamine control ($P < 0.05$, Dunnett's Analysis of Variance).

changes in the output of H^+ and K^+, an effect most likely due to inhibition of gastric H^+/K^+-ATPase.

Discussion

We have demonstrated that SCH 32651 potently inhibited the resting and stimulated acid secretion in the guinea-pig isolated fundic mucosa, thus indicating a direct effect on acid-secreting cells without the need of vascular, neuronal or hormonal mediation.

SCH 32651 differs from cimetidine (histamine H_2-receptor antagonist), atropine (anticholinergic) and prostaglandin (Main & Pearce, 1978; Soll, 1980; Boughton-Smith & Whittle, 1981) in that it effectively abolishes the acid secretory responses by the fundic mucosa to the combination of db cyclic AMP and theophylline. This finding suggests that the site of action for SCH 32651 is distal to the adenylate cyclase-cyclic AMP system and presumably near or at the site of H^+/K^+-ATPase, which is localized at the apical and tubulovesical membranes of the parietal cell (Olbe *et al.*, 1979; Fellenius *et al.*, 1981; Ray & Fromm, 1981). The enzyme is believed to be the final effector pump mediating the acid secretory process. Moreover, the rapid onset and the high reversibility of its action, in conjunction with its ability to affect the basal secretion by mucosal or serosal application, provide additional support for the suggestion that SCH 32651 acts at a superficial site on the membrane, most likely the H^+/K^+ exchange pump (H^+/K^+-ATPase) (Wallmark *et al.*, 1983).

The inhibitory effects of SCH 32651 on the resting and stimulated acid secretion by the isolated fundic mucosa were readily reversed by washing. This finding corresponds to our results in a separate study that acid secretion in rats returned promptly upon withdrawal of a 14 day treatment with SCH 32651 at 100 mg kg^{-1} (p.o.) ($\times$ 4 ID_{50}; Chiu *et al.*, unpublished observations).

It has been postulated that the ATP-driven H^+/K^+ exchange pump operates through a recycling of K^+ to cytoplasm in exchange for H^+, with Cl^- to provide for the return limb of (lumen to cell) the K^+ circuit to permit net flow of HCl into the gastric lumen (Forte *et al.*, 1980). Inhibition of the H^+/K^+-ATPase would concomitantly impair the K^+-pumping activity of the enzyme and K^+ translocation into the parietal cell. The gastric H^+/K^+-ATPase is sensitive to inhibition by mercurial compounds that react with sulphhydryl groups. Ray & Tague (1980) demonstrated in the chambered fundic mucosa from *Rana catesbeiana* that inhibition of histamine-, pentagastrin- or urecholine-induced H^+ secretion by *p*-chloromercuribenzene sulphonic acid caused an increased efflux of K^+. Furthermore, it was recently shown in the guinea-pig isolated fundic mucosa that during histamine stimulation omeprazole, a potent inhibitor of H^+/K^+-ATPase, increased the net efflux rate of K^+ into the mucosal solution whereas cimetidine and SCN^-, which are inactive against H^+/K^+-ATPase, did not alter the rate of K^+ efflux despite marked inhibition of H^+ secretion (Wallmark *et al.*, 1983). That SCH 32651 displays the same effect on K^+ efflux as omeprazole is consistent with the hypothesis that it acts as an inhibitor of H^+/K^+-ATPase.

In conclusion, SCH 32651, a potent antisecretory agent, appears to act directly on the parietal cell, near or at the site of H^+/K^+-ATPase which is a final step in the secretion of acid by all secretory stimuli.

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