

Effect of substituted benzimidazoles on acid secretion in isolated and enriched guinea pig parietal cells*

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SUMMARY The inhibitory effect of the three benzimidazole derivatives timoprazole, picoprazole, and omeprazole on histamine and dbcAMP stimulated ^{14}C -aminopyrine accumulation ($=\text{H}^+$ secretion) has been studied in isolated and enriched guinea-pig parietal cells. All compounds tested inhibited H^+ secretion in a concentration dependent manner with IC_{50} values of $8.5 \pm 1.9 \mu\text{mol/l}$ for timoprazole, $3.9 \pm 0.7 \mu\text{mol/l}$ for picoprazole, and $0.13 \pm 0.03 \mu\text{mol/l}$ for omeprazole. The IC_{50} of timoprazole, when dbcAMP was used as a stimulus, did not differ significantly from that of histamine stimulation. The type of inhibition was of a non-competitive nature. The full acid response to histamine after temporary exposure of the cells to the benzimidazoles could be restored by washing the cells twice; this suggests that the inhibition is reversible. The data – among others – indicate that the properties of the benzimidazoles described here would allow these compounds to be used as effective antisecretagogues.

Substituted benzimidazoles represent a new class of potent inhibitors of gastric acid secretion. Typical representatives of this chemical structure have been shown to block acid secretion in experimental animals¹ and man,² in isolated rabbit gastric glands,³ and in the isolated guinea-pig gastric mucosa.^{4,5} Substituted benzimidazoles have a mechanism of action which is different from the other anti-secretagogues known so far. There is strong evidence that this new class of compounds interferes with the gastric proton pump, the $\text{H}^+ \text{K}^+$ -ATPase,⁶ and thereby inhibits acid secretion.

It is the purpose of the present investigation to study the relative potencies of the three substituted benzimidazoles timoprazole, picoprazole, and omeprazole, in isolated and enriched guinea-pig parietal cells and to elucidate the type and stability of inhibition in this preparation.

Methods

PREPARATION OF PARIETAL CELLS

Guinea-pig isolated parietal cells were essentially prepared and enriched according to Soll.⁷ Normally

fed guinea-pigs of either sex (300–400 g) were killed by a blow on the neck, the abdominal cavity was opened, the stomach excised, opened along the major curvature, rinsed in ice cold buffer (see below), and fixed on a cork plate. The fundic mucosa of two animals per preparation was scraped off and placed in 20 ml albumin free buffer (pH 7.4) solution of 37°C which was subsequently incubated under gentle stirring with 0.5 g/l collagenase for 15 minutes to remove mucus and damaged superficial cells. The pH was kept constant at pH 7.4 by permanent autotitration with 5% NaHCO_3 .

The mucosal fragments were subsequently centrifuged for one minute at $250 \times g$ at room temperature. The supernatant was discarded and the remaining tissue pieces were resuspended in 20 ml buffer containing 1 g/l albumin, 0.5 g/l collagenase, and 0.3 g/l pronase and incubated for a further 80 minutes at 37°C and pH 7.4 under constant gassing with 95% $\text{O}_2/5\% \text{CO}_2$. The incubation mixture was then filtered through a nylon cloth and centrifuged for 10 minutes at $400 \times g$. The supernatant was discarded and the sediment was washed and recentrifuged twice with 30 ml enzyme free but albumin containing buffer at room temperature. The buffer composition was: NaCl 132.4, KCl 5.4, Na_2HPO_4 5, NaH_2PO_4 1, MgSO_4 1.2, CaCl_2 1, and glucose 5.5 mmol/l. Separation of the different cell populations was achieved by zonal centrifugation in the Beckman elutriation system. The

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system was loaded with the total amount of harvested cells. Cells were elutriated at constant speed (1390 rpm) and stepwise increasing flow of 5, 10, 15, and 35 ml/min resulting in an elutriation volume of 180, 250, 350, and 200 ml respectively. The final fraction contained the highest proportion of parietal cells (on average 70–80%) and was used for further processing. This fraction was counted and the proportion of parietal cells determined by size and granulation in a Türk counting chamber using light microscopy. The cells were tested for viability by the Trypan blue exclusion test and were found to be viable in >95%. The other fractions, containing mainly mucus and chief cells, were used for other purposes.

MEASUREMENT OF ACID SECRETION

Acid secretion in these cells was determined by measuring the cellular uptake and accumulation of ^{14}C -aminopyrine. Four hundred microlitres of a cell suspension containing $0.9\text{--}1.4 \times 10^6$ cells/ml and 8.3×10^{-6} mol/l ^{14}C -aminopyrine were incubated for 20 minutes at 37°C . At the end of the incubation period 100 μl of the cell suspension were pipetted as top layer into a polyethylene centrifuge tube partially filled with 50 μl 3N KOH on the bottom, 100 μl of a mixture of equal parts of silicone oil AR 20 and AR 200 in the middle, and 100 μl buffer (pH 7.4) on the top. Centrifugation of the tubes in a Beckman microfuge for 30 seconds brought the cells into KOH leaving behind the suspension medium with the ^{14}C -aminopyrine not taken up. The bottom of the polyethylene tubes containing the cells in KOH were cut off and dropped into a liquid scintillation counting vial which was filled one hour later with 10 ml scintillator (Quickszint^R). After thorough mixing counting was started after another hour. Values are generally expressed as pmol ^{14}C -aminopyrine/ 10^5 parietal cells. In a few cases the data were normalised to maximal stimulation of ^{14}C -aminopyrine-accumulation by a given stimulus.

To study the effect of different drugs on ^{14}C -aminopyrine-accumulation the following protocol was used: 40 minutes before the start of incubation substituted benzimidazoles in the desired concentration were added to the cell suspension; histamine or dbcAMP in the necessary concentration was added 30 minutes later. During the whole 40 minutes the incubation-mixture was kept at room temperature. The final reaction was started by adding ^{14}C -aminopyrine and transferring the test tubes into a shaking water bath kept at 37°C . Concentrations of agonists and antagonists should be taken from the relevant figures and legends. All determinations were made in duplicate. N represents the number of different cell preparations.

WASHOUT EXPERIMENTS

Standardised parietal cell preparations were incubated for 30 minutes at room temperature in the usual buffer containing 25 $\mu\text{mol/l}$ timoprazole, 10 $\mu\text{mol/l}$ picoprazole, 0.25 $\mu\text{mol/l}$ omeprazole, or an adequate volume of buffer as control. The incubation mixture was subsequently centrifuged for five minutes at room temperature and $335 \times g$. The supernatant was removed and replaced by a fresh benzimidazole free buffer. After 10 minutes at room temperature and the second identical centrifugation step and replacement of fresh buffer, the cells were counted, identified, and tested for viability. Adequate aliquots (400 μl containing $0.9\text{--}1.4 \times 10^6$ cells/ml) were then added to test tubes containing increasing concentrations ($10^{-7}\text{--}10^{-3}$ mol/l) histamine and kept for 10 minutes at room temperature before ^{14}C -aminopyrine was added and the incubation at 37°C started as described before.

COMPOUNDS

^{14}C -aminopyrine (New England Nuclear Corp, Dreieich) (spec act 60–120 mCi/mmol), bovine serum albumin (lyophilised, >92% pure) (Serva/Heidelberg), collagenase 125 U/mg (Sigma, Munich), pronase E (70,000 PUK/g) (Merck, Darmstadt), silicone oil AR 20 and AR 200 (Wacker-Chemie, Munich), histamine dihydrochloride (Merck, Darmstadt), dibutylryl-A-3:5-MP cyclic, monosodium salt, crystallised (Boehringer, Mannheim), timoprazole (kindly supplied by Professor Klemm, Byk Gulden, Konstanz), picoprazole and omeprazole (kindly supplied by Dr E Carlsson, Hässle, Mölndal, Sweden).

Results

Histamine in the range of $10^{-7}\text{--}10^{-3}$ mol/l stimulated ^{14}C -aminopyrine uptake and accumulation of guinea-pig isolated and enriched parietal cells in a concentration dependent manner with an EC_{50} between 10^{-5} and 10^{-6} mol/l. Maximal stimulation was usually achieved with 10^{-4} or 10^{-3} mol/l. All three substituted benzimidazoles inhibited ^{14}C -aminopyrine-accumulation in response to maximal stimulation by histamine (10^{-3} mol/l) in a concentration dependent manner (Fig. 1). The IC_{50} values are: timoprazole 8.5 ± 1.9 $\mu\text{mol/l}$, picoprazole 3.9 ± 0.7 $\mu\text{mol/l}$, and omeprazole 0.13 ± 0.03 $\mu\text{mol/l}$. Therefore the relative potencies are 1:2:65. Dibutylryl-cAMP in the range of $10^{-5}\text{--}5 \times 10^{-3}$ mol/l also stimulated ^{14}C -aminopyrine-accumulation with an EC_{50} of approximately 2×10^{-4} mol/l. Timoprazole was almost equally effective ($\text{IC}_{50} = 7.9$ $\mu\text{mol/l}$) in inhibiting dibutylryl-cAMP (2×10^{-3} mol/l) stimulated ^{14}C -aminopyrine uptake as that

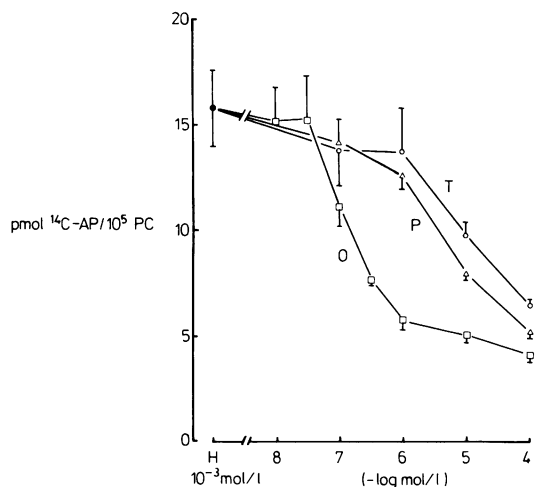


Fig. 1 Effect of increasing concentrations of timoprazole (T), picoprazole (P), and omeprazole (O) on ^{14}C -aminopyrine accumulation in isolated and enriched guinea-pig parietal cells (PC) stimulated by 10^{-3} mol/l histamine. $N = 5$, values $\bar{x} \pm s_x$.

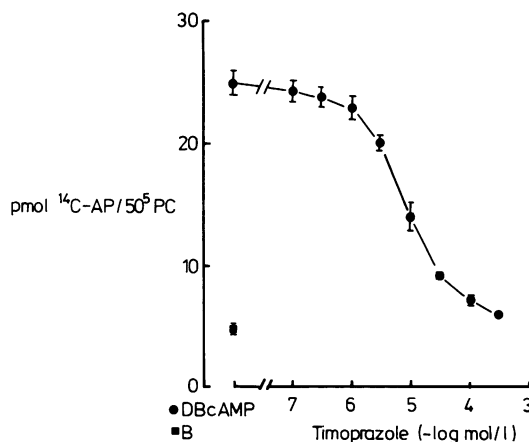


Fig. 2 Effect of an increasing concentration of timoprazole on ^{14}C -aminopyrine-accumulation in isolated and enriched guinea-pig parietal cells (PC) stimulated by 5×10^{-3} mol/l dibutyryl-cAMP (DBcAMP). B = Basal. $N = 4$, values $\bar{x} \pm s_x$.

stimulated by histamine (Fig. 2). Stimulation of ^{14}C -aminopyrine uptake by histamine in the presence of $8 \mu\text{mol/l}$ timoprazole or $2 \mu\text{mol/l}$ picoprazole and the transformation of the concentration response curves according to Eadie⁸ and Hofstee⁹ (not shown) revealed that in either case the inhibition type was of a non-competitive nature (Fig. 3). All compounds could be readily and completely washed out by two washings, as indicated by the restoration of the original concentration response relationship of histamine (Fig. 4).

Discussion

It is generally accepted that an accumulation of ^{14}C -aminopyrine in isolated parietal cells in response to a secretagogue reflects H^+ secretion by these cells. This is because aminopyrine as a weak base freely diffuses across membranes when in an unionised state. Aminopyrine becomes ionised in an acidic environment and so is trapped in an acid compartment when the cells start to secrete H^+ into the intracellular canaliculi. The responsiveness of different cell populations may vary tremendously and this occasionally results in considerable variations between groups of experiments. As the number of cells harvested per preparation is large enough, any drug effect can be related to a control value in the same preparation. Substituted benzimidazoles like the ones studied here have been

shown to be potent inhibitors of gastric acid secretion *in vitro* and *in vivo*.^{1-4,5}

The use of an isolated system consisting almost exclusively of parietal cells allows studies on the relative potencies and the formal type of inhibition of this class of compounds. From previous studies in other laboratories it is known that substituted

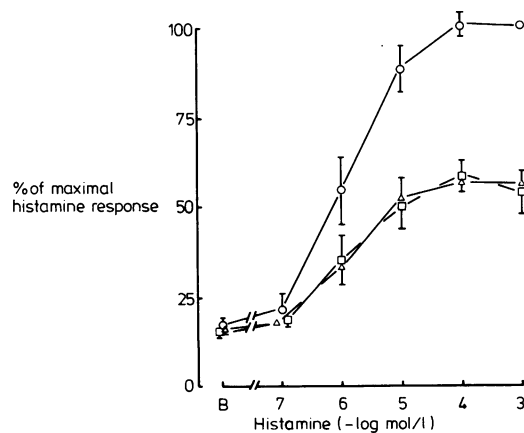


Fig. 3 Effect of $8 \mu\text{mol/l}$ timoprazole (Δ) or $2 \mu\text{mol/l}$ picoprazole (\square) on histamine stimulated (10^{-7} – 10^{-3} mol/l) (\circ) ^{14}C -aminopyrine-accumulation in isolated and enriched guinea-pig parietal cells. B = Basal. $N = 3$, values $\bar{x} \pm s_x$.

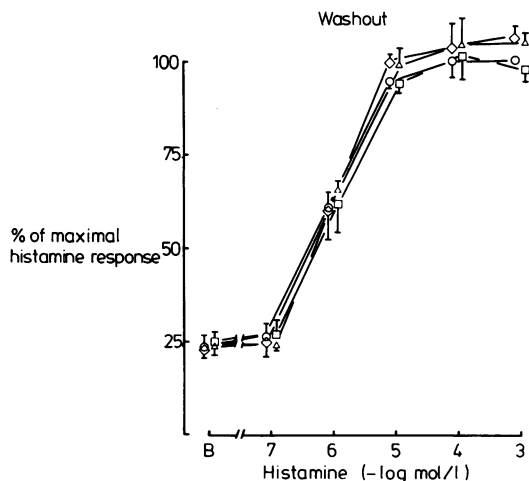


Fig. 4 Stimulation of ^{14}C -aminopyrine-accumulation by 10^{-7} – 10^{-3} mol/l histamine, in isolated and enriched guinea-pig parietal cells (\circ = controls) previously exposed to 25 $\mu\text{mol/l}$ timoprazole (\square), 10 $\mu\text{mol/l}$ picoprazole (\triangle), or 0.25 $\mu\text{mol/l}$ omeprazole (\diamond). B = Basal. $N = 3$, values $\bar{x} \pm s_x$.

benzimidazoles are likely to interfere with the parietal cell proton pump, the K^+/H^+ -ATPase.⁶ The evidence is mainly based upon the finding that, in isolated rabbit glands, substituted benzimidazoles inhibit ^{14}C -aminopyrine-accumulation on three different excitable levels: the histamine H_2 -receptor (stimulation by histamine), the protein kinase (stimulated by dibutyryl-cAMP), and the K^+/H^+ -ATPase as proton pump (stimulated by high K^+). The inhibition is clearly not of competitive nature. The mechanism by which substituted benzimidazoles interact with the proton pump is virtually unknown. Therefore any attempt to discuss the inhibitory mechanism would be purely speculative. It is important to note that the inhibitory action of timoprazole and its congeners is fully reversible after removal of the compounds, as two washings of the cells after previous exposure to substituted benzimidazoles restore the full stimulatory action of histamine. This is in contrast with *in vivo* conditions.¹⁰ It has been demonstrated that in mice after administration of radiolabelled omeprazole the label is detectable in the gastric mucosa for at least 16 hours.

Furthermore, one single dose of timoprazole causes a long-lasting suppression of gastric acid secretion. These discrepancies need to be solved. The sequence of potency in ascending order is: timoprazole < picoprazole < omeprazole, indicating that the principal structure gains in

potency by substitution on the benzimidazole moiety of the molecule. The number of compounds is still too small to allow clear-cut conclusions on structure activity relationships. If it holds true that the only mechanism of action of this class of compounds is an inhibition of the proton pump, then these compounds are potentially useful as anti-ulcer drugs. A K^+ -ATPase indistinguishable from that of the gastric mucosa has also been found in brush border membranes from the rabbit descending colon epithelium.¹¹ It is not known, however, whether substituted benzimidazoles block this enzyme and if so what the consequences are of such a blockade. Furthermore, little is known about side effects of substituted benzimidazoles not related to the principal mechanism of action.

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