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COMMUNICATIONS

In-vitro potencies of histamine H_2-receptor antagonists on tetraethylammonium uptake in rat renal brush-border membrane vesicles

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Abstract—The histamine H_2 antagonists cimetidine, ranitidine and famotidine are organic bases that are cleared from the body by active renal tubular secretion involving the organic cation transporter in the proximal tubule. To determine the potential for competition for the transporter between these drugs and other drugs, their inhibitory potencies were assessed in-vitro, using rat renal brush-border membrane vesicles and tetraethylammonium as the substrate. The concentration-dependent effect of cimetidine, ranitidine and famotidine on the 15-s proton-stimulated uptake of tetraethylammonium into the membrane vesicles was studied using five different rat kidneys. The order of inhibition potencies was: cimetidine (mean IC50 = 1·07 μM) > famotidine (2·43 μM) > ranitidine (55·4 μM). The results indicate the potential for drug interactions in the kidney, especially for cimetidine and famotidine.

The histamine H_2-receptor antagonists are used widely for the treatment of gastrointestinal ulcer disease. The most commonly used agents are cimetidine, ranitidine and famotidine. All are organic bases with pK_a values of 6·8, 8·2 and 6·7, respectively (Fig. 1), and are primarily excreted unchanged in urine (Kirch et al 1984; Lin 1991). Drug-drug interactions have been well described for cimetidine (Somogyi & Muirhead 1987), occur less frequently with ranitidine (Kirch et al 1984) and rarely occur with famotidine (Echizen & Ishizaki 1991). These interactions have been mainly described at the level of inhibition of hepatic oxidative drug metabolism.

Renal clearance is the most important pathway of elimination of the histamine H_2 antagonists and all three compounds are cleared by active renal tubular secretion via the organic cation transporter. Competition for this pathway is a well described phenomenon, and cimetidine, to a lesser extent ranitidine and least of all famotidine, compete with other organic cationic drugs for renal tubular secretion in man (Somogyi & Muirhead 1987; Muirhead et al 1987; Klotz & Kroemer 1991; Echizen & Ishizaki 1991). Animal models have also been used to examine the relative potencies of inhibition of tubular secretion between these three compounds. Using dog kidney brush-border membrane vesicles and N^1-methylnicotinamide as the substrate, cimetidine and famotidine have similar inhibitory potencies with K_i values of 2 and 3 μM, respectively, whereas ranitidine is less potent with a K_i value of 70 μM (Bendayan et al 1990). Muirhead & Somogyi (1991) using the rat isolated perfused-kidney model and triamterene as the substrate, showed that cimetidine and ranitidine had similar inhibitory potencies, whereas famotidine was less potent by a factor of at least two. Wong et al (1991) using rat renal cortical slices and amantadine as the substrate showed that at high cimetidine concentrations, uptake of amantadine was reduced to about the same degree as ranitidine, whereas in renal proximal tubules, cimetidine augmented amantadine, and ranitidine attenuated uptake to a similar degree as in cortical slices. Those authors speculated that the site of interaction for ranitidine was on the basolateral membrane and not on the brush-border membrane.

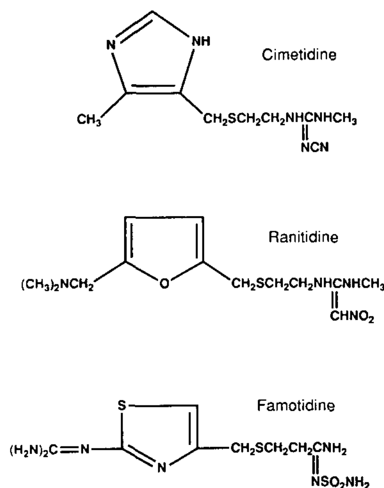

FIG. 1. Structural formulae of cimetidine, ranitidine and famotidine.

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Tetraethylammonium (TEA) is a commonly used probe for investigating in-vitro the uptake of other organic cations at the brush-border membrane of the proximal tubule (Takano et al 1984). Studies using TEA, which has a higher affinity for the organic cation transporter than the other commonly used organic cation substrate N^1-methylnicotinamide, and the H_2 antagonists have not been reported. The aim of the present study was to characterize the in-vitro potency of the three histamine H_2 antagonists cimetidine, ranitidine and famotidine on the uptake of TEA in rat renal brush-border membrane vesicles, and to clarify the divergent results so far reported.

Materials and methods

[1-^{14}C]Tetraethylammonium bromide (sp. act. 3·7 mCi $mmol^{-1}$) was obtained from Du Pont—New England Nuclear (Boston, MA, USA). Cimetidine was obtained from Sigma Chemical Company (St Louis, MO, USA), ranitidine HCl from Glaxo Australia Pty Ltd (Boronia, Australia) and famotidine from Merck Sharpe and Dohme (Australia) Pty Ltd (Granville, Australia). Tris(hydroxymethyl)aminomethane HCl (Tris HCl), ethylene glycolbis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), *N'*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulphonic acid (HEPES), 2-(*N*-morpholino)ethanesulphonic acid (MES) were obtained from Sigma Chemical Company. All other reagents were of the highest purity available. Deionized water (Milli-Q: Millipore Corporation, Bedford, MA, USA) was used throughout.

The study was approved by the Animal Ethics Committee of the University of Adelaide. The method for the preparation of brush-border membrane vesicles was that described by Gross & Somogyi (unpublished) based on the method of Biber et al (1981). Briefly, the renal cortex (1-2 g) of 3-month-old male

Hooded Wistar rats was finely sliced and homogenized in buffer (2·5 mM EGTA, 6 mM Tris HCl, 150 mM mannitol) for 2 min. After addition of $MgCl_2$ and stirring for 15 min at 4°C, the homogenate was centrifuged for 15 min at 2450 *g* and 0°C. The supernatant was centrifuged and the pellet of brush-border membranes resuspended in the buffer. The magnesium precipitation and centrifugation steps were repeated and the brush-border membranes were resuspended in an intravesicular pH 6·0 buffer (100 mM mannitol, 10 mM MES, 100 mM KCl).

Protein concentration and alkaline phosphatase activity were determined by the method of Lowry et al (1951) and a commercial kit (Sigma Diagnostics Procedure 104, Sigma Chemical Company), respectively.

Uptake experiments. The uptake of [^{14}C]TEA (250 μM) across the brush-border membrane vesicle preparation was studied by rapid filtration using a 1225 Sampling Manifold (Millipore Corporation) attached to a constant source of vacuum. Vesicle incubations were performed using 85 × 10 mm borosilicate glass tubes maintained in a water bath at 25°C. Vesicles (20 μL containing between 90 and 200 μg protein) suspended in pH 6·0 buffer were equilibrated for 10 min at 25°C. The experiment was initiated by adding 90 μL pH 7·4 buffer (100 mM D-mannitol, 10 mM HEPES, 100 mM KCl) containing [^{14}C]TEA and cimetidine, ranitidine, famotidine or *p*-aminohippuric acid (as a negative control) (equilibrated at 25°C). At predefined times, the incubations were halted by adding 3 mL ice-cold buffer. The tube contents were immediately poured onto pre-wetted 0·45 micron filters (Millipore Corporation) and the filters washed with a further two 3-mL aliquots of ice-cold buffer. The filters were air-dried, solubilized in 10 mL scintillant (Ready Value, Beckman Instruments Inc.) and the radioactivity associated with the filters determined by liquid scintillation counting (LS 3801 Liquid Scintillation System, Beckman Instruments Inc.), using the external standard channels-ratio technique. In parallel with each experimental run, the nonspecific binding of [^{14}C]TEA was determined by adding 3 mL pH 7·4 buffer to 90 μL [^{14}C]TEA/buffer solution and filtering as described above. Radioactivity associated with the vesicle experiments was corrected for the nonspecific [^{14}C]TEA binding to the filter. For each membrane preparation, all experiments were performed in triplicate.

Influence of the H_2 antagonists. The effect of cimetidine, ranitidine and famotidine on the 15-s proton-stimulated (pH_{in} 6·0:pH_{out} 7·4) uptake of [^{14}C]TEA (250 μM) was investigated in brush-border membrane vesicles from five rats. The final concentrations of cimetidine ranged from 0·25 to 25 μM, ranitidine from 20 to 250 μM and famotidine from 0·5 to 25 μM. Three or four concentrations of each inhibitor were used. The uptake of [^{14}C]TEA was calculated (% control) relative to the control uptake in the absence of the three compounds.

Statistics. The concentration of H_2 antagonist causing 50% inhibition of TEA uptake (IC50) was calculated from the regression of percentage uptake vs logarithm of concentration. Comparison of this variable amongst the three compounds was assessed using the non-parametric Friedman two-way analysis of variance and a multiple comparison test (Daniel 1978). Data are given as mean and s.d.

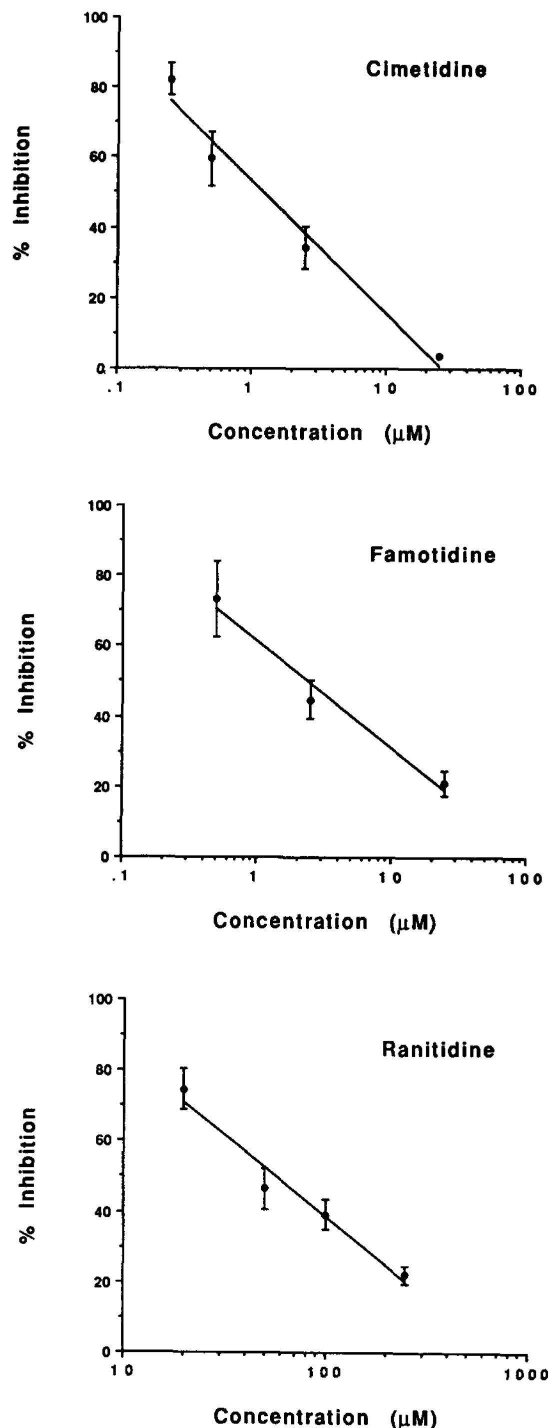

FIG. 2. Inhibition of TEA uptake in rat renal brush-border membrane vesicles by the H_2 antagonists cimetidine, famotidine and ranitidine.

Results

Alkaline phosphatase activity was enriched 5·9- to 9·2-fold (median = 7·0) in the brush-border membrane vesicles relative to the initial kidney homogenate. Fig. 2 summarizes the inhibitory effect of the three compounds on TEA uptake. There were significant ($P < 0·01$) differences between cimetidine, ranitidine and famotidine in inhibition of TEA uptake. Cimetidine was the most potent inhibitor (IC50 = 1·07 ± 0·27 μM), with famotidine (IC50 = 2·43 ± 0·70 μM) next and ranitidine (IC50 = 55·4 ± 9·7 μM) least. In all five experiments, *p*-aminohippuric acid (90 μM) had no significant ($P > 0·05$) effect on TEA uptake (105 ± 11% of control).

Discussion

The results from this study indicate that cimetidine and famotidine are potent inhibitors of TEA uptake into rat renal brush-border membrane vesicles with IC50 values in the low micromolar range. In contrast, ranitidine was more than an order of magnitude less potent. These data are similar to those reported using N^1-methylnicotinamide in dog kidney brush-border membrane vesicles (Bendayan et al 1990), indicating a lack of interspecies and substrate difference in the uptake process. Evidence from the investigators' laboratory indicates that a heterocyclic nitrogen confers high potency of inhibition of TEA uptake. Both cimetidine and famotidine share this common chemical structural property; in contrast, ranitidine contains a heterocyclic oxygen atom. However, the data are dissimilar to the more intact preparation studies. In the rat isolated perfused kidney, cimetidine and ranitidine were found to be equipotent, but famotidine was less potent (Muirhead & Somogyi 1991). Wong et al (1991) found no effect of cimetidine but that ranitidine inhibited amantadine uptake into isolated proximal tubules. Those authors speculated on a basolateral site of uptake inhibition of ranitidine and studies are being conducted in this laboratory to characterize the action of these compounds on this membrane. The divergent results could also be explained by the existence of subsystems of the organic cation transporter in the kidney.

Evidence for this has been accruing over the past few years. In in-vivo studies in rats, it has been shown that cimetidine at high concentrations (112–250 mg L^{-1}) inhibited the tubular secretion of famotidine, whereas the reverse did not occur even at very high famotidine concentrations (50–94 g L^{-1}) and low cimetidine concentrations of 1·2–3·8 mg L^{-1} (Lin et al 1988). Furthermore, famotidine at low doses inhibited the tubular secretion of TEA. Those authors suggested that their data provided evidence for subsystems of the renal organic cation transporter. Using rabbit renal brush-border membrane vesicles (Miyamoto et al 1989), the organic cation guanidine showed high and low affinity for uptake into the membrane. Differential inhibition of uptake by other organic cations such as cimetidine and choline was also observed, although the effect was not specific. It would thus appear that compounds could have different affinities for various transporters in the kidney. Whether similar phenomena occur in man is not known.

Clinically, it is likely that the reason why cimetidine and not famotidine inhibits the tubular secretion and hence the renal clearance of other organic cation drugs is that sufficiently high concentrations are achieved in plasma to approach or exceed its K_i value. In contrast, famotidine plasma concentrations are lower than cimetidine and are likely not to approach its K_i value with normal clinical doses. A different scenario could occur following overdose.

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