Gastric mucosal blood flow and acid secretory changes in man with impromidine: a new specific histamine-H₂-receptor agonist

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SUMMARY The effect of impromidine, a new histamine-H₂-receptor agonist, on gastric mucosal blood flow (neutral red clearance) and acid secretion was studied in nine volunteers. Impromidine stimulated a dose-dependent increase in neutral red clearance and acid secretion. Simultaneous cimetidine in three doses caused a parallel shift to the right for both acid output and clearance with unchanged maxima compatible with simple surmountable antagonism. There were small cardiovascular changes: an increased heart rate with a decreased diastolic pressure during infusion of impromidine. These changes were antagonised by cimetidine.

The involvement of histamine in the control of gastric acid secretion was supported by the discovery of the specific inhibitors of H₂-receptors on the parietal cell.¹ Studies in animals have also shown that the gastric vasculature is sensitive to histamine through a combination of H₁- and H₂-receptors although with different relative contributions.^{2,3} In man histamine stimulates a dose-dependent increase in both acid secretion and mucosal blood flow but the blood flow component is relatively resistant to inhibition by cimetidine.⁴ A specific histamine-H₂-receptor agonist would be of value in the investigation of the role of histamine in the control of gastric acid secretion and blood flow in both healthy man and in patients with peptic ulcer disease. Recent studies have indicated that impromidine is a potent stimulant of gastric secretion in man with relatively fewer side-effects than histamine.^{5,6} The purpose of the present investigation was to study the stimulation of acid secretion and blood flow in healthy volunteers using increasing doses of impromidine and to compare these effects with our previous results using histamine.⁴

Methods

In the study group there were nine volunteers, mean age 22 years (range 21-26 years). The study

was approved by the local ethical committee and informed written consent was obtained from each volunteer in accordance with the Declaration of Helsinki.⁷ Gastric mucosal blood flow was measured by the neutral red clearance technique.⁸

GASTRIC FUNCTION TESTS AND ESTIMATION OF MUCOSAL BLOOD FLOW

After an overnight fast each volunteer was intubated with a double lumen nasogastric tube (Sherwood 12-14 French gauge), the position being determined by a water recovery test.⁹ Overnight secretion was aspirated and discarded. A 15 minute basal sample was collected as a reference for neutral red standards (see below). Winged needles were inserted into a vein in each forearm; one for drug infusion and the other for blood sampling. A 15 ml venous sample was taken for processing of neutral red standards in whole blood and then a loading dose of neutral red (NR, 125 µg/kg) was given followed by a constant infusion (250 µg/kg per h). Blood samples were taken at 15 minute intervals for 45 minutes and then at 30 minute intervals thereafter. After a basal period (45 minutes) gastric acid secretion was stimulated by an infusion of impromidine. Dose-response curves were constructed on two separate days at least one week apart. On day one, impromidine was given in four sequential doses (1.25, 2.5, 5.0, and 10 µg/kg per h) each for one hour (Fig. 1). Two volunteers received impromi-

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Fig. 1 Mean ($\pm$ S.E.M.) acid output in volunteers during infusion of graded doses of impromidine (1.25–20 $\mu\text{g/kg per h}$). Basal secretion (B) $54.4 \pm 13.3 \mu\text{mol/min}$ has been subtracted. The theoretical maximum was calculated to be $530 \pm 60.5 \mu\text{mol/min}$ and is shown by the dotted line.

dine in the dose range 2.5–20 $\mu\text{g/kg per h}$. On day two, the test was repeated with cimetidine (0.25, 0.5, or 1.0 mg/kg) which was randomised among three groups of the volunteers (three, three, and two volunteers respectively). The infusion of cimetidine was begun 30 minutes before impromidine (dose-range 5–80 $\mu\text{g/kg per h}$).

Gastric juice was collected every 10 minutes. The volume of the aspirate was recorded and the concentration of H^+ measured by titration of 3 ml gastric juice to pH 7.0 with 0.1M NaOH using an automatic titrator (Radiometer, Copenhagen). Acid secretion was expressed as $\mu\text{mol H}^+/\text{min}$. NR concentration was determined in 3 ml blood and 1 ml gastric juice after alkalisation and extraction into diethyl ether (10 ml Analar grade, BDH Ltd) with further extraction into 0.1M HCl. The aqueous layer was read in a spectrophotometer (Pye-Unicam SP 1800) at 540 nm against serial standards of NR made up in whole blood or gastric juice and processed along with the samples. Neutral red clearance (NRC) in ml/min was calculated from the equation $\text{NCR} = (\text{NR})_g \times V / (\text{NR})_b$, where $(\text{NR})_g$ and $(\text{NR})_b$ are gastric and whole blood concentrations of NR and V is the volume of gastric aspirate in ml/min.

Dose-response curves were constructed using the mean of the last two 10 minute periods at each dose level of impromidine. The data were used to fit non-linear curves by the method of least squares¹⁰ so as to calculate a best fit maximum for acid secretion and NCR as well as obtaining calculated values for the dose of impromidine producing a half-maximal response

Fig. 2 Mean ($\pm$ S.E.M.) neutral red clearance in the same volunteers as in Fig. 1. Basal NRC (B) $20 \pm 4.3 \text{ ml/min}$ has been subtracted. The calculated maximum was $56 \pm 9.6 \text{ ml/min}$ and is shown by the dotted line.

(ED_{50}) and an inhibitory dose₅₀ (ID_{50}) for cimetidine. Standard errors for these calculations were also derived from the least squares fit.

Blood pressure and heart rate were measured electronically by inflating a cuff around the arm over the brachial artery (Digital 8000, Ueda Electronics Ltd) and changes were analysed by means of Student's paired *t* test.

Results

IMPROMIDINE DOSE-RESPONSE STUDIES

Impromidine (1.25–10 $\mu\text{g/kg per h}$) caused a dose-dependent increase in both acid output and NRC (Figs 1 and 2). In the two volunteers who received an infusion of impromidine 20 $\mu\text{g/kg per h}$, this dose caused a small increase in acid output above that induced by 10 $\mu\text{g/kg per h}$, but NRC at the high dose began to decrease. The best fit curves for these data give the following calculated values (omitting the data for 20 $\mu\text{g/kg per h}$). Maximal acid output was 530 ± 60.5 (SEM) $\mu\text{mol H}^+/\text{min}$ and the ED_{50} $2.3 \pm 0.26 \mu\text{g/kg per h}$. For NRC the calculated maximal rate was $56 \pm 9.6 \text{ ml/min}$ and the ED_{50} $1.7 \pm 0.34 \mu\text{g/kg per h}$.

EFFECT OF CIMETIDINE ON IMPROMIDINE-INDUCED ACID SECRETION AND NRC: COMPARISON WITH PREVIOUS HISTAMINE DATA

In a previous study, cimetidine (0.6 mg/kg per h) reduced acid secretion induced by histamine but had no effect on neutral red clearance.⁴

Fig. 3 The effect of cimetidine given in three doses 0.25 (■), 0.5 (▲), and 1.0 (◆) mg/kg per h on impromidine-induced gastric acid secretion. The calculated ID_{50} for cimetidine inhibition was 0.10 ± 0.014 mg/kg per h.

In the present study cimetidine caused a parallel, dose-dependent displacement of the curves for both acid secretion and NRC to the right (Fig. 3, Table). The data are also tabulated for comparison of the effect of cimetidine on both histamine- and impromidine-induced acid secretion and NRC (Table). On a molar basis impromidine is some five times more potent than histamine on acid output and NRC. The calculated ID_{50} for cimetidine-inhibition of acid secretion was the same whether histamine or impromidine was used as stimulant but the effects on NRC differed.

CARDIOVASCULAR EFFECTS OF IMPROMIDINE

During the infusion of impromidine (5 and 10 μ g/kg per h) there was an increase in pulse rate of 12 ± 3 beats per minute and this was statistically different from the pretreatment periods or the lower doses of impromidine ($P < 0.05$). This increase in pulse rate was accompanied by a

Table Values for dose of agonist producing half maximal rates (ED_{50}) for impromidine and histamine on acid secretion and neutral red clearance (NRC) and inhibitory dose $_{50}$ (ID_{50}) for cimetidine against these agonists

	Impromidine	Histamine
ED_{50} (μ g/kg per h)		
ACID	2.3 ± 0.213	11.1 ± 2.15
NRC	1.7 ± 0.339	7.9 ± 1.27
ID_{50} (mg/kg per h)		
ACID	0.10 ± 0.014	0.3 ± 0.15
NRC	0.10 ± 0.027	No effect†

The data for histamine are taken from Knight *et al.*⁴

†Cimetidine 0.6 mg/kg per h had no effect on the histamine-induced NRC.

Fig. 4 Comparison of the acid secretory and NRC responses during infusion of graded doses of histamine (Δ) and impromidine ($\blacklozenge$). The calculated regression line for histamine was $Y = 0.10X + 19$, $r^2 = 0.61$, $n = 38$ ($P < 0.001$) and for impromidine $Y = 0.14X + 23$, $r^2 = 0.57$, $n = 44$ ($P < 0.001$).

decrease in diastolic pressure of 12 ± 4.1 mm Hg ($P < 0.05$ compared with resting levels). Only small changes in systolic pressure were seen, so that the mean arterial blood pressure fell from 92 ± 3.3 mm Hg to 80 ± 2.3 mm Hg was due entirely to the above changes in diastolic pressure. Concomitant infusion of cimetidine prevented these changes but the cardiovascular responses reappeared at higher doses of impromidine 20, 40, and 80 μ g/kg per h.

Relationship between NRC and acid secretion during impromidine and histamine infusion

There was a significantly linear relationship between neutral red clearance and acid output during impromidine infusion and this is compared with histamine infusion (Fig. 4). The intercepts of the two regression lines were not different but there was a difference in slopes (slope $\pm 95\%$ confidence limits: impromidine 0.14 ± 0.06 and histamine 0.10 ± 0.04 , $P < 0.05$), impromidine producing a higher NRC relative to secretion than histamine.

Discussion

In 1966, Jacobson, Linford, and Grossman¹¹ described the use of aminopyrine clearance for the estimation of gastric mucosal blood flow. Since that time experience with the clearance markers has provided evidence that mucosal blood flow and acid secretion are in some way linked, although this relationship is not entirely clear.¹² In addition, some limitations to the use

of clearance markers have been uncovered (although perhaps not widely recognised by those who use such agents) and have prompted more sceptical interpretations of results.¹³⁻¹⁵ An element of uncertainty remains that the clearance markers may not accurately reflect mucosal blood flow during low secretory rates or when there are rapid changes in flow. It is important to keep this in mind when studies are confined to man and where other methods are not yet available for comparison.^{15 16}

The newest clearance marker, neutral red, has been used in both animals and in man.⁸ In animals during brisk acid secretion blood flow as estimated by neutral red clearance is of the same order as that using other methods.¹² We have used the clearance of neutral red to estimate gastric mucosal blood flow in man during stimulation of acid secretion by the new agent, impromidine, which acts specifically at the histamine- H_2 -receptor. Impromidine increased neutral red clearance in a dose-dependent manner in addition to its previously described effect to increase acid secretion.⁵ As expected, the H_2 -receptor antagonist cimetidine produced a parallel shift to the right of the dose-response curves for both acid secretion and NRC but did not change the maximum. This agrees with our previous studies in the dog in which cimetidine and ranitidine inhibited NRC and acid secretion to both histamine and impromidine.⁸ However, in man, cimetidine had no effect on the NRC response to histamine while reducing the secretory response.⁴ Initially, this suggested to us that the vasculature was less sensitive than the parietal cell to cimetidine and that the unchanged response of the vasculature during infusion of cimetidine was due to residual stimulation of H_1 -receptors by histamine. However, in that study the histamine- H_1 -receptor antagonist, mepyramine, also failed to alter the vascular response to histamine. The study with impromidine and cimetidine reported here indicated that the above suggestion is untenable and that the sensitivity of the vasculature is probably not different from that of the parietal cell, although the relationship between the two is still not completely clear. The effect of histamine on the parietal cell is undoubtedly *via* stimulation of the H_2 -receptor but the response of the vasculature is more complex. The increased blood flow seen with histamine may reflect partly the energy requirements of increased acid secretion and partly a direct interaction with histamine- H_1 and H_2 -receptors on the vasculature. Using more direct methods in

animals the histamine-induced vasodilatation in the mesenteric vascular beds has been described as biphasic and time-dependent—the H_1 -response characterised as immediate but short lived and the H_2 -response as slow in onset and of longer duration.² More difficult to understand is the fact that the H_1 -antagonists can actually increase histamine-induced blood flow and acid secretion in some animals and perhaps in man.¹² Two explanations for this are possible: first an inhibitory action of the H_1 -receptor on the gastric vasculature to restrain blood flow and hence secretion; second, the systemic hypotensive action of histamine which leads to a reduction in splanchnic blood flow may limit the gastric mucosal blood flow and secretory responses to histamine.¹⁷ In either case, inhibition of the H_1 -mediated effects of histamine or use of a specific H_2 -agonist would then allow unfettered blood flow and secretion. In the present study in man a comparison of the blood flow responses to histamine with those of impromidine showed that for the same level of acid secretion histamine-induced blood flow was less. Also in another study in man¹⁸ again using neutral red clearance we found that pentagastrin-induced blood flow was greater than histamine-induced flow and that both cimetidine and ranitidine inhibited these responses to pentagastrin. It may be that blood pressure changes which occur on infusion of histamine in animals and in man but which are less marked with impromidine or pentagastrin^{5, 19-21} could account for the lesser blood flow, but clearly histamine-mediated responses of the vasculature are complex in man. The specific H_2 -receptor agonist, impromidine, has some advantages over histamine as a potent gastric secretagogue in healthy man, producing readily definable acid secretory and vascular responses which can be competitively inhibited by cimetidine. Thus, it may have a place in the routine testing of gastric function in patients with peptic ulcer diseases and will also be useful for the study of mechanisms of gastric acid secretion and mucosal blood flow in these patients.

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