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Mediation by CCK_B receptors of the CCK-evoked hyperaemia in rat gastric mucosa

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1 Cholecystokinin octapeptide (CCK-8) and gastrin-17 augment gastric mucosal blood flow in the rat. The present study examined whether the gastric vasodilator effect of these peptides is mediated by CCK_A or CCK_B receptors.

2 Intravenous injection of CAM-1481 (1 mg kg^{-1}), a dipeptoid antagonist of CCK_A receptors, or CAM-1028, a dipeptoid CCK_B receptor antagonist (1 mg kg^{-1}), had no effect on basal gastric mucosal blood flow as determined by the clearance of hydrogen in urethane-anaesthetized rats.

3 Intravenous infusion of CCK-8 or gastrin-17 (8–200 pmol min^{-1}) increased gastric mucosal blood flow in a dose-dependent fashion. The CCK_B receptor antagonist, CAM-1028, significantly attenuated the hyperaemic response to CCK-8 and gastrin-17 whereas the CCK_A receptor antagonist, CAM-1481, did not antagonize CCK-8 but caused a slight attenuation of the vasodilator response to gastrin-17.

4 The selectivity of the two antagonists was proved by the findings that CAM-1028, but not CAM-1481, inhibited gastric acid secretion evoked by CCK-8 or gastrin-17 (CCK_B receptor assay) while CAM-1481, but not CAM-1028, inhibited the CCK-8-induced contraction of guinea-pig isolated gall bladder strips (CCK_A receptor assay).

5 These data show that the actions of CCK-8 and gastrin-17 to increase mucosal blood flow in the rat stomach are primarily mediated by CCK_B receptors.

Keywords: Cholecystokinin (CCK); cholecystokinin octapeptide (CCK-8); gastrin; gastrin-17; CCK receptors; CCK_A receptors; CCK_B receptors; CCK receptor antagonists; CAM-1028; CAM-1481; gastric mucosal blood flow; vasodilatation; hyperaemia; gastric acid secretion; gall bladder contraction

Introduction

Cholecystokinin (CCK) causes intestinal vasodilatation (Bowen *et al.*, 1975; Fara *et al.*, 1975) and has been implicated as an endocrine messenger of postprandial hyperaemia (Chou *et al.*, 1977). CCK also increases blood flow in the human (Demling & Classen, 1968) and feline (Guth & Smith, 1976) gastric mucosa but the receptors responsible for this effect have not yet been identified. Both CCK_A and CCK_B/gastrin receptors exist in the digestive tract, with CCK_A receptors being present in the pancreas and on the gall bladder and intestinal muscle (Bishop *et al.*, 1992; Gully *et al.*, 1993; Hughes *et al.*, 1993), CCK_B receptors being expressed by gastric enterochromaffin-like and parietal cells (Hughes *et al.*, 1993; Prinz *et al.*, 1993; Soll & Berglindh, 1994) and both CCK_A and CCK_B receptors occurring on vagal nerve fibres (Lin & Miller, 1992; Corp *et al.*, 1993). Apart from its secretory effect which is mediated by CCK_B receptors (Forster *et al.*, 1990), many of the gastric effects of CCK including inhibition of gastric emptying (Forster *et al.*, 1990), inhibition of gastric acid secretion via somatostatin release (Lloyd *et al.*, 1992) and gastric mucosal protection (Evangelista & Maggi, 1991) are predominantly mediated by CCK_A receptors. The systemic haemodynamic alterations caused by CCK are also inhibited by a CCK_A receptor antagonist (Janssen *et al.*, 1991).

In view of this information it was the aim of the current study to investigate whether CCK octapeptide (CCK-8) increases mucosal blood flow in the rat stomach to a similar extent as rat gastrin-17 and whether the gastric vasodilator responses to CCK-8 and gastrin-17 are mediated by CCK_A or CCK_B receptors. This question was addressed by the use of two novel water-soluble dipeptoid compounds, CAM-1481 and CAM-1028, which have been characterized as potent and selective CCK_A (Boden *et al.*, 1993) and CCK_B (Hughes *et al.*, 1990) receptor antagonists, respectively.

Methods

Measurement of gastric mucosal blood flow

All experiments of this study were approved by the Ministry of Science and Research of the Federal Republic of Austria. Female Sprague-Dawley rats, weighing 180–220 g, were fasted for 20 h but were allowed free access to water. After the induction of anaesthesia with urethane (1.5 g kg^{-1} subcutaneously) the rats were placed on a heated table, to maintain their rectal temperature at 37°C, and fitted with a tracheal cannula, to facilitate spontaneous respiration and to allow for the administration of hydrogen. Mean arterial blood pressure was recorded from a cannula in the right carotid artery by means of a pressure transducer (ISOTEC; HSE, March, Germany) and displayed on a chart recorder (SERVOGOR 464; ABB-GOERZ, Vienna, Austria). A second cannula was placed in the left jugular vein for continuous infusion of saline (1.5 ml h^{-1}), to avoid dehydration, and for the intravenous administration of drugs. The stomach was exposed by a midline laparotomy and fitted with an inflow cannula placed in the forestomach and an outflow cannula inserted through the pylorus (Holzer *et al.*, 1991). Saline (kept at room temperature) was perfused through the stomach at a rate of 0.7–0.8 ml min^{-1} throughout the whole experiment.

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Gastric mucosal blood flow was measured with the hydrogen clearance technique (Leung *et al.*, 1984). The washout of hydrogen gas was estimated by a needle-type platinum electrode inserted from the serosa into the basal portion of the gastric corpus mucosa and positioned at the submucosal border of the muscularis mucosae (Holzer *et al.*, 1991). Determination of blood flow was discontinuous, as the experimental protocol involved alternating 15 min periods of saturation and desaturation of the tissue with hydrogen. The current representing the actual hydrogen concentration at the site of the electrode was fed into a personal computer via an A/D converter. The washout curve was then fitted to a monoexponential curve, the power of which was used to calculate the average gastric mucosal blood flow (ml min^{-1} 100 g^{-1}) during the 15 min period of desaturation (Livingston *et al.*, 1989).

The alternating cycle of hydrogen saturation and desaturation was begun after a 45 min period of equilibration (0 min). In the experiments, in which the dose-dependency of the effects of CCK-8 and gastrin-17 on gastric mucosal blood flow was studied, basal gastric mucosal blood flow was determined during the period of 15–30 min, and the effects of CCK-8 and gastrin-17 on blood flow were recorded during the period of 45–60 min. When the antagonistic actions of CAM-1481 and CAM-1028 on the hyperaemic responses to CCK-8 and gastrin-17 were tested, basal gastric mucosal blood flow was determined during the periods of 15–30 min (before the administration of CAM-1481 or CAM-1028) and 45–60 min (after the administration of CAM-1481 or CAM-1028), and the effects of CCK-8 and gastrin-17 on blood flow were recorded during the period 75–90 min.

CAM-1481, CAM-1028 (1 mg kg^{-1}) or their vehicle (saline, 1 ml kg^{-1}) was injected intravenously 15 min before the administration of CCK-8 or gastrin-17 was started. CCK-8 or gastrin-17 was infused intravenously at a rate of 15 μl min^{-1}, the appropriate doses being selected in preliminary experiments. Since the hyperaemic effect of CCK-8 was rapid in onset, it was sufficient to start the infusion of this peptide 5 min before a desaturation curve was recorded. In contrast, gastrin-17 was infused for a period of 15 min until a measurement of gastric mucosal blood flow was taken in order to account for the slow onset of action of this peptide. Only one dose of either CCK-8 or gastrin-17 was examined in each experiment. The gastric mucosal blood flow measured before the perfusion of any peptide was set as 100%, and the hyperaemia caused by CCK-8 or gastrin-17 was expressed as percentage increment of gastric mucosal blood flow (peak values in percent minus 100%).

Measurement of gastric acid secretion

Gastric acid secretion was measured in some experiments in order to test CAM-1481 and CAM-1028 for their activity on gastric CCK_B receptors stimulated by CCK-8 or gastrin-17. The rats used for these experiments were handled as described for the measurement of gastric mucosal blood flow. The gastric effluent was collected in 4 fractions of 15 min, beginning 30 min prior to the administration of CCK-8 or gastrin-17. The first fraction represented basal gastric acid secretion, and the second fraction basal gastric acid secretion measured immediately after intravenous injection of CAM-1481, CAM-1028 (1 mg kg^{-1} each) or their vehicle (saline, 1 ml kg^{-1}). The third and fourth fractions accounted for the acid output stimulated by the intravenous infusion of CCK-8 or gastrin-17. The volumes of the gastric perfusate fractions were determined, 3 ml aliquots added to 3 ml of saline and then titrated to a pH of 7.0 with 0.01 M NaOH. After correction for the acidity of the blank perfusion medium (saline) determined in a reference sample, the gastric secretion of H^+ was computed and expressed in μmol min^{-1}. Total gastric acid output was calculated as the net increment of acid secretion evoked by CCK-8 or gastrin-17 during the 30 min period after the start of the peptide infusion (stimulated over baseline acid secretion) and expressed in μmol.

Contraction of the guinea-pig isolated gall bladder

CAM-1481 and CAM-1028 were tested for their activity on CCK_A receptors by studying their antagonism of CCK-8-induced contractions of the guinea-pig isolated gall bladder. The assay was based on the method described by Bishop *et al.* (1992). In brief, guinea-pigs of either sex (350–450 g) were killed by cervical dislocation and bleeding. Longitudinal strips of smooth muscle were dissected from the gall bladder and suspended in organ baths containing 2 ml of Krebs solution (mM: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.0). This medium was gassed with 95% O_2 and 5% CO_2 to give a pH of 7.4 and maintained at 29°C. The motor activity of the strips was recorded with an isometric tension transducer (HSE, March, Germany) and displayed on a chart recorder (ABB-GOERZ, Vienna, Austria). After the initial application of a tension of 0.1 mN the tissues were allowed to equilibrate for 1 h. Then the strips were challenged by a depolarizing K^+ concentration (122.7 mM, KCl replacing all NaCl in the bath medium), and the ensuing contraction was used to normalize the subsequent contractile responses to CCK-8. After washout of the high K^+ concentration the strips were incubated with either CAM-1481, CAM-1028 (1 μM each) or an equivalent volume of their vehicle (saline). Five minutes later cumulative concentration-response curves for CCK-8 (0.1 nM–1 μM) were recorded by administering increasing doses of CCK-8 to the bath, the applications being spaced apart by intervals of 2–4 min after which a stable response to each dose had been achieved. Only one concentration-response curve was constructed with each strip. The responses to CCK-8 were expressed as a percentage of the contraction evoked by the high K^+ concentration.

Substances

The saline solution used here was 0.15 M NaCl. Urethane (Fluka, Buchs, Switzerland) was dissolved in water at a concentration of 25% (wt/wt). Rat gastrin-17 (gastrin-I, Peninsula, Heidelberg, Germany) and CCK-8 (sulphated, Bachem, Bubendorf, Switzerland) were dissolved in 5% $NaHCO_3$ at a concentration of 100 μM. Further dilutions were made with saline. CAM-1481 (N-[α-methyl-N-[(tricyclo[3.3.1.$1^{3,7}$]dec-2-yloxy)carbonyl]-L-tryptophyl]-D-3-(phenylmethyl)-β-alanine) and CAM-1028 (4-{[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[[1.7.7-trimethyl-bicyclo(2.2.1)hept-2-yl]-oxy]carbonyl]amino]propyl]amino]-1-phenylethyl} amino-4-oxo-{1S-1α.2β[S*(S) 4α]}-butanoate N-methyl-D-glucamine) (Parke-Davis, Cambridge, U.K.) were dissolved in saline.

Statistical analysis

All data are presented as mean ± s.e.mean. Statistical evaluation of the results was performed with the Wilcoxon test for matched pairs, the Mann-Whitney U test or the Kruskal-Wallis H test as appropriate. Probability values $P<0.05$ were regarded as significant.

Results

Gastric mucosal blood flow

The basal values of gastric mucosal blood flow and mean arterial blood pressure measured before administration of any drug are shown in Table 1. Intravenous infusion of CCK-8 or gastrin-17 resulted in a dose-dependent increase in gastric mucosal blood flow, the increments evoked by the highest peptide doses amounting to over 300% (Figure 1). The hyperaemic responses to either peptide took place in the absence of any change in mean arterial blood pressure, with the exception of the highest dose of CCK-8 (0.2 nmol min^{-1}) which caused a slight but significant ($P<0.05$) rise of mean arterial blood pressure from 93 ± 4 to 99 ± 2 mmHg ($n=7$).

Compared with the vehicle, neither the CCK_A receptor antagonist, CAM-1481 nor the CCK_B receptor antagonist, CAM-1028 (1 mg kg^{-1} each) had any significant effect on gastric mucosal blood flow and mean arterial blood pressure under basal conditions (Table 1). CAM-1481 also failed to alter the CCK-8-induced increment of gastric mucosal blood flow, whereas the hyperaemia caused by gastrin-17 appeared to be attenuated by CAM-1481 (Figure 2). In contrast, CAM-1028 inhibited the gastric hyperaemic responses to all doses of CCK-8 and gastrin-17 to a significant extent when compared with the responses recorded after injection of the vehicle (Figure 2).

Gastric acid secretion

The secretory effect of gastrin-17 and CCK-8 was used as CCK_B receptor bioassay to study the selectivity and effectiveness of CAM-1028, at the dose used here (1 mg kg^{-1}), as a CCK_B antagonist versus CAM-1481 as a CCK_A antagonist. The ability of gastrin-17 to stimulate gastric acid secretion (Figure 2) was shared by CCK-8 (Table 2). CAM-1028 had no significant effect on basal gastric acid secretion but inhibited the gastric acid secretory response to gastrin-17 (Figure 2) and CCK-8 (Table 2). Conversely, neither basal gastric acid se-

Table 1 Gastric mucosal blood flow (GMBF) and mean arterial blood pressure (MAP) before and after administration of CAM-1481 and CAM-1028

Parameter	*Treatment vehicle*	*CAM-1481*	*CAM-1028*
GMBF before	43.3±3.7	44.0±3.0	43.0±3.3
GMBF after	40.5±3.7	45.6±2.7	44.1±3.6
MAP before	100.9±2.3	102.4±2.5	100.8±3.1
MAP after	88.7±2.5	91.3±2.5	95.2±2.4

Gastric mucosal blood flow (ml min^{-1} 100 g^{-1}) and mean arterial blood pressure (mmHg) were measured immediately before, and 5 min after, the intravenous injection of vehicle (saline, 1 ml kg^{-1}), CAM-1481 or CAM-1028 (1 mg kg^{-1} each). Data are mean±s.e.mean, $n=17$–19.

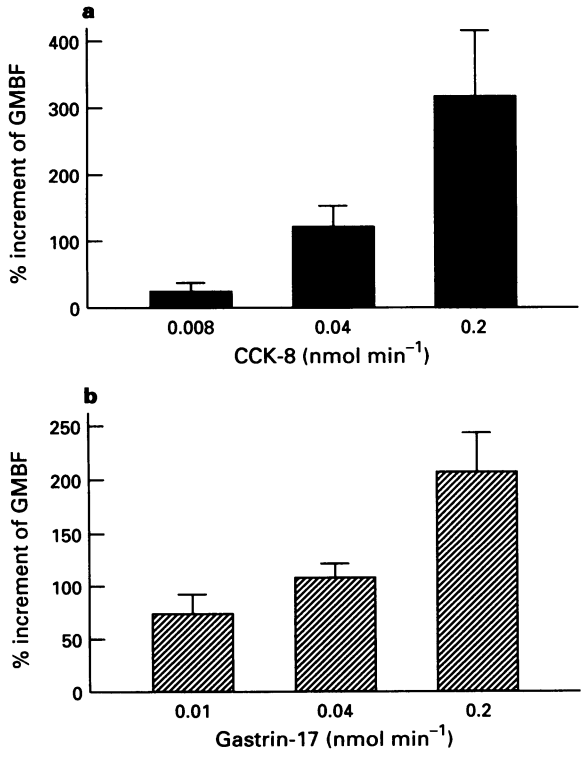

Figure 1 Increase in gastric mucosal blood flow (GMBF) caused by (a) CCK-8 and (b) gastrin-17. The intravenous infusion of CCK-8 and gastrin-17 was started 5 and 15 min prior to the measurement of stimulated blood flow, respectively. The hyperaemic effects are expressed as % increments of gastric mucosal blood flow. The values shown are mean±s.e.mean, $n=6$–7 for each group.

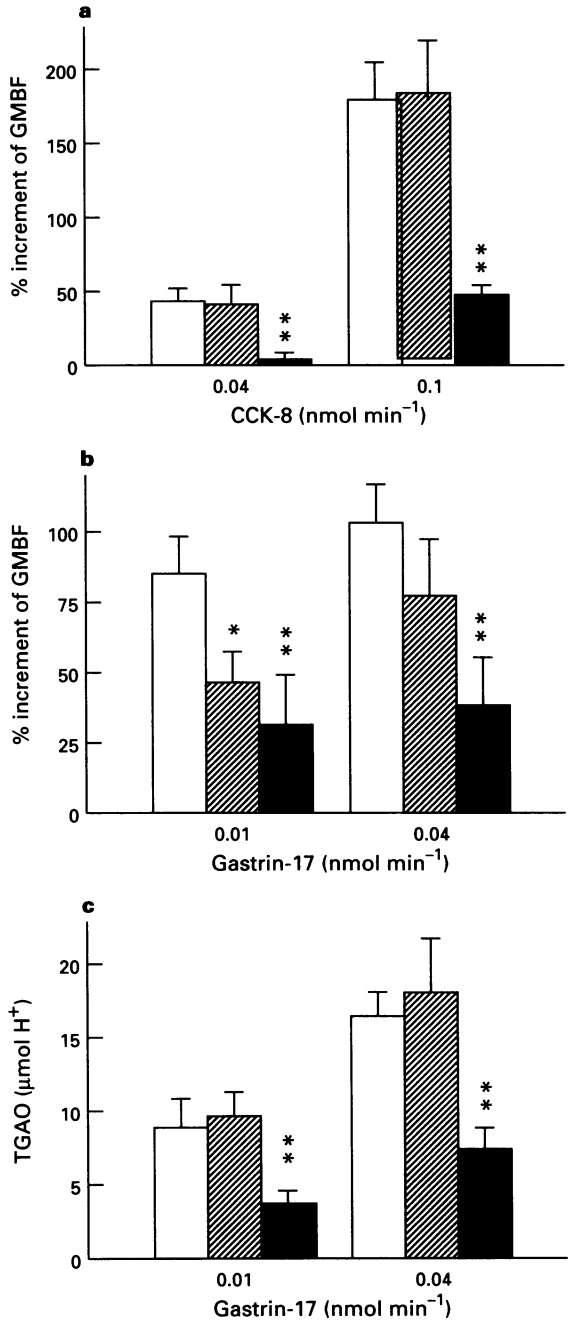

Figure 2 Effects of vehicle (open columns, saline, 1 ml kg^{-1}), CAM-1481 (hatched columns) and CAM-1028 (solid columns) (1 mg kg^{-1} each) on (a) the CCK-8- and (b) gastrin-17-induced increments of gastric mucosal blood flow (GMBF) and on (c) gastrin-17-stimulated total gastric acid output (TGAO). The infusion of CCK-8 and gastrin-17 was started 5 and 15 min prior to the measurement of stimulated blood flow, respectively. The hyperaemic effects are expressed as % increments of gastric mucosal blood flow, and total gastric acid output is calculated as the net secretion evoked by gastrin-17 during the 30 min period after the start of the peptide infusion (stimulated over baseline acid secretion). The vehicle, CAM-1481 or CAM-1028 was injected intravenously 15 min before the infusion of CCK-8 or gastrin-17 was begun. The values shown are mean±s.e.mean, $n=8$–11 per group. $^*P<0.05$, $^{**}P<0.01$ versus vehicle.

Table 2 Basal gastric acid secretion (BGAS) before and after injection of vehicle, CAM-1481 or CAM-1028 and total gastric acid output (TGAO) evoked by CCK-8 after these treatments

Parameter	*Treatment vehicle*	*CAM-1481*	*CAM-1028*
BGAS before	0.09 ± 0.03	0.06 ± 0.02	0.07 ± 0.02
BGAS after	0.08 ± 0.02	0.04 ± 0.02	0.07 ± 0.01
TGAO after CCK-8	10.7 ± 1.4	11.0 ± 1.2	3.6 ± 0.4**

Basal gastric acid secretion is expressed as μmol min^{-1}. Total gastric acid output (μmol H^+) is the net secretion evoked by CCK-8 (0.04 nmol min^{-1}) during the 30 min period after the start of the peptide infusion (stimulated over baseline acid secretion). Vehicle (saline, 1 ml kg^{-1}), CAM-1481 or CAM-1028 (1 mg kg^{-1} each) was injected intravenously 15 min before the intravenous infusion of CCK-8 was begun. Data are mean ± s.e.mean, $n = 8$ for each group.** $P < 0.01$ versus vehicle.

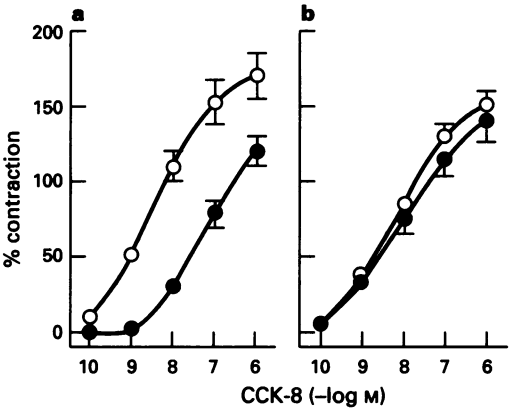

Figure 3 Effects of vehicle (○, saline), (a) CAM-1481 (●) and (b) CAM-1028 (●) (1 μM each) on the CCK-8-induced increments of isometric tension of guinea-pig isolated gall bladder strips. The responses to CCK-8 are expressed as a percentage of the response to 122.7 mM K^+, which was determined at the beginning of each experiment and used to standardize the strips. The values shown are mean ± s.e.mean, $n = 6$ for each group.

cretion (Table 2) nor the secretory response to gastrin-17 (Figure 2) and CCK-8 (Table 2) was altered by CAM-1481 (1 mg kg^{-1}).

Gall bladder contraction

The CCK-8-induced contraction of the guinea-pig isolated gall bladder was used as a CCK_A receptor assay to prove the selectivity and effectiveness of CAM-1481 as a CCK_A antagonist versus CAM-1028 as a CCK_B antagonist. The concentration-response curve for the contractile effect of CCK-8 was left unaltered by CAM-1028 (1 μM) but was shifted to the right by a factor of approximately 70 when CAM-1481 (1 μM) was present in the bath (Figure 3).

Discussion

The present data show that CCK-8 is very potent and effective in increasing gastric mucosal blood flow in the rat, a finding that extends previous observations in the human (Demling & Classen, 1968) and feline (Guth & Smith, 1976) gastric microcirculation. CCK-8 appeared to be roughly equipotent as, but somewhat more effective than, gastrin-17 in causing hyperaemia in the rat gastric mucosa. Since mean arterial blood pressure either did not change or rose slightly in response to CCK-8 or gastrin-17 it is inferred that the hyperaemic action of either peptide results primarily from dilatation of gastric mucosal arterioles. It follows that CCK-8 is not only able to reduce vascular resistance in the small intestine of dogs and cats (Bowen *et al.*, 1975; Fara *et al.*, 1975; Chou *et al.*, 1977) and to dilate the canine hepatic artery (Richardson & Withrington, 1977) but can also relax gastric arterioles to increase effectively mucosal blood flow in the rat stomach. In the intestine, CCK has been implicated as a mediator of postprandial hyperaemia (Chou *et al.*, 1977), but the physiological role of the peptide in the regulation of gastric blood flow has not yet been explored. Because the potency with which CCK-8 increases gastric mucosal blood flow is comparable with that of gastrin-17 it might be argued that gastric hyperaemia is among the physiological actions of endogenous CCK released during digestive activity.

The major aim of the current study was to characterize the type of receptor by which CCK-8 and gastrin-17 increase gastric mucosal blood flow. This question was addressed by use of two novel receptor-selective dipeptoid antagonists of CCK_A and CCK_B receptors, CAM-1481 (Boden *et al.*, 1993) and CAM-1028 (Hughes *et al.*, 1990), respectively. These substances were chosen because they are, unlike benzodiazepine-derived CCK antagonists, readily solouble in water and hence pose no problem in their intravascular administration. They were furthermore shown to be effective antagonists of CCK in appropriate CCK_A and CCK_B receptor bioassays. Thus, the CCK-8-induced contraction of the guinea-pig gall bladder, which is mediated by a homogeneous population of CCK_A receptors (Bishop *et al.*, 1992), was inhibited by the CCK_A receptor antagonist, CAM-1481 but not by the CCK_B receptor antagonist, CAM-1028. Conversely, the gastric acid secretion induced by gastrin-17 or CCK-8 was selectively blocked by the CCK_B receptor antagonist, CAM-1028 but was not affected by the CCK_A receptor antagonist, CAM-1481. This observation is consistent with the reported findings that the secretory responses to CCK and gastrin are solely mediated by CCK_B receptors (Forster *et al.*, 1990; Hughes *et al.*, 1993; Soll & Berglindh, 1994; Walsh, 1994).

With the proven receptor selectivity of CAM-1028 and CAM-1481 it was possible to demonstrate that the gastric hyperaemic response to CCK-8 is solely due to activation of CCK_B receptors and does apparently not involve CCK_A receptors. This result is unexpected inasmuch as it is the so-called brain type of CCK receptor that mediates the gastric vasodilator effect of CCK-8 while many other effects of CCK in the stomach are brought about via activation of CCK_A receptors, the previously termed peripheral type of CCK receptors (Walsh, 1994). CCK_B receptors do likewise play an important role in the rise of gastric mucosal blood flow evoked by gastrin-17, since the gastric vasodilatation evoked by this peptide was also inhibited by CAM-1028. Unlike the hyperaemia caused by CCK-8, however, the hyperaemia induced by gastrin-17 was also attenuated by CAM-1481. It is unlikely that this effect reflects a nonspecific action of CAM-1481, because this antagonist had no inhibitory influence on the secretory response to gastrin-17 and on the hyperaemic reaction to CCK-8. It follows that CCK_A receptors seem to play some role in the gastric vasodilatation caused by gastrin-17, but this conjecture awaits further confirmation.

In summary, the current study has shown that intravascular administration of CCK-8 increases blood flow in the rat gastric mucosa through a local vasodilator action and that the potency and effectiveness of CCK-8 in causing gastric mucosal hyperaemia are comparable to that of gastrin-17. The vasodilator action of CCK-8 is exclusively mediated by CCK_B receptors as shown by the use of receptor-selective antagonists of CCK. It remains to be established whether CCK is involved in the physiological regulation of gastric mucosal blood flow and under which conditions CCK becomes operative in this respect.

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References

BISHOP, L.A., GERSKOWITCH, V.P., HULL, R.A.D., SHANKLEY, N.P. & BLACK, J.W. (1992). Combined dose-ratio analysis of cholecystokinin receptor antagonists, devazepide, lorglumide and loxiglumide in the guinea-pig gall bladder. *Br. J. Pharmacol.*, **106**, 61–66.

BODEN, P.R., HIGGINBOTTOM, M., HILL, D.R., HORWELL, D.C., HUGHES, J., REES, D.C., ROBERTS, E., SINGH, L., SUMAN-CHAUHAN, N. & WOODRUFF, G.N. (1993). Cholecystokinin dipeptoid antagonists: design, synthesis and anxiolytic profile of some novel CCK-A and CCK-B selective and mixed CCK-A/CCK-B antagonists. *J. Med. Chem.*, **36**, 552–565.

BOWEN, J.C., PAWLIK, W., FANG, W.-F. & JACOBSON, E.D. (1975). Pharmacologic effects of gastrointestinal hormones on intestinal oxygen consumption and blood flow. *Surgery*, **78**, 515–519.

CHOU, C.C., HSIEH, C.P. & DABNEY, J.M. (1977). Comparison of vascular effects of gastrointestinal hormones on various organs. *Am. J. Physiol.*, **232**, H103–H109.

CORP, E.S., MCQUADE, J., MORAN, T.H. & SMITH, G.P. (1993). Characterization of type A and B CCK receptor binding sites in rat vagus nerve. *Brain Res.*, **623**, 161–166.

DEMLING, L. & CLASSEN, M. (1968). The influence of gastrointestinal hormones on local blood flow and H-ion concentration of the human gastric mucosa. In *The Physiology of Gastric Secretion*. ed. Semb. L.S. & Myren, J. pp. 18–22. Oslo: Universitetsforlaget.

EVANGELISTA, S. & MAGGI, C.A. (1991). Protection induced by cholecystokinin-8 (CCK-8) in ethanol-induced gastric lesions is mediated via vagal capsaicin-sensitive fibres and CCK_A receptors. *Br. J. Pharmacol.*, **102**, 119–122.

FARA, J.W. & MADDEN, K.S. (1975). Effect of secretin and cholecystokinin on small intestinal blood flow distribution. *Am. J. Physiol.*, **229**, 1365–1370.

FORSTER, E.R., GREEN, T., ELLIOT, M., BREMNER, A. & DOCKRAY, G.J. (1990). Gastric emptying in rats: role of afferent neurons and cholecystokinin. *Am. J. Physiol.*, **258**, G552–G556.

GULLY, D., FRéHEL, D., MARCY, C., SPINAZZé, A., LESPY, L., NELIAT, G., MAFFRAND, J.-P. & LE FUR, G. (1993). Peripheral biological activity of SR 27897: a new potent non-peptide antagonist of CCK-A receptors. *Eur. J. Pharmacol.*, **232**, 13–19.

GUTH, P.H. & SMITH, E. (1976). The effect of gastrointestinal hormones on the gastric microcirculation. *Gastroenterology*, **71**, 435–438.

HOLZER, P., LIVINGSTON, E.H. & GUTH, P.H. (1991). Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology*, **101**, 416–423.

HUGHES, J., BODEN, P., COSTALL, B., DOMENEY, A., KELLY, E., HORWELL, D.C., HUNTER, J.C., PINNOCK, R.D. & WOODRUFF, G.N. (1990). Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 6728–6732.

HUGHES, J., WOODRUFF, G., HORWELL, D.C., MCKNIGHT, A. & HILL, D. (1993). Gastrin/cholecystokinin-B receptor pharmacology. In *Gastrin*. ed. Walsh, J.H. pp. 169–186. New York: Raven Press.

JANSSEN, P.J.J.M., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991). Mechanisms contributing to the differential haemodynamic effects of bombesin and cholecystokinin in conscious. Long Evans rats. *Br. J. Pharmacol.*, **102**, 123–134.

LEUNG, F., GUTH, P.H., SCREMIN, O., GOLANSKA, E. & KAUFFMAN, G. (1984). Regional gastric mucosal blood flow measurements by hydrogen gas clearance in the anesthetized rat and rabbit. *Gastroenterology*, **87**, 28–36.

LIN, C.W. & MILLER, T.R. (1992). Both CCK-A and CCK-B/gastrin receptors are present on rabbit vagus nerve. *Am. J. Physiol.*, **263**, R591–R595.

LIVINGSTON, E.H., REEDY, T., LEUNG, F.W. & GUTH, P.H. (1989). Computerized curve fitting in the analysis of hydrogen gas clearance curves. *Am. J. Physiol.*, **257**, G668–G675.

LLOYD, K.C.K., RAYBOULD, H.E. & WALSH, J.H. (1992). Cholecystokinin inhibits gastric acid secretion through type A cholecystokinin receptors and somatostatin in rats. *Am. J. Physiol.*, **263**, G287–G292.

PRINZ, C., KAJIMURA, M., SCOTT, D.R., MERCIER, F., HELANDER, H.F. & SACHS, G. (1993). Histamine secretion from rat enterochromaffin cells. *Gastroenterology*, **105**, 449–461.

RICHARDSON, P.D.I. & WITHRINGTON, P.G. (1977). The effect of glucagon, secretin, pancreozymin and pentagastrin on the hepatic arterial vascular bed of the dog. *Br. J. Pharmacol.*, **59**, 147–156.

SOLL, A.H. & BERGLINDH, T. (1994). Receptors that regulate gastric acid-secretory function. In *Physiology of the Gastrointestinal Tract., Third Edition*. ed. Johnson, L.R. pp. 1139–1169. New York: Raven Press.

WALSH, J.H. (1994). Gastrointestinal hormones. In *Physiology of the Gastrointestinal Tract. Third Edition*. ed. Johnson, L.R. pp. 1–128. New York: Raven Press.

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