Effect of α_2 -adrenoceptor agonists on gastric pepsin and acid secretion in the rat

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1 The purpose of the present study was to analyze the effects of the α_2 -adrenoceptor agonists clonidine, guanabenz, detomidine and medetomidine on pepsin secretion in conscious rats provided with gastric chronic fistula and to compare this with acid secretion.

2 Basal interdigestive gastric secretion, which is mainly neurally driven in the rat, and the secretion directly stimulated by the two main stimulants of chief cells, cholecystokinin octapeptide (CCK8) and methacholine, were studied.

3 Basal secretion of pepsin and acid was inhibited by all four drugs with comparable EC_{50} s.

4 CCK-stimulated pepsin and acid secretion was less sensitive than basal pepsin and acid secretion to α_2 -adrenoceptor inhibition.

5 Methacholine-stimulated pepsin and acid secretion was not changed by clonidine and guanabenz; methacholine-stimulated acid was even marginally increased by clonidine.

6 These results do not favour the presence of α_2 -receptors on chief cells in the rat stomach. They rather suggest that pepsin inhibition by α_2 -adrenoceptor agonists is indirect and due to central or peripheral inhibition of the discharge of nerve fibres activating pepsin secretion.

Keywords: Gastric fistula rats; pepsin secretion; acid secretion; α_2 -adrenoceptors; clonidine; guanabenz; detomidine; medetomidine

Introduction

Both direct inhibition and indirect stimulation of gastric acid secretion by α_2 -adrenoceptor agonists have been reported. Inhibition had both central and peripheral components (Taylor & Mir, 1982; Pascaud *et al.*, 1983), whereas stimulation might take place through activation of peripheral histamine receptors (Del Tacca *et al.*, 1982; Soldani *et al.*, 1984). By comparison, the influence of α_2 -adrenoceptor agonists on pepsin secretion is poorly documented and the presence of α_2 -adrenoceptors on chief cells has not been reported.

The purpose of this study was to determine the effects of four α_2 -adrenoceptor agonists, clonidine, guanabenz, detomidine and medetomidine, on gastric pepsin secretion *in vivo*, and compare it with acid secretion. The experiments were conducted in gastric fistula rats under basal conditions, a situation in which gastric secretion is mainly neurally driven, and after direct stimulation of chief cells by cholecystokinin octapeptide (CCK8) or methacholine, two potent stimulants of pepsin secretion (Tazi-Saad *et al.*, 1992).

Methods

Animals

Male Wistar rats (Iffa Credo, Les Oncins, F-69210 L'Arbresle, France), weighing 300 ± 20 g, were used in these studies. They were maintained in the laboratory on a commercial autoclaved pelleted chow (UAR 113, Alimentation Rationnelle, Villemoisson, F-91360 Epinay sur Orge, France).

Gastric chronic fistula

After premedication with 10 mg kg^{-1} of acepromazine i.p. (Vetranquil, Clin Midy, F-45142, St Jean de la Ruelle,

France), the rats were anaesthetized with 50 mg kg^{-1} ketamine i.p. (Imalgène 500, Rhône Mérieux, F-69002 Lyon, France). A gastric fistula was set up following the method of Emâs *et al.* (1981): a seromuscular purse was prepared in the ruminal part of the stomach, and a Thomas cannula was inserted and secured in place with the help of a square pad of synthetic felt $1.5 \times 1.5 \text{ cm}$ (Dacron felt, USCI, U.S.A.).

Salivary secretion

In experiments with methacholine, salivary secretion was also stimulated and gastric secretion was therefore contaminated by salivary bicarbonate. To correct for this contamination, salivary secretion was measured in separate experiments. Under ether anaesthesia, a polyethylene catheter (Biotrol No 13) was introduced in the lower oesophagus through a ruminal incision, secured in place by two ligatures at the level of the cardia and rumen, and conducted through the abdominal wall. The muscle and skin were sutured, the rat was placed in a Bollman cage, and an infusion catheter was inserted in a tail vein. After 2 h of post anaesthesia recovery, saliva was collected as 40 min fractions in 5 ml graduated tubes. Methacholine was infused in successive increasing doses under the same conditions as in gastric fistula rats. The volume of saliva samples was measured to the nearest 0.1 ml. Bicarbonate was determined by an auto-analyzer method (Chariot & Rozé, 1976).

Secretory tests

Two weeks of post-operative recovery were allowed before the secretory tests began. During this period, the rats were trained to stand quiet in the Bollman cages during three sham-experiments. Secretory tests were then performed no more than once a week. After a 24 h fasting period with drinking water *ad libitum*, the gastric cannula was opened and the stomach was rinsed with water prewarmed at 37°C . A plastic prolongator was screwed onto the gastric cannula

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and a small diameter polyethylene catheter with lateral holes was introduced and secured in the cannula. This catheter helped to collect the gastric juice safely, by guiding it drop by drop into the collecting tube. The rats were placed in Bollman cages, and an infusion catheter was inserted in a tail vein and secured with adhesive tape. A venous infusion of NaCl 0.9% w/v was then started. Gastric secretion was collected as 40 min samples in tared tubes. The weight of gastric juice was measured, and accepted as a measure of gastric volume, assuming that 1 ml = 1 g. Two 40 min samples were collected before injecting any substance. The mean of these two samples was taken as representative of basal secretion.

Acid secretion

Acid was titrated to pH = 7.0 with NaOH 4 mM, on 0.1 ml samples, with an automatic titrator (Metrohm AG, 9100 Herisau, Switzerland). When the pH of gastric samples was greater than or equal to 5.0, the samples were immediately acidified with 20 μ l HCl (6 M) per ml of gastric juice, in order to protect pepsin activity, which rapidly fades away in solutions of pH > 5.

Pepsin secretion

Pepsin activity was determined by a semi-automated method derived from the method proposed by Anson & Mirsky (1933), which involves two successive steps: (1) Pepsin present in the samples was allowed to digest an aqueous solution of 2.5% haemoglobin (Sigma, St Louis, MO, U.S.A.), pH = 1.7, for 3 min at 37°C. The reaction was then stopped by adding 5% trichloroacetic acid (Prolabo, Paris, France), and soluble hydrolysis products were separated by centrifugation. (2) Soluble hydrolysis products were determined with the Folin-Ciocalteu reagent (Merck, West Point, U.S.A.) in a Technicon auto-analyzer, with tyrosine as a standard. Volume of each sample was 50 μ l for the dosage and 50 μ l for the blank tube without hydrolysis. Pepsin activity of the gastric juice was determined relative to a control scale of standard purified pepsin (Fluka, Buchs, Switzerland, ref 77152, 0.2 to 1 mg ml⁻¹) and was expressed as pepsin units (PU). One unit corresponds to the amount of pepsin releasing 1 μ mol tyrosine min⁻¹ under the conditions adopted for the study.

Experimental schedules

Effect of α_2 -adrenoceptor agonists on basal secretion Clonidine, guanabenz, detomidine and medetomidine were administered as successive i.v. infusions of increasing doses (4.35, 13.7, 43.5 nmol kg⁻¹ h⁻¹), each infused for 80 min. The effect of each dose was measured during the second 40 min half of the 80 min infusion period.

The time course of the effect was analyzed on 240 min i.v. infusions of clonidine (13.7, 43.5 or 137 nmol kg⁻¹ h⁻¹) or guanabenz (13.7 or 43.5 nmol kg⁻¹ h⁻¹). To test the α_2 -specificity of the clonidine effect, the α_2 -adrenoceptor antagonist, idazoxan, was administered as a s.c. injection, 40 min before the infusion of clonidine had begun.

Effect of clonidine and guanabenz on CCK-stimulated secretion Clonidine (13.7, 43.5 or 137 nmol kg⁻¹ h⁻¹) or guanabenz (13.7 or 43.5 nmol kg⁻¹ h⁻¹) were first infused alone i.v. for 120 min, and then accompanied by CCK, 1.9 and 6 nmol kg⁻¹ h⁻¹ as two successive 40 min infusions. In antagonist experiments, idazoxan was injected s.c., 40 min before clonidine infusion.

Effect of clonidine and guanabenz on methacholine-stimulated secretion Clonidine or guanabenz were first infused alone i.v. for 120 min, and then accompanied by methacholine, 3100 and 9710 nmol kg⁻¹ h⁻¹, as two successive 40 min

infusions. Since salivary secretion (a source of fluid and bicarbonate) was stimulated by methacholine, acid secretion was underestimated and volume was overestimated in these experiments. Thus, volume and acid data were corrected for salivary secretion measured in separate experiments: the volume of saliva was subtracted from the total volume of juice collected from the gastric fistula, whereas salivary bicarbonate output was added to gastric acid output, assuming that gastric acid had been stoichiometrically neutralized by salivary bicarbonate. To ensure pepsin stability, all samples from methacholine experiments were immediately acidified with 6 M HCl 20 μ l ml⁻¹.

Substances tested

The following substances were used: CCK: Boc-Nle₂₈-Nle₃₁-CCK₂₆₋₃₃ (a gift of J. Martinez, Centre CNRS-INSERM de Pharmacologie Endocrinologie, Montpellier, France), a synthetic analogue of CCK8 the activity of which is equal to that of CCK8 in all models tested to date; methacholine: acetyl- β -methylcholine chloride (Pharmacie Centrale des Hopitaux, Paris, France); clonidine: a gift of Boehringer (Ingelheim, Germany); guanabenz acetate: Sigma (St Louis, MO, U.S.A.); detomidine hydrochloride and medetomidine hydrochloride: a gift of Farnos (Turku, Finland); idazoxan: a gift of Reckitt and Colman (Kingston upon Hull, GB). All drugs were dissolved in NaCl 0.9%. CCK solutions were prepared in 0.9% NaCl with 0.3% bovine serum albumin added.

Expression of results and statistics

The results are expressed as means $\pm$ standard error of the mean (s.e.mean). The doses causing 50% inhibition (ED₅₀) were calculated from the linear portion of the regression curve of the effect on log dose, as described by Tallarida & Murray (1984). Statistical significance of the differences between means was tested by one or two way ANOVA, followed when necessary by a multigroup comparison test (Dunnett or Newman-Keuls). A *P* value $\leq$ 0.05 was considered significant.

Results

Effect of α_2 -adrenoceptor agonists on basal gastric secretion

Dose-response curves by infusion of successive increasing doses Clonidine, guanabenz, detomidine and medetomidine inhibited in a dose-related way the basal output of acid and pepsin (Figure 1). The inhibition observed with the dose of 43.5 nmol kg⁻¹ h⁻¹ reached about 90% of the basal acid and pepsin outputs. The calculated ED₅₀ values (doses causing 50% inhibition) are shown in Table 1: all four drugs had similar ED₅₀s on volume, acid and pepsin outputs.

Time course of the inhibition produced by clonidine and guanabenz The time course of the inhibition produced by long lasting (240 min) i.v. infusions of 13.7 nmol kg⁻¹ h⁻¹ of clonidine or guanabenz was identical with both drugs. The peak inhibition (about 50% for volume, acid and pepsin) was observed after 120 min of infusion, and remained at this level until the end of the infusion (not shown). The time course was similar when the clonidine or guanabenz dose was increased to 43.5 nmol kg⁻¹ h⁻¹ (Figure 2): the peak inhibition was reached after 120 min of i.v. infusion, and remained approximately constant for the rest of the infusion period. With this latter dose of clonidine, the inhibition at 120 min was 80% for volume, and 90% for acid and pepsin outputs. With this latter dose of guanabenz, the inhibition at 120 min was 60% for volume, 90% for acid, and 80% for pepsin output (Figure 2). After clonidine (137 nmol kg⁻¹ h⁻¹), the

Figure 1 Effects of clonidine (○), guanabenz (●), detomidine (□) and medetomidine (■), on gastric juice volume (a) and on acid (b) and pepsin (c) outputs as a function of doses in conscious rats provided with gastric chronic fistula. The results are expressed as percentage inhibition of basal secretion. The data show the mean of 6 or 7 rats per group. To increase clarity, the vertical lines showing s.e.mean have been drawn only for the group receiving clonidine.

Table 1 The ED₅₀ values (dose causing 50% inhibition; nmol kg⁻¹ h⁻¹, 95% confidence limits in parentheses) of clonidine, guanabenz, detomidine and medetomidine on basal gastric secretion

Substance	Volume	Acid output	Pepsin output
Clonidine	9.0 (5.4–14.8)	10.2 (5.9–17.6)	4.7 (2.1–10.3)
Guanabenz	13.6 (10.2–18.2)	17.7 (10.7–29.4)	7.7 (5.5–10.8)
Detomidine	10.6 (7.5–14.2)	15.1 (9.6–23.7)	9.8 (7.1–13.6)
Medetomidine	8.9 (6.1–13.2)	12.2 (8.1–18.3)	4.8 (3.6–6.5)

Data are calculated from the experiments shown in Figure 1, *n* = 6 or 7 rats per group.

inhibition at 120 min was 87% for volume, 99% for acid, and 100% for pepsin output (Figure 3).

Pepsin concentration was significantly decreased by 43.5 nmol kg⁻¹ h⁻¹ of clonidine or guanabenz (Table 2).

Idazoxan alone did not change basal gastric secretion (not shown), but suppressed the clonidine inhibition of volume, acid and pepsin outputs (Figure 3).

Clonidine and guanabenz on CCK-stimulated gastric secretion

Volume, acid and pepsin outputs were stimulated in a dose-related way by CCK (Figure 4), while pepsin concentration was decreased (Table 2).

Figure 2 Time course of the inhibitory effect of clonidine (43.5 nmol kg⁻¹ h⁻¹, i.v., ○) and guanabenz (43.5 nmol kg⁻¹ h⁻¹, i.v., ●) on gastric juice volume (a) and on acid (b) and pepsin (c) outputs in conscious rats provided with gastric chronic fistula. The time course of gastric secretion in a control group receiving only NaCl (□) is shown for comparison. The results are expressed as percentage inhibition of basal secretion, shown as means of 8 rats per group; vertical bars represent s.e.mean. The average basal levels were: volume 980 ± 81.3 μl 40 min⁻¹, acid output 75.0 ± 7.68 μmol 40 min⁻¹, pepsin output 10.3 ± 0.66 PU 40 min⁻¹.

Figure 3 Antagonism by idazoxan (3 mg kg⁻¹, s.c. 40 min before beginning the infusion of clonidine) of the inhibitory effects of clonidine (137 nmol kg⁻¹ h⁻¹, i.v.) on basal volume juice, and on acid and pepsin outputs in conscious rats provided with gastric chronic fistula. Basal secretion = open columns; clonidine = solid columns; idazoxan + clonidine = hatched columns. Means of 8 rats per group. Vertical lines show s.e.mean. ****P* < 0.001 versus basal level (Dunnett's test)

Clonidine decreased CCK-stimulated gastric secretion (Figure 4). In Figure 4, the probability values refer to the significance of the difference between the three dose levels of clonidine (0, 13.7 and 43.5 nmol kg⁻¹ h⁻¹), under basal conditions (Control groups) and during the infusion of 1.9 or

Table 2 Effect of clonidine and guanabenz on pepsin concentration in the gastric juice (PU ml⁻¹), in basal conditions (control) and with cholecystokinin (CCK) stimulation

	Control	CCK 1.9	CCK 6	P (CCK effect)
Control	11.1 ± 0.9	7.6 ± 0.2	8.4 ± 0.2	<0.001
Clonidine [13.7*]	11.0 ± 0.6	7.5 ± 0.3	9.1 ± 0.5	<0.001
Clonidine [43.5]	5.6 ± 1.6	7.1 ± 0.9	9.1 ± 0.7	NS
P (Clonidine effect)	<0.01	NS	NS	
Guanabenz [13.7]	9.2 ± 0.2	7.8 ± 0.3	9.9 ± 0.4	<0.001
Guanabenz [43.5]	6.0 ± 1.2	8.5 ± 0.8	11.3 ± 1.3	<0.05
P (Guanabenz effect)	<0.01	NS	NS	

*Doses are in nmol kg⁻¹ h⁻¹. The values are shown as mean ± s.e.mean of 6 to 16 rats per group. The *P* levels correspond to the result of the ANOVA between doses of CCK, clonidine or guanabenz. NS: not significant.

Figure 4 Effects of clonidine (left panel) and guanabenz (right panel) on gastric juice volume (a) and on acid (b) and pepsin (c) outputs in basal conditions (Control) and under cholecystokinin (CCK) stimulation (1.9 and 6 nmol kg⁻¹ h⁻¹) in conscious rats provided with gastric chronic fistula. The stimulation consisted of two successive 40 min infusions of CCK 1.9 and 6 nmol kg⁻¹ h⁻¹ from 120 min after the beginning of clonidine or guanabenz infusion. The doses of clonidine and guanabenz were: zero = open columns: 13.7 nmol kg⁻¹ h⁻¹ = solid columns and 43.5 nmol kg⁻¹ h⁻¹ = hatched columns. Means of 6 to 16 rats per group. Vertical lines show s.e.mean.

The probability values, ***P* < 0.01 and ****P* < 0.001 refer to the significance between the dose levels of clonidine or guanabenz (0, 13.7 and 43.5 nmol kg⁻¹ h⁻¹) under basal conditions (control) and under CCK stimulation (1.9 and 6 nmol kg⁻¹ h⁻¹) (ANOVA).

6 nmol kg⁻¹ h⁻¹ of CCK. The clonidine ED₅₀ values were twice as great for CCK-stimulated (Table 3) as compared to the basal volume and acid secretion (Table 1, *P* < 0.05 to 0.01), and about four times as great for CCK-stimulated as compared to the basal pepsin output. Pepsin concentration was not significantly changed by clonidine in CCK-treated rats (Table 2). When using large doses of clonidine, CCK-stimulated secretion was almost abolished: after 137 nmol kg⁻¹ h⁻¹ of clonidine, volume was decreased by 70%, acid output by 99%, and pepsin output by 90% (Figure 5).

Guanabenz (13.7 and 43.5 nmol kg⁻¹ h⁻¹) was also given with CCK (1.9 and 6 nmol kg⁻¹ h⁻¹) (Figure 4). The effects of guanabenz were very similar to those of clonidine on the CCK-stimulated gastric juice volume, acid output and pepsin output. The ED₅₀ values of guanabenz were significantly greater on CCK-stimulated volume and pepsin output (Table 3) than on the basal secretion (Table 1). Pepsin concentration was not significantly changed by guanabenz in CCK-stimulated rats (Table 2).

Idazoxan antagonism of the clonidine inhibition of CCK-stimulated pepsin secretion

In these studies, a maximally effective dose of 137 nmol kg⁻¹ h⁻¹ of clonidine was used to ensure a nearly complete inhibition of pepsin secretion. The prior s.c. injection of idazoxan (3 mg kg⁻¹) totally suppressed the clonidine-induced inhibition of pepsin output and decreased the clonidine-induced inhibition of volume (*P* < 0.05) and acid output (*P* = 0.14) (Figure 5).

Effect of clonidine and guanabenz on methacholine-stimulated gastric secretion

Methacholine stimulated in a dose-related way the volume and the acid and pepsin outputs, as shown in Figure 6. The volume and acid values were corrected for salivary con-

Table 3 The ED₅₀ values (doses causing 50% inhibition; nmol kg⁻¹ h⁻¹, 95% confidence limits in parentheses) of clonidine and guanabenz on cholecystokinin (CCK)-stimulated gastric secretion

Substance	CCK dose (nmol kg ⁻¹ h ⁻¹)	Volume	Acid output	Pepsin output
Clonidine	1.9	21.5 ^b (16.4–28.2)	19.3 ^a (14.9–25.0)	19.4 ^c (15.3–24.5)
	6	20.9 ^b (16.1–27.0)	20.4 ^a (15.9–26.1)	22.7 ^c (17.5–29.4)
Guanabenz	1.9	26.8 ^a (16.2–44.0)	24.4 (15.4–38.7)	29.2 ^c (16.4–52.1)
	6	28.8 ^a (16.0–52.0)	24.1 (13.8–42.1)	43.3 ^c (15.6–120)

Data are calculated from the experiments shown in Figure 4, *n* = 6 to 14 rats per group, where ^a: *P* < 0.05, ^b: *P* < 0.01, ^c: *P* < 0.001 versus the corresponding ED₅₀ values on basal secretion (Table 1).

Figure 5 Antagonism by idazoxan (3 mg kg^{-1} , s.c. 40 min before beginning the infusion of clonidine) of the inhibitory effects of clonidine ($137 \text{ nmol kg}^{-1} \text{ h}^{-1}$, i.v.) on gastric juice volume, and on acid and pepsin outputs stimulated by CCK ($1.9 \text{ nmol kg}^{-1} \text{ h}^{-1}$, i.v.), accompanying the infusion of clonidine for 40 min from 120 min after the beginning of clonidine infusion) in conscious rats provided with gastric chronic fistula. CCK = open columns; CCK + clonidine = solid columns; CCK + idazoxan + clonidine = hatched columns. The results are expressed as percentage of the CCK-stimulated secretion. Means of 8 to 12 rats per group. Vertical lines show s.e.mean.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus group CCK (Dunnett's test).

Figure 6 Effects of clonidine (left panel) and guanabenz (right panel) on gastric juice volume (a) and on acid (b) and pepsin (c) outputs in basal conditions (Control) and under methacholine (M) stimulation (3100 and 9710 $\text{nmol kg}^{-1} \text{ h}^{-1}$) in conscious rats provided with gastric chronic fistula. The stimulation consisted of two successive 40 min infusions of methacholine 3100 and 9710 $\text{nmol kg}^{-1} \text{ h}^{-1}$ from 120 min after the beginning of clonidine or guanabenz infusion. The doses of clonidine and guanabenz were: zero = open columns; $13.7 \text{ nmol kg}^{-1} \text{ h}^{-1}$ = solid columns and $43.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$ = hatched columns. Means of 6 to 12 rats per group. Vertical lines show s.e.mean. NS = no significant difference.

* $P < 0.05$ and *** $P < 0.001$ = values of the difference between the doses of clonidine and guanabenz (0, 1.37, and $43.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) under basal conditions (Control) and under methacholine stimulation (3100 and 9710 $\text{nmol kg}^{-1} \text{ h}^{-1}$) (ANOVA).

tamination, by use of the average data of methacholine stimulation of salivary secretion, shown in Table 4. This correction was also made in clonidine treated groups, since

clonidine ($43.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) did not change the methacholine-induced stimulation of salivary volume and bicarbonate output (Table 4).

Clonidine and guanabenz (13.7 or $43.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$) did not decrease significantly gastric secretion (volume, acid and pepsin) stimulated either by a submaximal ($3100 \text{ nmol kg}^{-1} \text{ h}^{-1}$) or maximal ($9710 \text{ nmol kg}^{-1} \text{ h}^{-1}$) dose of methacholine (Figure 6). Clonidine ($13.7 \text{ nmol kg}^{-1} \text{ h}^{-1}$) actually caused a slight increase in methacholine-stimulated acid output.

Discussion

The effect of α_2 -adrenoceptor agonists on gastric acid secretion has been studied in a variety of species, mainly by use of clonidine (Hoefke & Kobinger, 1966; Cheng *et al.*, 1981; Del Tacca *et al.*, 1982; Pascaud *et al.*, 1983; Soldani *et al.*, 1984) but also with guanfacin, xylazine, guanabenz, detomidine and medetomidine (Kunchandy *et al.*, 1985; Bernardini *et al.*, 1986; Savola *et al.*, 1989; Blandizzi *et al.*, 1990). In those studies, small doses of clonidine usually induced an inhibition of gastric acid secretion when this secretion was stimulated by vagal activity or by pentagastrin. Such inhibition was also observed during basal and pentagastrin-stimulated secretion in conscious fistula rats (Pascaud *et al.*, 1983), conscious pylorus-ligated rats (Cheng *et al.*, 1981; Del Tacca *et al.*, 1982; Bernardini *et al.*, 1986), or 2-deoxyglucose stimulated secretion in rats and dogs (Cheng *et al.*, 1981; Soldani *et al.*, 1984). This inhibition has been interpreted as resulting from the action of clonidine at neural α_2 -adrenoceptors, located both peripherally (presynaptic α_2 -receptors on vagal acetylcholine-producing neurones, Del Tacca *et al.*, 1982) and centrally (Taylor & Mir, 1982; Pascaud *et al.*, 1983; Dettmar & Lord, 1986; Fornai *et al.*, 1990).

In contrast, basal gastric acid secretion in preparations without endogenous vagal tone, such as basal secretion in lumen-perfused anaesthetized rats (Bernardini *et al.*, 1986) or basal secretion in dogs (Soldani *et al.*, 1984) was moderately increased by clonidine. This effect was suppressed by histamine H_2 -receptor antagonists, and has been attributed either to direct activation of histamine receptors, or to histamine release by clonidine. The non-imidazole derivative, guanabenz, did not display such stimulating effect (Bernardini *et al.*, 1986). Recently, however, the selective α_2 -adrenoceptor agonist, detomidine, which lacks affinity for H_2 -receptors (Virtanen & Nyman, 1985), has been found to stimulate gastric acid secretion in urethane-anaesthetized, lumen-perfused, non-vagotomized or vagotomized rats (Blandizzi *et al.*, 1990). This effect was reversed by idazoxan and the authors suggested that it might correspond to the blockade by detomidine of an α_2 -mediated sympathetic inhibitory tone on the gastric secretion. In other studies, acid secretion stimulated by cholinergic agents was either unchanged (Pascaud *et al.*, 1983, conscious rats) or even increased (Soldani *et al.*, 1984, conscious dogs) by clonidine.

In contrast to these detailed reports of α_2 -adrenoceptor agonist effects on gastric acid secretion, no data have been published as yet concerning pepsin secretion. The present results show that basal pepsin secretion in conscious rats (an animal model submitted to permanent sustained vagal stimulant tone) was decreased in a dose-related way by the four α_2 -adrenoceptor agonists tested, with similar potencies (non significantly different ED_{50} values of 4.7 to 9.8 $\text{nmol kg}^{-1} \text{ h}^{-1}$ on pepsin secretion) and efficacies (approaching 100% inhibition at the dose of $43.5 \text{ nmol kg}^{-1} \text{ h}^{-1}$). This α_2 -mediated effect (suppressed by idazoxan), was comparable for volume, acid and pepsin, and we suggest that it may be due to the occupation of either central α_2 -adrenoceptors or presynaptic α_2 -adrenoceptors located on peripheral cholinergic neurones. Since this inhibition of basal secretion corresponds to the suppression of an endogenous cholinergic stimulus, it does not implicate the presence of inhibitory

Table 4 Effect of methacholine and methacholine plus clonidine on salivary secretion in conscious rats

Methacholine (nmol kg ⁻¹ h ⁻¹)	Clonidine (nmol kg ⁻¹ h ⁻¹)	Time (μ l min ⁻¹)	Bicarbonate	
			Concentration (mmol l ⁻¹)	Output (μ mol 40 min ⁻¹)
0	0	26 ± 26	21.6 ± 4.1	2.1 ± 1.2
3100	0	27 ± 470	39.5 ± 2.2	80.7 ± 14.4
3100	43.5	27 ± 470	31.1 ± 1.6	71.6 ± 11.1
9710	0	30 ± 240	42.0 ± 1.2	141 ± 13.2
9710	43.5	30 ± 410	38.1 ± 0.2	144 ± 15.3

P levels, 2 way ANOVA:

Between methacholine doses

<0.01

<0.01

<0.001

Clonidine/no clonidine

NS

<0.01

NS

Interactions clonidine/methach.

NS

NS

NS

Data are shown as the mean $\pm$ s.e.mean of 4 rats per group.

α_2 -adrenoceptors on chief cells. Thus, the simple decrease in acetylcholine efflux from nerve terminals to chief cells can explain the results.

The effect of clonidine and guanabenz was also tested on stimulated pepsin secretion, and we chose for these experiments CCK and methacholine, which probably are the two main physiological stimulants of pepsin secretion (Hersey, 1987; Tazi-Saad *et al.*, 1992). The results were different with these two stimulants: whereas clonidine and guanabenz both decreased CCK-stimulated acid and pepsin secretion, they did not change (or even marginally increased) methacholine-stimulated acid and pepsin secretion. Since pepsin concentration did not decrease with increasing doses of α_2 -adrenoceptor agonists (Table 2), it may be that only the volume flow was decreased by α_2 -adrenoceptor agonists in CCK-stimulated gastric glands, thus preventing pepsin from being washed out of the glands. If the inhibition of CCK-induced secretion is due to the absence of cholinergic tone in gastric glands, the effects observed may be attributed to the central decrease of vagal tone and/or to the presynaptic inhibition of acetylcholine release by α_2 -adrenoceptor stimulation. Previous experiments comparing clonidine and ST91 a clonidine-like drug (which crosses the blood-brain barrier poorly) indicated that clonidine inhibition of gastric acid secretion in conscious rats had a large central component (Pascaud *et al.*, 1983). Clonidine thus inhibited the effect of the central stimulant 2-deoxy-D-glucose, which acts through the vagus on muscarinic gastric receptors, whereas the peripheral cholinergic stimulant methacholine was not affected (Pascaud *et al.*, 1983). It has also been shown that the volume and acid responses to pentagastrin are much decreased in vagotomized rats, and that most of the response

to pentagastrin can be restored by infusing small doses of acetylcholine, able to mimic a pseudo-endogenous vagal tone (Chicau-Chovet *et al.*, 1988). This, however, has not been determined with CCK.

The effect of methacholine was not changed, or tended to increase, after clonidine or guanabenz administration. This suggests that the α_2 -adrenoceptor agonists did not interfere with the intracellular mechanisms activated by methacholine, and thus that the parietal and chief cell membranes do not bear α_2 -adrenoceptors able to interfere with the cellular processes when the muscarinic receptors of these cells are stimulated. Soldani *et al.* (1984) found in the dog an increase of both bethanechol- and histamine-stimulated acid secretion by clonidine. These authors suggested that this result was due to the histamine receptor stimulating effect of clonidine. The mechanism is probably different in our experiments, since guanabenz, which has no histamine-like effects (Bernardini *et al.*, 1986) produced the same effect as clonidine.

In conclusion, we suggest that the effect of the α_2 -adrenoceptor agonists on both pepsin and acid is probably due to the central and peripheral inhibition of the neural cholinergic input to gastric glands. Some cholinergic background is necessary for developing the stimulating effect of various agents, which explains why CCK-stimulation is also inhibited by the α_2 -adrenoceptor agonists. When methacholine is used, it compensates for the inhibition of cholinergic background, and does not allow α_2 -adrenoceptor agonists to inhibit this secretion.

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