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Short communication

Characterization of α_2-adrenoceptor subtypes involved in the modulation of gastric acid secretion

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Abstract

The effects of several α_2-adrenoceptor agonists and antagonists were examined on gastric acid secretion from reserpinized rats undergoing electrical stimulation of the left vagus nerve. Both detomidine and oxymetazoline inhibited vagal acid hypersecretion, their effects being fully prevented by idazoxan, 7,8-(methylenedioxy)-14-α-hydroxyalloberbane (CH 38083), or 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline (RX 821002), and partly antagonized by yohimbine or rauwolscine. 2-(2,4-(*O*-methoxy-phenyl)-piperazin-1-yl)-ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione (ARC 239) did not affect the antisecretory action of the two agonists, while prazosin at the highest dose was partly effective only against detomidine. Atropine markedly reduced vagally evoked acid output. It is suggested that acid secretion induced by vagal cholinergic stimulation is modulated by α_{2A}-like adrenoceptor subtypes.

Keywords: Presynaptic α_2-adrenoceptor; α_2-Adrenoceptor subtype; Gastric acid secretion; Vagus nerve; (Rat)

1. Introduction

It is well established that both central and peripheral α_2-adrenoceptors play an important role in the modulation of gastric acid secretion (Del Tacca et al., 1982; Pascaud et al., 1983). While the mechanisms by which central α_2-adrenoceptors promote the inhibition of acid output remain not completely understood, at the peripheral level it was shown that presynaptic α_2-adrenoceptors, located on vagus nerve cholinergic terminals, modulate gastric secretion through an inhibition of acetylcholine release (Del Tacca et al., 1982).

More recently, it was suggested that α_2-adrenoceptors do not represent a homogeneous population and that they can be classified in at least four subtypes, named α_{2A}, α_{2B}, α_{2C} and α_{2D} (Bylund, 1992; MacKinnon et al., 1994). In particular, α_{2A}-adrenoceptors show higher affinity for oxymetazoline than for prazosin and 2-(2,4-(*O*-methoxy-phenyl)-piperazin-1-yl)-ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione (ARC 239), whereas α_{2B}-adrenoceptors exhibit a converse selectivity for these drugs (Bylund, 1992). In addition, the pharmacological profiles of α_{2C}- and α_{2D}-adrenoceptors closely resemble those for α_{2B} and α_{2A} binding sites, respectively (MacKinnon et al., 1994).

As far as the digestive tract is concerned, the existence of α_2-adrenoceptor subtypes has been demonstrated through different experimental approaches in various segments of both small and large intestine (Zhang et al., 1982; Blandizzi et al., 1993; Hildebrand et al., 1993). However, no effort has been made so far to characterize the α_2-adrenoceptor subtypes involved in the regulation of gastric functions. In the present study, several α_2-adrenoceptor agonists and antagonists were tested in an attempt to functionally subclassify the presynaptic α_2-adrenoceptors located on peripheral vagus nerve terminals that are involved in the modulation of gastric acid secretion in the rat.

2. Materials and methods

2.1. Perfusion of the gastric lumen and evaluation of acid secretion

The experiments were carried out on male Wistar rats, weighing about 200 g and fasted for 24 h. Twenty

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hours before the experiments, the animals were treated with reserpine (2 mg/kg i.p.) in order to prevent the influence exerted by endogenous catecholamines on gastric α_2-adrenoceptors (Blandizzi et al., 1990).

Perfusion of the rat stomach in situ was carried out following a procedure previously reported (Blandizzi et al., 1990). Briefly, the animals were anaesthetized with urethane (1.2 g/kg i.p.), and the rectal temperature was monitored and maintained between 37 and 39° C with an infrared lamp. The gastric lumen was perfused continuously with 154 mM NaCl solution at 37° C, at a rate of 1 ml/min, and 15-min effluent fractions were collected. The acidity of the gastric perfusate was measured with an autotitrator pH meter (PHM85, Radiometer, Copenhagen, Denmark) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH. At the time of the experiment, the vagus nerves of each rat were carefully separated from the carotid arteries and cut at the cervical level. The peripheral end of the left vagus nerve was then placed in a platinum electrode. After stabilization of the basal acid output, the gastric acid secretion was evoked by continuous electrical stimulation of the vagus nerve (150 min). The stimulus parameters were square wave pulses of 0.5-ms duration, delivered at 3 Hz with supramaximal intensity (10 V). All test drugs were administered by i.v. route at 60 min; in agonist-antagonist interaction experiments, the antagonist was administered 10 min before the agonist. The acid secretory values for the gastric perfusate were expressed as μEq H^+/15 min. The acid output obtained during the last 90 min of electrical vagal stimulation was also calculated, and the effects of test drugs were expressed as percentages of the vagally induced acid output detected in control animals.

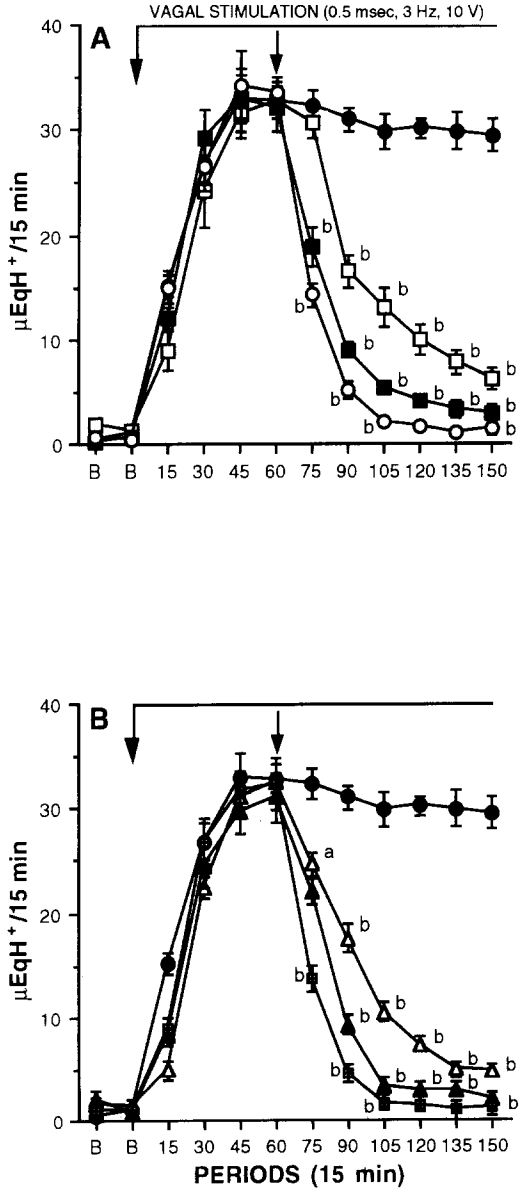

Fig. 1. Anaesthetized rats with perfused gastric lumen. Effects of detomidine 1.5 (□), 5 (■) and 15 (○) μmol/kg i.v. (A) or oxymetazoline 1.5 (△), 5 (▲) and 15 (⊠) μmol/kg i.v. (B) on gastric acid secretion (μEq H^+/15 min) evoked by electrical vagal stimulation. The arrow indicates the time of agonist administration. B = basal. Each point represents the mean value obtained from 8 experiments ± S.E.M. (vertical lines). Significant differences from control values (●): [a] $P < 0.01$; [b] $P < 0.001$.

2.2. Drugs

The following drugs were used: oxymetazoline hydrochloride, prazosin hydrochloride, urethane ethylcarbamate and reserpine (all from Sigma Chemical Co., St. Louis, MO, USA), detomidine hydrochloride (Farmos, Turku, Finland), idazoxan hydrochloride (Reckitt and Colman, Hull, UK), CH 38083 (7,8-(methylenedioxy)-14-α-hydroxyalloberbane hydrochloride; Chinoin, Budapest, Hungary); RX 821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline; RBI, Natick, MA, USA); yohimbine hydrochloride (Lilly, Indianapolis, IN, USA); rauwolscine hydrochloride (Care Roth, Karlsruhe, Germany); atropine sulphate (BDH Chemicals, Poole, UK). ARC 239 (2-(2,4-(*O*-methoxy-phenyl)-piperazin-1-yl)-ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione hydrochloride) was kindly provided by Karl Thomae, Biberach, Germany.

2.3. Statistical analysis

Results are given as means ± S.E.M. The significance of differences was evaluated by Student's *t*-test for paired or unpaired data and *P* values lower than 0.05 were considered significant; *n* indicates the number of experiments.

3. Results

In control reserpinized rats, electrical stimulation of the left vagus nerve caused a marked and rapid in-

crease in gastric acid secretion (from 1.1 ± 0.4 to 33.2 ± 2.3 μEq H^+/15 min; $n = 8$; $P < 0.001$) which reached a steady level within 30–45 min and lasted until the end of the experiment (Fig. 1). The acid output measured during the last 90 min of the vagal stimulation was 182.8 ± 12.2 μEq H^+/90 min ($n = 8$). The same control values were used when comparing the effects of both agonists tested in the present study to those of controls (Fig. 1). The hypersecretory response elicited by vagal activation was markedly inhibited by atropine 0.1 μmol/kg i.v. (30.3 ± 3.6 μEq H^+/90 min, $n = 6$; $P < 0.001$ versus control animals; data not shown).

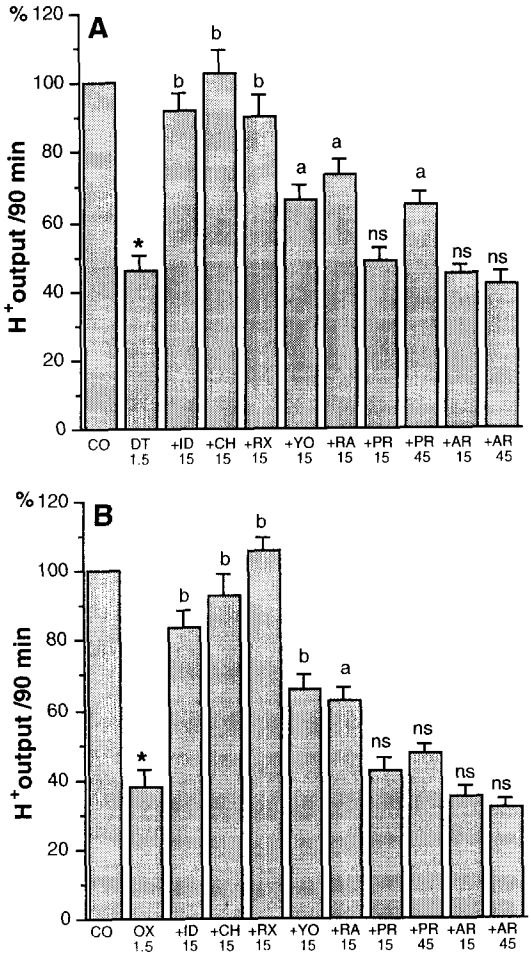

Fig. 2. Anaesthetized rats with perfused gastric lumen. Effects of detomidine 1.5 μmol/kg i.v. (DT1.5) (A) or oxymetazoline 1.5 μmol/kg i.v. (OX1.5) (B), given alone or following pretreatment with i.v. idazoxan 15 μmol/kg (+ID15), CH 38083 15 μmol/kg (+CH15), RX 821002 15 μmol/kg (+RX15), yohimbine 15 μmol/kg (+YO15), rauwolscine 15 μmol/kg (+RA15), prazosin 15 μmol/kg (+PR15) and 45 μmol/kg (+PR45), or ARC 239 15 μmol/kg (+AR15) and 45 μmol/kg (+AR45), on gastric acid secretion (% of H^+ output/90 min) evoked by electrical vagal stimulation (0.5 ms, 3 Hz, 10 V). Columns indicate the mean values obtained from 6–8 experiments ± S.E.M. (vertical lines). Significant differences from control value (CO): $^*P < 0.001$. Significant differences from detomidine or oxymetazoline alone: $^a P < 0.01$; $^b P < 0.001$; ns = not significant.

Under these experimental conditions, the α_2-adrenoceptor agonists detomidine and oxymetazoline, both administered at 1.5, 5 and 15 μmol/kg ($n = 8$ for each dose of agonist), did not affect basal acid secretion (not shown), but they induced a significant and dose-dependent inhibition of acid output evoked by vagal stimulation (Fig. 1). When tested alone, the α_2-adrenoceptor antagonists idazoxan, 7,8-(methylenedioxy)-14-α-hydroxyalloberbane (CH 38083), 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline (RX 821002), yohimbine, rauwolscine (all tested at 15 μmol/kg), prazosin or ARC 239 (both tested at 15 and 45 μmol/kg) ($n = 4$ for each dose of antagonist) failed to modify either basal or vagally stimulated acid secretion (not shown).

In agonist-antagonist interaction experiments, idazoxan, CH 38083, RX 821002, yohimbine or rauwolscine, all administered at the dose of 15 μmol/kg ($n = 6$ for each drug), significantly antagonized the inhibitory effect exerted by detomidine or oxymetazoline (both administered at 1.5 μmol/kg i.v.) on vagally induced acid secretion (Fig. 2). Prazosin partly antagonized the antisecretory action induced by detomidine 1.5 μmol/kg when tested at the dose of 45 μmol/kg ($n = 6$), whereas it was without effect against oxymetazoline 1.5 μmol/kg ($n = 6$) (Fig. 2). By contrast, ARC 239 (15 and 45 μmol/kg; $n = 6$ for each dose) did not significantly modify the depressant action exerted by detomidine or oxymetazoline on gastric acid output elicited by vagal stimulation (Fig. 2).

4. Discussion

α_2-Adrenoceptors have been subdivided in different subtypes on the basis of their differential affinity for several drugs (Bylund, 1992; MacKinnon et al., 1994). However, very few studies have made a functional characterization of α_2-adrenoceptor subtypes in in vivo models. In the present study, we attempted to make a subclassification of the presynaptic α_2-adrenoceptor subtypes involved in the modulation of gastric acid secretion at the peripheral level and, for this purpose, a gastric hypersecretory response was elicited by electrical stimulation of the vagus nerve in reserpinized rats. In this respect, our data, showing that the vagally induced acid output was markedly reduced by atropine or detomidine, confirm the results of previous studies showing that pre-junctional α_2-adrenoceptors on vagal cholinergic endings mediate an inhibitory control of gastric secretion through a decrease in acetylcholine release (Del Tacca et al., 1982).

Under the experimental conditions adopted in the present study, oxymetazoline was nearly as effective as detomidine in inhibiting the acid secretory response elicited by vagal stimulation. The antisecretory effects of both these agonists were significantly prevented by

several α_2-adrenoceptor antagonists, including RX 821002, which was found to be an appropriate ligand for labelling all α_2-adrenoceptor subtypes in binding studies (O'Rourke et al., 1994), although it shows relatively higher affinity for α_{2A} than for α_{2B} sites (Uhlen and Wikberg, 1991). In addition, the inhibitory effect of detomidine or oxymetazoline was not affected by ARC 239, even when administered at relatively high doses, whereas prazosin was partly effective at the highest dose against detomidine. According to the currently proposed criteria (Bylund, 1992), our results suggest that the pharmacological profile of α_2-adrenoceptors located on vagal nerve terminals of the rat stomach resembles more that of α_{2A} or α_{2D} than that of α_{2B} or α_{2C} binding sites. This view is in full agreement with previous data showing that presynaptic α_2-adrenoceptor subtypes involved in the modulation of acetylcholine release from both submucosal and myenteric plexus of guinea-pig ileum exhibit high sensitivity for oxymetazoline and low affinity for prazosin and ARC 239 (Shen et al., 1990; Blandizzi et al., 1993).

Although four different α_2-adrenoceptor subtypes have been identified by means of pharmacological tools, genes coding only three subtypes have been cloned from humans and rats (MacKinnon et al., 1994). However, genes for human α_{2A} and rat α_{2D} subtype share very high sequence identity and their products exhibit full immunological cross-reactivity (Kurose et al., 1993). In addition, the rat α_{2D} subtype differs pharmacologically from the human α_{2A}-adrenoceptor mainly because of a relatively lower affinity for yohimbine and rauwolscine (MacKinnon et al., 1994). On these bases, it is now accepted that the α_{2A}- and α_{2D}-adrenoceptors represent species homologues of the same receptor subtype, and that the rat α_{2D}-adrenoceptor should be better classified as 'α_{2A}-like adrenoceptor' (MacKinnon et al., 1994). In this regard, it appears interesting to mention that, in our experiments, both yohimbine and rauwolscine were less effective than RX 821002, idazoxan and CH 38083 in antagonizing the antisecretory effects of detomidine or oxymetazoline.

In conclusion, the present study suggests that peripheral α_{2A}-like adrenoceptors modulate vagally induced acid secretion from rat stomach, and confirms that drugs such as oxymetazoline, prazosin and ARC 239 represent valuable tools for the characterization of α_2-adrenoceptor subtypes also in in vivo models under appropriate experimental conditions.

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