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Determination on functional basis of presynaptic α_2-adrenoceptor subtypes in guinea-pig duodenum

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Abstract

The effects of several α_2-adrenoceptor agonists and antagonists were examined on the cholinergic twitch contractions evoked by electrical field stimulation of guinea-pig duodenum. Oxymetazoline, xylazine, noradrenaline, α-methyl-noradrenaline or medetomidine (0.01–30 μM) were nearly equieffective in inhibiting duodenal twitch responses. The effects of xylazine were competitively counteracted by antagonists tested (0.03–10 μM) with the following order of potency: RX 821002 = idazoxan > rauwolscine = yohimbine = BRL 44408 >> prazosin = ARC 239 = BRL 41992. According to the current classification, it is suggested that α_2-heteroadrenoceptors involved in the modulation of duodenal cholinergic neurotransmission belong to the α_{2D} subtype.

Keywords: Presynaptic α_2-adrenoceptors; Receptor subtypes; Cholinergic nerves; Intestinal motility; Duodenum

The current classification of α_2-adrenoceptors distinguishes four subtypes, named α_{2A}, α_{2B}, α_{2C} and α_{2D}, on the basis of their different pharmacological characteristics [5]. α_{2A}-Adrenoceptors display higher affinity for oxymetazoline and BRL 44408 (2-[2*H*-(1-methyl-1,3-dihydroisoindole)-methyl]-4,5-dihydroimidazoline) than for prazosin, ARC 239 (2-(2,4-(*O*-methoxy-phenyl)-piperazin-1-yl)-ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione HCl) and BRL 41992 (1,2-dimethyl-2,3,9,13*b*-tetrahydro-1*H*-dibenzo[*c*,*f*]imidazol[1,5-*a*]azepine HCl), while α_{2B}-adrenoceptors exhibit a converse selectivity for these drugs [5,16]. The pharmacological profiles of α_{2C}- and α_{2D}-adrenoceptors closely resemble those for α_{2B} and α_{2A} binding sites, respectively [5]. It is noteworthy also that homogeneous populations of native prototypic α_2-adrenoceptor subtypes are expressed in various tissues and cell lines, including human platelets and HT29 cells (α_{2A}), neonatal rat lung (α_{2B}) [4, 13], opossum OK cells (α_{2C}) [3], rat submaxillary gland and RINm5F cells (α_{2D}) [12,13].

Although four different α_2-adrenoceptor subtypes were identified by means of pharmacological tools, genes coding only three subtypes have been cloned so far [5]. In particular, α_2-adrenoceptors cloned from human libraries, designated as α_2-C10, α_2-C2 and α_2-C4, exhibit pharmacological characteristics corresponding to those of prototypic α_{2A}, α_{2B} and α_{2C} subtypes, respectively [6], whereas the binding properties of α_2-adrenoceptors encoded by rat genes, named α_2-RNG, α_2-RG10 and α_2-RG20, show high correlation with those of native α_{2B}, α_{2C} and α_{2D} sites, respectively [8,10,15]. Therefore, since genes coding for human α_{2A}- and rat α_{2D}-adrenoceptors share very high sequence identity, and their co-existence within the same species has not been demonstrated, it is now accepted that α_{2A} and α_{2D} sites represent species homologues of the same receptor subtype [5].

At intestinal level, efforts to define α_2-adrenoceptor subtypes involved in the modulation of cholinergic neurotransmission were performed on ileal preparations by means of neurochemical approaches [1,7]. In the present study, several α_2 ligands were tested on the cholinergic motor responses of guinea-pig duodenum, in an attempt to subclassify the α_2-adrenoceptor subtypes located on cholinergic nerve terminals of myenteric plexus.

Experiments were carried out according to the technique previously described by Poli et al. [11], with minor

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modifications. Male albino guinea-pigs, 300–400 g body weight, were killed by cervical dislocation; the whole duodenum was rapidly removed and placed in Krebs solution aerated with 95%O_2 + 5% CO_2 gas mixture. The Krebs solution had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.5 (pH 7.4 ± 0.1). Segments of duodenum (15–20 mm long) were set up in 10-ml organ baths at 37°C containing oxygenated Krebs solution, and suspended vertically from isometric transducers under an initial resting tension of 0.5 g. The contractile activity of the longitudinal muscle was recorded by a poligraph. Electrical field stimulation (1 ms, 200–250 mA, 0.1 Hz) of the duodenal segments evoked recurrent muscle contractions (twitch responses), which became stable in their amplitude within 30–60 min and lasted up to 3–4 h. This contractile activity was completely abolished by tetrodotoxin (1 μM) or atropine (0.01 μM), but was unaffected by hexamethonium (10 μM), indicating an involvement of postganglionic cholinergic nerves.

Agonists were added cumulatively to the bathing fluid in 0.5 log unit increments. It was possible to construct at least three concentration-response curves for a given agonist in the same preparation without the occurrence of significant desensitization phenomena, provided that a 60-min interval elapsed between two subsequent concentration-response curves. Because tissues were observed to recover rapidly from maximally effective concentrations of xylazine after washing, it was possible to study the interaction of this agonist with at least two different concentrations of a given antagonist in the same preparation. For this purpose, 60 min were allowed to elapse between two consecutive concentration-response curves, the antagonists were added to the bath 20 min before xylazine, and one of two duodenal segments taken from each animal served as control and received xylazine alone in order to correct for possible time-dependent changes of the agonist efficacy. The agonist potencies were expressed as IC_{50} (concentration required to produce 50% of the maximal inhibitory effect); the percent maximum inhibition of the control twitch response (E_{max}) was also evaluated. The Schild analysis was applied to data obtained from agonist-antagonist interaction experiments and, after verifying that the slope of Schild plot was not significantly different from unity, the slope was constrained to unity with consequent estimation of the antagonist potencies as pK_B values [9]. Results are given as means ± SEM. The significance of differences was evaluated by Student's t-test. P values lower than 0.05 were considered to be significant.

The following drugs were used: oxymetazoline HCl, xylazine HCl, noradrenaline bitartrate, prazosin HCl, atropine sulphate, tetrodotoxin (Sigma Chemical Co., St. Louis, MO); α-methyl-noradrenaline, RX 821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline), idazoxan HCl, rauwolscine HCl, yohimbine HCl, hexamethonium

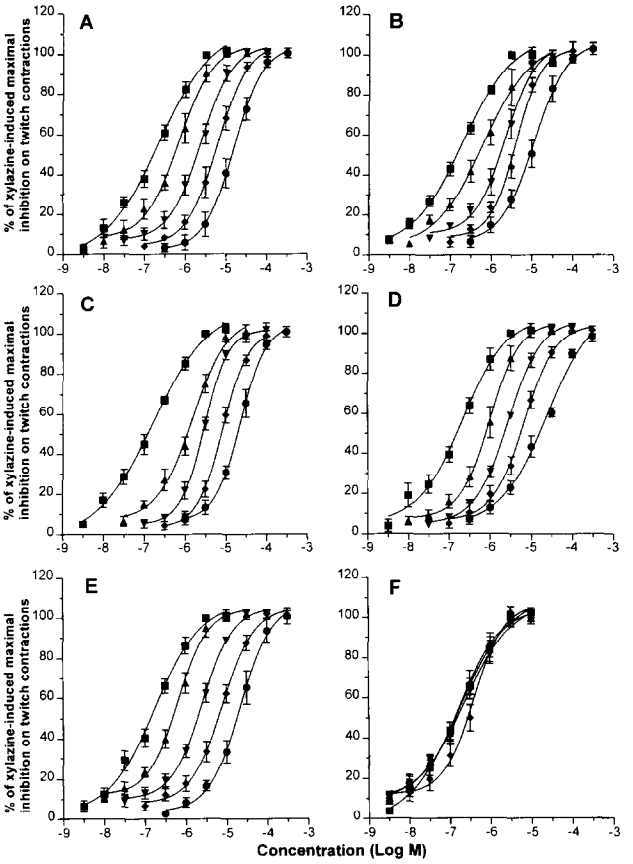

Fig. 1. Inhibitory effect of xylazine on cholinergic twitch contractions evoked by electrical field stimulation (1 ms, 200–250 mA, 0.1 Hz) of guinea-pig duodenum either in the absence (■) or in the presence of: RX 821002 0.03 (▲), 0.1 (▼), 0.3 (◆), 1 (●) μM (A); idazoxan 0.03 (▲), 0.1 (▼), 0.3 (◆), 1 (●) μM (B); rauwolscine 0.3 (▲), 1 (▼), 3 (◆), 10 (●) μM (C); yohimbine 0.3 (▲), 1 (▼), 3 (◆), 10 (●) μM (D); BRL 44408 0.3 (▲), 1 (▼), 3 (◆), 10 (●) μM (E); prazosin 10 μM (▲), ARC 239 10 μM (▼) or BRL 41992 10 μM (◆) (F). Each concentration-response curve represents the mean of 6–12 experiments.

2HCl (RBI, Natick, MA); medetomidine HCl (Farmos, Turku, Finland); ARC 239 (kindly provided by Karl Thomae, Biberach, Germany); BRL 44408 and BRL 41992 (both kindly provided by Smith Kline Beecham, Frythe, Welwyn, UK).

Under the experimental conditions adopted in the present study, oxymetazoline (0.01–10 μM), xylazine (0.01–10 μM), noradrenaline (0.01–10 μM), α-methyl-noradrenaline (0.01–10 μM), or medetomidine (0.01–30 μM) caused a concentration-dependent decrease in twitch contractions. The following IC_{50} and E_{max} values were calculated for each agonist tested: 0.11 ± 0.04 μM, 87.6 ± 3.9% (oxymetazoline, n = 15); 0.13 ± 0.03 μM, 86.4 ± 3.5% (xylazine, n = 30); 0.20 ± 0.01 μM, 84.6 ± 6.8% (noradrenaline, n = 10); 0.15 ± 0.01 μM, 86.2 ± 4.3% (α-methyl-noradrenaline, n = 10); 0.81 ± 0.06 μM, 88.6 ± 7.2% (medetomidine, n = 10).

In the presence of different concentrations of RX 821002 (0.03–1 μM), idazoxan (0.03–1 μM), rauwolscine

Table 1

Effects of α_2-adrenoceptor antagonists on xylazine-induced inhibition of cholinergic twitch contractions evoked by low frequency electrical field stimulation of guinea-pig duodenum

Antagonist	pK_B	Slope	P
RX 821002	8.07 (8.17–7.96)	−0.98 (1.27–0.68)	0.789
Idazoxan	8.02 (8.25–7.79)	−0.85 (1.36–0.34)	0.347
Rauwolscine	7.27 (7.47–7.06)	−0.89 (1.40–0.39)	0.467
Yohimbine	7.14 (7.30–6.98)	−0.89 (1.23–0.56)	0.307
BRL 44408	7.11 (7.28–6.94)	−1.12 (1.45–0.80)	0.239
Prazosin	<5.00	ND	ND
ARC 239	<5.00	ND	ND
BRL 41992	<5.00	ND	ND

Values reported for pK_B and slope, with 95% confidence limits in brackets, are the mean of a minimum of six determinations. P, level of probability for slope of Schild plot to be significantly different from −1. ND, not determinable.

(0.3–10 μM), yohimbine (0.3–10 μM) or BRL 44408 (0.3–10 μM) there was a parallel displacement to the right of the concentration-response curve to xylazine (Fig. 1A–E). Schild plot analysis showed that slopes were not significantly different from negative unity, thus suggesting that the antagonist action was competitive in nature (Table 1). By contrast, prazosin, ARC 239 or BRL 41992, all applied at concentrations up to 10 μM, failed to affect the inhibitory action of xylazine on cholinergic twitch contractions (Fig. 1F). Data obtained from correlation analysis between pK_B values obtained in the present study and pK_d values reported by other authors for native α_2 binding sites or α_2-adrenoceptor genes transfected into COS cells are shown in Table 2.

The results of the present study, showing that the electrically-induced duodenal motility was concentration-dependently decreased by different α_2 agonists, are in agreement with data obtained by Poli et al. [11], who demonstrated that the activation of prejunctional α_2-adrenoceptors in the guinea-pig duodenum is associated with a restriction of Ca^{2+} access into cholinergic nerve terminals through N-type Ca^{2+} channels, thus resulting in a reduction of acetylcholine release.

In our experiments, oxymetazoline was nearly as potent and effective as xylazine, or other α_2 agonists, in decreasing the twitch responses of duodenal longitudinal muscle. The inhibitory action of xylazine was significantly counteracted by several α_2 antagonists, including BRL 44408, which was found to be an appropriate ligand for discriminating between α_{2A}- and α_{2B}-adrenoceptor subtypes [5,16]. In addition, the inhibitory effect of xylazine was not affected by prazosin, ARC 239 or BRL 41992, even when these antagonists were applied at relatively high concentrations. These results, taken together with data provided by correlation analysis (Table 2), suggest that α_2-adrenoceptors located on cholinergic nerve endings of guinea-pig duodenum do not belong to the α_{2B} or α_{2C} subtype, and that their pharmacological profile

Table 2

Correlation between pK_B values of antagonists at α_2-heteroadrenoceptors of guinea-pig duodenum and pK_d values at other α_2-adrenoceptor binding sites

Binding sites	r	Slope	Number of antagonists	P
Native α_2-adrenoceptors				
Human platelets (α_{2A}) [4,13]	0.912**	0.69	7	0.004
HT29 cells (α_{2A}) [4]	0.928*	0.78	5	0.023
Neonatal rat lung (α_{2B}) [4,13]	0.001	0.01	7	0.974
OK cells (α_{2C}) [3]	0.865	0.58	5	0.058
Rat submaxillary gland (α_{2D}) [13]	0.909**	0.71	7	0.004
RINm5F cells (α_{2D}) [12]	0.932**	0.79	7	0.002
Human α_2-adrenoceptor genes transfected into COS cells				
α_2-C10 (α_{2A}) [6]	0.756*	0.61	7	0.048
α_2-C2 (α_{2B}) [6]	0.077	−0.05	7	0.872
α_2-C4 (α_{2C}) [6]	0.380	0.19	7	0.399
Rat α_2-adrenoceptor genes transfected into COS cells				
α_2-RNG (α_{2B}) [8,15]	0.077	0.01	6	0.881
α_2-RG10 (α_{2C}) [10]	0.780	0.40	4	0.220
α_2-RG20 (α_{2D}) [8]	0.980**	0.64	5	0.003

Shown are correlation coefficients (r) and slopes of the regressions 'pK_d at α_2-adrenoceptor binding sites' on 'pK_B at guinea-pig duodenal α_2-heteroadrenoceptors'. pK_B values at guinea-pig duodenal α_2-heteroadrenoceptors from Table 1 (4.5 for <5). pK_d values at α_2-adrenoceptor binding sites from studies quoted in the reference list.
*Significantly different from 0, $P < 0.05$.
**Significantly different from 0, $P < 0.01$.

rather fits with that of α_{2A} or α_{2D} binding sites. This view is in line with findings of previous studies showing that α_2-adrenoceptor subtypes, mediating the prejunctional inhibition of cholinergic neurotransmission at gastric or ileal level, exhibit high sensitivity to oxymetazoline and very low affinity for prazosin and ARC 239 [1,2,7].

The comparison between our pK_B values and affinities of the same antagonists for prototypic native or recombinant α_2 binding sites revealed a significant correlation with both α_{2A} and α_{2D} subtypes, thus suggesting that the presynaptic α_2-adrenoceptors examined in the present study would possess pharmacological characteristics intermediate between those of human α_{2A} and rat α_{2D} subtypes. However, it must be noted that the main pharmacological difference between α_{2A}- and α_{2D}-adrenoceptors lies in their different affinities towards yohimbine and rauwolscine [5]. In this regard, it is worthy of mention that the pK_B values obtained for yohimbine (7.14) and rauwolscine (7.27) in our experiments are more closely related to the affinities of these drugs for α_{2D} (7.15 and 7.28, respectively) than for α_{2A} (8.50 and 8.72, respectively) binding sites [13]. In addition, Svensson et al. [14] have recently identified the guinea-pig gene homologue of the human α_2-C10 adrenoceptor subtype, and found

that yohimbine binds to this receptor, transiently expressed in COS cells, with a pK_d value of 7.68. Overall, according to these findings, it appears that α_2-heteroadrenoceptors of guinea-pig duodenum may be classified as α_{2D} subtypes.

In conclusion, our data provide evidence in support of the heterogeneity of prejunctional α_2-adrenoceptors and suggest that the inhibitory adrenergic activity on cholinergic duodenal motility is mediated by α_{2D}-adrenoceptor subtypes.

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