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Evidence for functional α_{2D}- adrenoceptors in the rat intestine

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1 Peristaltic contractions were induced in segments of rat ileum by raising the intramural pressure. A mean pressure of 4.3 ± 0.2 cmH_2O ($n=112$) was required to initiate rhythmic contractions of the longitudinal muscle (preparatory phase) and associated volume expulsions caused by circular muscle contraction. The frequency of peristalsis remained constant over two 15 min periods of stimulation.

2 The α_2-adreoceptor agonists, clonidine, UK 14,304, B-HT 920, B-HT 933 and the selective α_{2A}-agonists, oxymetazoline and guanfacine, caused concentration-dependent inhibition of peristaltic contractions. The potency order and IC_{50} values (nM) were: clonidine (2.81) $\geqslant$ oxymetazoline (4.23) $\geqslant$ UK 14,304 (4.48) $\geqslant$ guanfacine (5.51) > B-HT 920 (78.72) > B-HT 933 (442.48).

3 High concentrations of clonidine, amounting to more than 10 and 100 times the IC_{50} value in the peristaltic reflex (30 and 300 nM respectively), failed to inhibit the cholinergic contractile response to transmural electrical stimulation over the range of 2.5 to 40 Hz.

4 α_2-Adrenoceptor antagonists were used to determine the subtype of presynaptic α_2-adrenoceptor in rat ileum. All the antagonists tested caused parallel shifts to the right of the concentration-response regression line to clonidine and none, except ARC 239, influenced the rate of peristalsis. However, ARC 239 significantly decreased the frequency of control period peristaltic contractions. The order of affinity of the antagonists against clonidine (pK_B values in parentheses) was RX 821002 (8.99) > phentolamine (8.07) > BRL 44408 (7.43) $\geqslant$ rauwolscine (7.41) $\geqslant$ yohimbine (7.28) > prazosin (5.86) $\geqslant$ ARC 239 (5.74).

5 These results, when compared with binding and functional data from various other tissues and cell lines, are consistent with the presence of presynaptic α_{2D}-adrenoceptors in rat ileum. Further evidence is provided that this subtype of α_2-adrenoceptor is probably located proximal to the final cholinergic neurones in the reflex arc.

Keywords: Rat ileum; peristalsis; transmural stimulation; α_2-adrenoceptor subtypes; α_2-adrenoceptor agonists; α_2-adrenoceptor antagonists; pA_2; pK_B

Introduction

α_2-Adrenoceptors have been classified into four subtypes, named α_{2A}, α_{2B}, α_{2C} and α_{2D}. The basis for this classification is on receptor cloning and expression, correlation of radioligand binding affinity and antagonist affinity in functional assays (Bylund, 1988; Bylund *et al.*, 1988; 1991; Harrison *et al.*, 1991; Lanier *et al.*, 1991; MacKinnon *et al.*, 1994). Three genes on chromosomes 2, 4 and 10, code for α_2-adrenoceptors in man (Lorenz *et al.*, 1990), and three rat genes, RG10, RG20 and RNG have also been expressed (Lanier *et al.*, 1991). The gene located on human chromosome 10 (α_2-C10) corresponds to the α_{2A} subtype, and is 89% homologous with rat RG20 gene. The gene on human chromosome 2 (α_2-C2) has marked similarities to the α_{2B} subtype and may be the equivalent of rat RNG gene. The profile of the gene on human chromosome 4 (α_2-C4) most closely resembles the α_{2C} receptor and may be the equivalent of rat RG10 gene (Lorenz *et al.*, 1990; Lanier *et al.*, 1991; MacKinnon *et al.*, 1994).

The α_2-antagonists, yohimbine and rauwolscine, display a high affinity for α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors. The α_{2A} subtype occurring in human platelets and in the colon adenocarcinoma cell line HT29 can be distinguished by the low affinity of prazosin and high affinity of oxymetazoline (Bylund *et al.*, 1988). The α_{2B} subtype, which is expressed in neonatal rat lung, has a relatively high affinity for prazosin (Bylund *et al.*, 1988). The α_{2C} receptor, which was found in the opossum kidney (OK cell line), is similar to the α_{2B} with a relatively high affinity for prazosin and over a 60 fold higher affinity for BAM 1303 (Murphy & Bylund, 1988; Blaxall *et al.*, 1991). Various rodent tissues, for instance, rat submaxillary gland and rat vas deferens (Michel *et al.*, 1989; Smith & Docherty, 1992) and bovine pineal gland (Simonneaux *et al.*, 1991) express a peculiar α_2-adrenoceptor, which is characterized by a 20 fold lower affinity for the classical antagonists, yohimbine and rauwolscine. It has been suggested on the basis of these properties, that this receptor should be named the α_{2D}-adrenoceptor or an 'α_{2A}-like-adrenoceptor' (MacKinnon *et al.*, 1994). Since there is no evidence to suggest that both α_{2A} and α_{2D} subtypes exist in the rat, it is considered that the RG20 gene, and therefore the α_{2D}-adrenoceptor, may be the rat homologue of the human α_{2A}-receptor. However, it is sufficiently distinct pharmacologically, so the α_{2D} designation is used here.

Previous studies on guinea-pig ileum and rabbit jejunum have demonstrated that the peripheral α_2-adrenoceptors are located presynaptically on postganglionic neurones innervating intestinal smooth muscle cells. Further, α_2-adrenoceptors mediate inhibition of small intestinal contraction by inhibiting acetylcholine release from enteric cholinergic nerves (Doherty & Hancock, 1983; Wikberg, 1977; Andrejak *et al.*, 1980).

The aims of the present study were to determine whether the α_2-adrenoceptors are a functional part of the peristaltic reflex in the rat ileum and to determine the α_2-adrenoceptor subtype to which they belong. For these purposes, the effects of α_2-adrenoceptor agonists in suppressing the peristaltic reflex were measured and pA_2 values for antagonists against clonidine were determined.

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Methods

Peristalsis

Hooded Wistar rats of either sex, 250–350g, were stunned by a blow to the head and killed by exsanguination. Segments of ileum (5 cm proximal to the caecum) were excised and tied at the aboral end onto a glass J tube. Each tissue was placed in a 30 ml jacketed organ bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, D-(+)-glucose 11, maintained at 37°C and continuously gassed with 95% oxygen: 5% carbon dioxide. Segments were tied off at the oral end to give lengths of between 7–9 cm and the cotton thread was connected to an isotonic transducer (Ugo Basil) to monitor length changes of the longitudinal smooth muscle under a resting tension of 1 g. The free end of the J tube was connected by flexible tubing to a 5 ml float chamber half filled with Krebs-Henseleit solution.

The float was connected to a second isotonic transducer to record volume changes related to circular muscle contraction. Both the reservoir and transducer (volume meter) were attached to a worm screw stand, enabling them to be raised gradually above the fluid level of the organ bath to increase intraluminal pressure in the ileum and initiate peristaltic contractions. The frequency and intensities of the longitudinal and circular muscle contractions were recorded on a Grass model 79D polygraph.

The ileum segments remained quiescent with only occasional contractions of the longitudinal muscle when the height of the Krebs-Henselet solution in the reservoir was set at the same level of liquid as in the organ bath. After a 45 min equilibration period, the intraluminal pressure was increased gradually at approximately 3 mm s^{-1} until it initiated a peristaltic wave. This was recorded as a contraction of the longitudinal muscle (preparatory phase) closely followed by expulsion of fluid (circular muscle contraction). The pressure resulting in a peristaltic contraction was maintained for two periods of 15 min separated by a rest period of 10 min at zero pressure. In each period, 3–4 control peristaltic contractions were recorded before one of agonists was added cumulatively to the bath fluid until an obvious decrease in the rate of peristaltic contractions occurred. The antagonist, added 10 min before the control contraction, was present in the bathing fluid for approximately 15 min before addition of clonidine. Three concentrations of each antagonist were tested with the exception of ARC-239 which was tested at 1 and 3 μM.

Transmural stimulation

Segments of rat ileum (3–4 cm) were mounted in 30 ml jacketed organ baths containing Krebs-Henseleit solution (see above) at 37°C and allowed to equilibrate under a resting load of 1 g for 45 min. Changes in the length of the longitudinal smooth muscle were recorded with isotonic transducers (Ugo Basil) and displayed on a multichannel Grass model 79D polygraph. The segments of rat ileum were stimulated transmurally at supramaximal voltage with pulses of 1 ms duration at frequencies of 2.5, 5, 10, 20 and 40 Hz in trains lasting 8 s. The trains of pulses were delivered 3 min apart.

Drugs

The following drugs were used: ARC-239 hydrochloride (2-(2,4-(O-methoxyphenyl)-piperazine-1-yl)-ethyl-4, 4-dimethyl-1, 3-(2H,4H)-isoquinolinedine; Boehringer Ingelheim, Artarmon, Australia), B-HT 920 dihydrochloride (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine; Boehringer Ingelheim, Germany), B-HT 933 dihydrochloride (2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-(5,4-d)-azepine; Boehringer Ingelheim, Artarmon, Australia), BRL 44408 (2-(2H-(1-methyl-1,3-dihydroisoindole) methyl)-4, 5-dihydro-imidazoline; SmithKline Beecham, Welwyn, U.K.), clonidine hydrochloride (Sigma, St. Louis, U.S.A.), guanfacine hydrochloride (Sandoz, Basel, Switzerland), oxymetazoline hydrochloride (ICN Biochemicals, Cleveland, U.S.A.), phentolamine mesylate (Ciba-Geigy, Basel, Switzerland), prazosin hydrochloride (Pfizer, Sydney, Australia), rauwolscine hydrochloride (Research Biochemical Inc., Natick, U.S.A.), RX 821002 hydrochloride (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline; Research Biochemical, Natick, U.S.A.), UK 14,304, (5-bromo-6-(2-imidazolin-2-yl-amino)-quinoxaline; Research Biochemical Inc., Natick, U.S.A.), yohimbine hydrochloride (ICN Biochemicals, Aurora, U.S.A.).

UK 14,304 and prazosin were dissolved in ethanol and then diluted in distilled water so that the final concentration of ethanol in the bath was less than 0.05%. BRL 44408 was dissolved in 10 mM HCl and then diluted in distilled water. All other drugs were dissolved in distilled water.

Statistic analysis

The inhibitory effect of the α_2-adrenoceptor agonists was expressed as a percentage of the contraction rate present before addition of the agonist. Semilogarithmic linear regression analysis was used to determine the potencies of the agonists which are expressed as IC_{50} values defined as the concentration causing a 50% inhibition in the rate of peristalsis. The negative logarithm of receptor dissociation constants of the antagonists (pK_B) were the means of multiple pK_B values calculated from the equation: $pK_B = -\log [B] + \log (DR-1)$, (Furchgott, 1972), where [B] is the one point molar concentration of antagonist and DR (dose-ratio) is the ratio of the clonidine IC_{50} in the presence of antagonist, divided by the geometric mean IC_{50} in the absence of antagonist. pA_2 values and slopes of the regressions were estimated using three point concentrations of antagonists according to the method of Arunlakshana & Schild (1959) with unconstrained slopes. Results are given as the mean with the 95% confidence limits. The data from the clonidine dose-response curve in the presence and absence of all the antagonists were subjected to the test for parallelism by the computer programme described by Tallarida & Murray (1987). The significance of differences were analysed by Student's paired or unpaired t test where appropriate. The criterion for statistical significance was set at $P<0.05$.

Results

Peristalsis

A mean pressure of 4.3 ± 0.2 cmH_2O ($n=112$) was required to initiate rhythmic contractions of the longitudinal muscle and associated volume expulsions. The frequency of peristalsis remained the same over 15 min at 0.89 ± 0.09 peristaltic waves per min ($n=6$), and each resulted in an 18 ± 2% shortening of the longitudinal muscle and expulsion of 70 ± 9 μl of fluid per cm of tissue length ($n=6$) in the control preparations of rat ileum.

Inhibitory effect of α_2-adrenoceptor agonists on peristalsis

The α_2-adrenoceptor agonists all induced concentration-dependent decreases in the rate of peristalsis with the following order of potency: clonidine $\geqslant$ oxymetazoline $\geqslant$ UK 14304 $\geqslant$ guanfacine > B-HT 920 > B-HT 933 (Figure 1). The individual IC_{50} values and potency ratios relative to clonidine are summarized in Table 1 and the inhibitory effect of clonidine on peristaltic contraction is shown in Figure 2.

Influence of α-adrenoceptor antagonists on the inhibitory effect of clonidine on peristalsis

The addition (10 min incubation) of three different concentrations of the α-adrenoceptor antagonists, BRL 44408,

phentolamine, prazosin, rauwolscine, RX 821001 and yohimbine to the bathing fluid did not influence the frequency of peristaltic contractions compared to the control rate of 0.89 ± 0.09 peristaltic waves per min ($n=6$). However, ARC 239 at concentrations of 1 and 3 μM decreased the frequency of control period peristaltic contractions to 0.69 ± 0.04 ($n=4$) and 0.54 ± 0.06 ($n=6$) min^{-1} respectively. The latter frequency was significantly slower than that of the control group (Student's t test, $P<0.05$). ARC 239, at a concentration of 10 μM completely abolished peristaltic contractions.

The influence of one of the antagonists, rauwolscine on the inhibitory effect of clonidine is shown in Figure 3. All antagonists caused a parallel rightward shift of the concentration-response to clonidine. Schild plots gave regression lines with slopes not different from unity with the exception of RX 821002. The pA_2 and pK_B values for the antagonists are summarized in Table 2.

Correlation between affinities of antagonists for the rat ileum presynaptic α_2-adrenoceptor and various other α_2 sites

Correlation between functional α_2-adrenoceptor affinities of antagonists in the rat ileum and α_2 binding or functional receptor affinities of antagonists in other tissues and cell lines is shown in Table 3. The rank order of potency of these antagonists markedly resembles the affinities displayed by the α_{2D} receptor subtype where RX 821002 > phentolamine > rauwolscine ≈ yohimbine > prazosin ≈ ARC 239 (Bylund *et al.*, 1991; Remaury *et al.*, 1992). A strong correlation of affinities was obtained when the data from rat ileum was compared with bovine pineal gland, rat cerebral cortex and rat submaxillary gland, tissues known to contain the α_{2D}-receptor subtype (Table 3).

Transmural stimulation

Transmural stimulation of rat isolated ileum induces frequency-related cholinergic contractions which are threshold at 2.5 Hz and peak at around 20 Hz (Coupar & DeLuca, 1994). Clonidine at 30 nM and 300 nM (10 min incubation) did not alter the responses of the rat ileum longitudinal muscle to stimulation at 2.5, 5, 10, 20 and 40 Hz delivered in trains of 8s duration compared to the control responses ($P>0.05$, Student's paired t test, $n=4$).

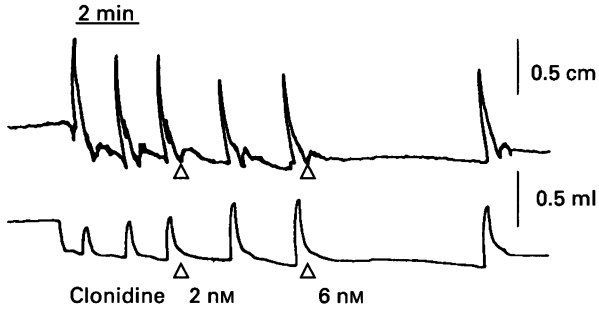

Figure 2 Inhibition by clonidine of the peristaltic reflex in a segment of rat ileum. The upper trace shows the contraction of the longitudinal muscle and the lower trace the corresponding volume expulsion as a result of circular muscle contraction in response to raising the intramural pressure to 4 cmH_20. Clonidine at 2 nM slowed the reflex while 6 nM virtually abolished it.

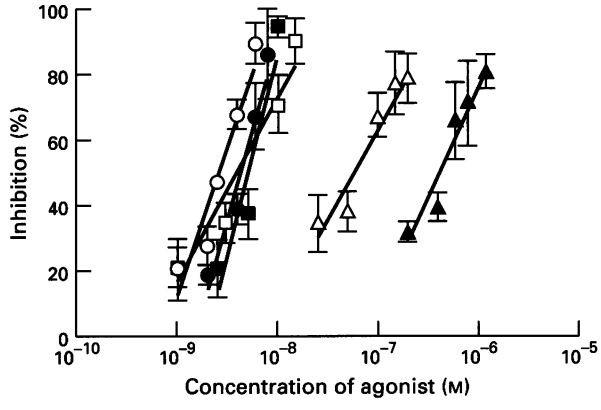

Figure 1 The effect of α_2-adrenoceptor agonists at inhibiting the peristaltic reflex of the rat ileum; (○) clonidine; (●) oxymetazoline; (□) UK 14304; (■) guanfacine; (△) B-HT920; (▲) B-HT933. Means with s.e.mean are shown.

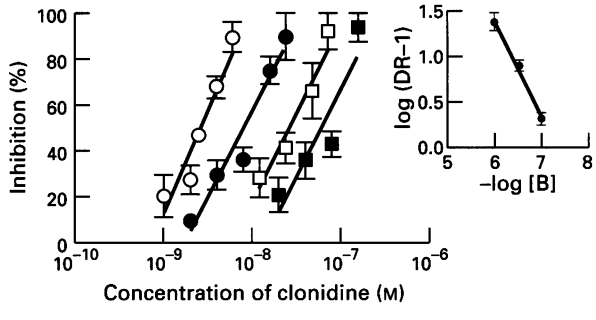

Figure 3 The inhibitory effect of rauwolscine on the response to clonidine in the peristaltic reflex of the rat ileum: (○) 0; (●) 0.1; (□) 0.3 and (■) 1 μM rauwolscine. Rauwolscine 10 min incubations caused parallel rightward shifts to the clonidine concentration-response regression lines. Insert: Schild plot of rauwolscine data. Mean values ± s.e.mean are shown.

Table 1 Potencies relative to clonidine and IC_{50} values (nM) of α_2-adrenoceptor agonists for inhibition of peristalsis in the rat ileum

Agonist	IC_{50} (95% CL)	*Potency*	n
Clonidine	2.81 (2.14–3.48)	1	6
Oxymetazoline	4.23 (2.81–5.65)	0.66	6
UK 14304	4.48 (2.98–5.98)	0.63	7
Guanfacine	5.51 (3.95–7.07)	0.51	5
B-HT 920	78.72 (55.18–102.26)	0.04	6
B-HT 933	442.48 (368.35–516.71)	0.006	5

Discussion

This study shows that the α_2-adrenoceptors of the rat small intestine have a functional role in suppressing the peristaltic reflex. The presence of functional α_2 receptors is shown by the pronounced activity of highly selective α_2-agonists, UK 14,304, B-HT 920 and B-HT 933 (Cambridge, 1981; Kobinger, 1986; Timmermans *et al.*, 1990). It is interesting to compare IC_{50} values and relative potencies of agonists used in our experiments to those obtained in previous work. The IC_{50} values of UK 14,304 and B-HT 920 for inhibition of peristalsis correspond to the values for UK 14,304 at inhibiting the twitch response induced by electrical stimulation of rat vas deference (4.48 vs 5.7 nM; Lattimer & Rhodes, 1985) and for B-HT 920 at causing α_2-adrenoceptor-mediated inhibition in the firing rate of rat locus coeruleus cells (78.72 vs 71 nM, Seutin *et al.*, 1990). In the present experiments, the IC_{50} value of clonidine is slightly lower than values from the two studies mentioned above (2.81 vs 4.9 and 5.3 nM respectively), but is close to the value for inhibiting the stimulation-evoked contraction in prostatic portions of the rat vas deferens (2.81 vs 2 nM, Salles *et al.*, 1994). Also, B-HT 933 was found to be the least potent agonist with a potency relative to clonidine of 0.006. This relative potency is virtually the same as the previously obtained value of 0.007 for inhibition of electrically-induced contraction of the rat vas deferens (Smith & Docherty, 1992).

The present experiments have established that clonidine does not inhibit transmitter release from the final cholinergic neurones innervating the longitudinal muscle of the rat ileum and hence this is not likely to be the site of action of the α_2-agonists. This finding contrasts to the location of α_2-adrenoceptors in the guinea-pig ileum. It has been well established that the guinea-pig ileum contracts in response to single pulses of electrical field stimulation (0.1 Hz) and that strong tetanic contraction is induced by high frequency (10 Hz; Wikberg, 1977). In comparison, the longitudinal muscle of the rat isolated small intestine does not respond to single pulses of transmural stimulation, but does respond to repetitive stimulation where the threshold for contractions occurs at around 2.5 Hz peaks at 10 to 20 Hz (Coupar & De Luca, 1994). High concentrations of clonidine, over 100 fold higher than its IC_{50} value, failed to show any inhibitory effect on cholinergic contractions induced by frequencies ranging from 2.5 to 40 Hz. However, in the guinea-pig ileum, clonidine inhibits contraction of longitudinal muscle in response to single pulses (0.1 Hz) with an IC_{50} of 10 nM (Doherty & Hancock, 1983) as well as at the considerably higher frequency of 5 Hz, where inhibition is apparent at concentrations as low as 4 nM (Andrejak *et al.*, 1980). Therefore, the present results showing that clonidine is a potent antagonist of the peristaltic reflex but not

Table 2 Affinity values of α_2-adrenoceptor antagonists as measured by ability to inhibit the response to clonidine in rat ileum

Antagonist (concentration; μM)	*Slope*	*pA_2*	*pK_B*
RX 821001 (0.001, 0.01, 0.1)	0.90* (0.82–0.98)	9.07 (8.95–9.19)	8.99 (8.70–9.28)
Phentolamine (0.1, 0.3, 1)	1.11 (0.96–1.26)	7.92 (7.75–8.09)	8.07 (7.82–8.32)
BRL 44408 (0.1, 0.3, 1)	1.07 (0.88–1.26)	7.36 (7.18–7.54)	7.43 (7.31–7.55)
Rauwolscine (0.1, 0.3, 1)	1.07 (0.85–1.29)	7.31 (7.12–7.50)	7.41 (7.26–7.56)
Yohimbine (0.1, 0.3, 1)	1.09 (0.81–1.37)	7.20 (7.0–7.40)	7.28 (7.15–7.41)
Prazosin (1, 1.8, 3)	1.09 (0.53–1.65)	5.82 (5.71–5.93)	5.86 (5.76–5.96)
ARC239 (1,3)			5.74 (5.55–5.93)

Slopes and pA_2 values were calculated by regression analysis of Schild plots, 95% confidence limits shown in parentheses, each concentration $n=4-8$. pK_B values are the means of 3 estimated values (2 for ARC239) calculated by using the equation of Furchgott (1972).
*Slope significantly different from 1 ($P<0.05$).

Table 3 Correlation between pK_B values of the antagonists in the rat ileum and various other α_2-adrenoreceptor sites

Tissue (cell line)	r	*Slope*	*Number of antagonists*
Correlation with functional presynaptic α_2-receptors			
Rabbit brain cortex (α_{2A})[a]	0.87*	0.93	6
Rat brain cortex (α_{2D})[a]	0.99***	1.02	5
Rat submaxillary gland (α_{2D})[b]	1.0***	1.01	4
Correlation with radioligand binding α_2 sites			
HT29 cell line (α_{2A})[c]	0.81	1.05	5
Human platelets (α_{2A})[d]	0.76	0.94	5
Human C10 clone (α_{2A})[e]	0.81	1.08	6
Neonatal rat lung (α_{2B})[c]	0.27	0.11	5
Human C2 clone (α_{2B})[e]	0.60	0.47	6
OK cell (α_{2C})[c]	0.45	0.58	5
Human C4 clone (α_{2C})[e]	0.59	0.43	6
Bovine pineal gland (α_{2D})[f]	0.96**	0.81	5
Rat brain cortex (α_{2D})[g]	0.96***	0.91	7
Rat submaxillary gland (α_{2D})[g]	0.97***	0.97	7
Rat jejunum epithelial cells (α_{2D})[h]	1.0***	1.07	5

Shown are correlation coefficients and slopes of the regression of 'pK_B at functional presynaptic α_2-adrenoceptors' or 'pK_i at α_2 binding sites' on 'pK_B at rat ileum presynaptic α_2-adrenoceptors'. pK_B values at rat ileum presynaptic α_2-adrenoceptors are from Table 2. Significant differences from 0: *$P<0.05$; **$P<0.01$; ***$P<0.001$.
[a]Trendelenburg *et al.* (1993); [b]Limberger *et al.* (1992); [c]Blaxall *et al.* (1991); [d]Bylund *et al.* (1988); [e]Devedjian *et al.* (1994); [f]Simonneaux *et al.* (1991); [g]Renouard *et al.* (1994); [h]Paris *et al.* (1990).

of transmural stimulation is evidence that the α_2-adrenoceptors that mediate inhibition of the preparatory phase of the peristaltic reflex are probably located proximal to the final cholinergic neurones in the reflex arc.

Seven antagonists were used to identify the subtype to which the rat ileum presynaptic α_2-adrenoceptors belong. Using clonidine as a 'non-selective' α_2-agonist, the slopes of Schild plots for the antagonists were shown to be close to unity and the affinity estimates as quantified by pA_2 and pK_B values agreed well, the maximal difference amounting to 0.15. However, we observed that ARC 239 at 3 μM significantly decreased the frequency of control period peristalsis, and 10 μM completely abolished peristalsis. The reason is unclear, especially since it has been reported that ARC 239 acted as neither agonist nor antagonist of the neurally evoked vasoconstriction in submucosal arterioles. In this tissue low concentrations of ARC 239 (1 and 3 nM) shifted the concentration-effect curves to clonidine and UK 14,304 to the left (Shen *et al.*, 1990).

Two comparisons have been made between the present results and previously published data. The first, was correlation analysis between the antagonist pK_B values found in the present experiments (Table 2) with literature values of pK_B or pK_i at the known subtypes of α_2-adrenoceptors in functional studies as well as radioligand binding sites (see Table 3). No significant correlation was shown between antagonist affinities in the rat ileum and affinity values at α_{2B}-binding sites of rat neonatal lung and human C2 clone ($r=0.27-0.60$) nor for affinity at α_{2C}-binding sites of the OK cell line and human C4 clone ($r=0.45-0.59$) (Blaxall *et al.*, 1991; Devedjian *et al.*, 1994). However, there was strong positive correlation of the present results with the affinity values of antagonists acting at α_{2D} receptors of bovine gland, rat brain cortex, rat submaxillary gland and rat jejunum epithelial cells both in functional α_2-receptors or binding sites ($r=0.96-1.0$) (Simonneaux *et al.*, 1991; Trendelenburg *et al.*, 1993; Limberger *et al.*, 1992; Renouard *et al.*, 1994; Paris *et al.*, 1990). There was also correlation, although not as strong, with the α_{2A}-sites of human platelet, HT29 cell line, human C10 clone and rabbit brain cortex ($r=0.76-0.87$) (Bylund *et al.*, 1988; Blaxall *et al.*, 1991; Trendelenburg *et al.*, 1993 Devedjian *et al.*, 1994;).

The second comparison is of antagonist K_B ratios for rat ileum α_2-adrenoceptors from the current experiments and K_i ratios at known binding sites as shown in Table 4. The ratios clearly differentiate the rat ileum presynaptic α_2-adrenoceptors from α_{2A}, α_{2B} and α_{2C} subtypes. For example, the phentolamine/yohimbine ratio at rat ileum α_2-adrenoceptors is 0.16 but 9.2 at α_{2A} sites; a 58 fold difference. Similarly, the difference in prazosin/yohimbine ratios for the rat ileum and the α_{2A}-site is 19 fold; the difference in oxymetazoline/yohimbine ratios is 888 fold at α_{2B}-sites; and the difference in prazosin/phentolamine ratios is 108 fold at both α_{2B} and α_{2C} sites. In contrast, the K_B ratios of the pairs of antagonists listed in Table 4 for rat ileum presynaptic α_2-adrenoceptors display less than 6 fold differences from the corresponding K_i ratios at α_{2D} sites (Blaxall *et al.*, 1991; Simonneaux *et al.*, 1991; Renouard *et al.*, 1994).

Three closely related genes, named RG-20, RNG, RG-10, have been discovered in the rat which encode for α_2-adrenoceptors (Lorenz *et al.*, 1990). Each of these genes is analogous to one of the human adrenoceptor genes. The rat analogue of human α_2C10 is RG-20 (89% identical), the rat analogue of α_2C2 is RNG (82%), and the rat analogue of α_2C4 is RG-10 (90%) (Harrison *et al.*, 1991). Comparing the present results with the results of ligand binding studies of the expressed RG-20, RNG and RG-10 receptors in rat (Harrison *et al.*, 1991), reveals that α_2-adrenoceptors in rat ileum correspond to the RG-20 receptor (see Table 4). For instance, the ratio of K_B values for phentolamine/yohimbine is 0.16 for the rat ileum α_2-adrenoceptors and the ratio of K_i values is 0.08 at the RG-20 binding site, only a 2 fold difference. However, the differences at RNG and RG-10 binding sites amount to 11 and 54 fold respectively. When the ratios of prazosin/phentolamine are compared it shows less than a 5 fold difference with the RG-20 binding site, but 56 and 46 fold-differences at RNG and RG-10 binding sites respectively. The ratios for oxymetazoline/yohimbine comparing the RG-20 binding site shows only a 4 fold difference, but the difference is considerably larger at 775 and 612 fold at RNG and RG-10 binding sites respectively.

It has been demonstrated that the rat RG-20 cloned receptor is identical to the α_2-adrenoceptor expressed in the rat submaxillary gland (Lanier *et al.*, 1991; Kurose *et al.*, 1993) and has similar ligand binding properties to the putative α_{2D} binding site found in bovine pineal gland (Bylund *et al.*, 1991). The presynaptic α_2-adrenoceptors identified in the present study, therefore, probably represent the receptor encoded by the rat RG-20 gene. Whether the RG-20 gene is a species homologue of the human α_{2A} gene or whether it represents a novel α_{2D} receptor remains to be investigated.

Furthermore, the high potency of oxymetazoline and guanfacine in the rat ileum rules out the possibility that the presynaptic α_2-adrenoceptors in this tissue are of the α_{2B} or α_{2C} subtypes. Oxymetazoline is a useful drug for discriminating the three human α_2-adrenoceptor subtypes, where it displays 108 and 13 fold higher affinities for the α_2C10 than the α_2C2 and α_2C4 respectively (Devedjian *et al.*, 1994). Guanfacine was also shown to have 60 fold selectivity for α_{2A} compared to α_{2B}-adrenoceptors (Uhlen & Wikberg, 1991).

All of the above comparisons are entirely consistent with assigning the presynaptic α_2-adrenoceptor of the rat ileum to the α_{2D} subtype. As a result, it is suggested that the rat ileum peristaltic reflex preparation could be used to develop drugs

Table 4 Ratios of dissociation constants at α_2-adrenoceptors

Tissue	*Phentolamine/ yohimbine*	*Prazosin/ yohimbine*	*Prazosin/ phentolamine*	*Oxymetazoline/ yohimbine*
Rat ileum	0.16	26	162	0.08
HT 29 cell (α_{2A})[a]	9.2	486	53	1.2
Neonatal rat lung (α_{2B})[a]	3.6	5.4	1.5	71
RNG (α_{2B})[b]	1.8	5.3	2.9	62
OK cell (α_{2C})[a]	52	91	1.5	166
RG-10 (α_{2C})[b]	8.7	31	3.5	49
Bovine pineal gland (α_{2D})[c]	0.91	29	33	0.4
Rat submaxillary gland (α_{2D})[d]	0.38	14	37	0.31
RG-20 (α_{2D})[b]	0.08	59	790	0.29

The values are ratios of K_B values for the pairs of antagonists in rat ileum (clonidine as agonist) with the exception of the agonist oxymetazoline where the IC_{50} value for inhibiting rat ileum peristalsis is used, and K_i ratios for various binding sites. [a]Blaxall *et al.* (1991); [b]Harrison *et al.* (1991); [c]Simonneaux *et al.* (1991); [d]Renouard *et al.* (1994).

with activity at α_{2D}-adrenoceptors. In addition, it is suggested that the agonist order of potencies and relative potencies established in this study could be incorporated into a framework for characterizing tissues with functional α_{2D}-adrenoceptors.

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