British Journal of Pharmacology (1998) 125, 127–135
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Rilmenidine reveals differences in the pharmacological characteristics of prejunctional α_2-adrenoceptors in the guinea-pig, rat and pig

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1 The α_{2A} and α_{2D}-adrenoceptor subtypes are thought to be species homologs most easily differentiated on the basis of the potency of antagonists. In the present study we have compared the effect of rilmenidine with two other selective α_2-adrenoceptor agonists, UK-14304 (5-bromo-6- [2-imidazolin-2-ylamino]-quinoxaline) and clonidine, against electrically-evoked contractions in five isolated preparations from the rat, guinea-pig and pig, and, where possible, determined the receptor subtype involved.

2 UK-14034, clonidine and rilmenidine produced concentration-dependent inhibition of the electrically-evoked contractions of the rat isolated vas deferens and tail artery and the guinea-pig ileum. These inhibitory effects were reversed by the selective α_2-adrenoceptor antagonist, RX-811058 (1 μM), except in the rat tail artery preparations where the remaining neurogenic response was inhibited; evidence for the involvement of 'innervated' α_2-adrenoceptors. Both clonidine and UK-14304 produced concentration-dependent inhibition of responses in the porcine isolated tail artery and urinary bladder but clonidine was markedly less efficacious in these preparations. In contrast, rilmenidine failed to inhibit the neurogenic contractions in either preparation.

3 Although rilmenidine failed to elicit a detectable response in either the porcine isolated tail artery or urinary bladder, it (10 μM and 30 μM, respectively) competitively antagonised the inhibitory effects of UK-14304 with an estimated dissociation constant of (pK_B) 5.82 and 5.93, respectively.

4 Prazosin (1 μM) failed to alter the effect of UK-14304 against neurogenic contractions in the porcine isolated urinary bladder, while rauwolscine (pK_B 8.87) was 10 fold more potent than phentolamine (pK_B 7.56). On the other hand, phentolamine (pK_B 8.42) was only marginally more potent than rauwolscine (pK 8.05) against clonidine-induced inhibition of electrically-evoked contractions of the guinea-pig isolated ileum. This pharmacological evidence with antagonists supports the presence of α_{2D}-adrenoceptors in the rat and guinea-pig and the α_{2A}-adrenoceptors in the pig.

5 We have demonstrated that rilmenidine, unlike clonidine and UK-14304, is devoid of any agonist activity at prejunctional α_{2A}-adrenoceptors in the pig, but is an efficacious agonist at α_{2D}-adrenoceptors in the rat and guinea-pig.

Keywords: Rilmenidine; clonidine; UK-14304; α_{2A}-adrenoceptors; α_{2D}-adrenoceptors; neurotransmission; urinary bladder; guinea-pig ileum; vas deferens; tail artery

Introduction

α_2-Adrenoceptors are currently subdivided into four subtypes, α_{2A}, α_{2B}, α_{2C} and α_{2D}, on the basis of their pharmacological properties (Bylund *et al*., 1994; Mackinnon *et al*., 1994). α_{2B}- and α_{2C}-adrenoceptors can be most easily distinguished from α_{2A}- and α_{2D}-adrenoceptors on the basis of the affinity of the selective α_1-adrenoceptor antagonist prazosin: this antagonist is approximately 30 fold more potent at $\alpha_{2B/C}$-adrenoceptors (pK_1 7) than at $\alpha_{2A/D}$-adrenoceptors (Bylund & Ray-Prenger, 1989; Bylund *et al*., 1994). α_{2A}- and α_{2D}-adrenoceptors are thought to represent species homologs of the same receptor, with the α_{2A}-present in man, pigs and rabbits, while the α_{2D}-adrenoceptor subtype is present in the rat, mouse, guinea-pig and cattle (Bylund *et al*., 1994). However, pharmacological differences have been revealed either by determining the rank order of potency of an extensive range of antagonists, followed by comparison with known preparations based on correlation coefficient (Renouard *et al*., 1994; O'Rourke *et al*., 1994; Trendelenburg *et al*., 1995), or establishing the potency ratio for pairs of antagonists (Molderings & Göhert, 1995; Trendelenburg *et al*., 1996a). For example, phentolamine has been shown to be approximately 5 fold more potent than rauwolscine at α_{2D}-adrenoceptors (Funk *et al*., 1995; Trendelenburg *et al*., 1996b; Wahl *et al*., 1996), while the reverse is true at α_{2A}-adrenoceptors (Trendelenburg *et al*., 1994; Molderings & Göhert, 1995). To date, however, there have been no studies concerning the comparative effect of putative agonists at α_{2A}- and α_{2D}-adrenoceptors.

Rilmenidine is an antihypertensive agent with pharmacological similarities to clonidine, i.e. a non-catecholamine derivative with agonist activity at pre- and post-junctional α_2-adrenoceptors in the rat, rabbit and dog, albeit with lower potency (Verbeuren *et al*., 1986, 1989; Marsault *et al*., 1996). The antihypertensive action is characterised by a reduction in central sympathetic drive, leading to a reduction in peripheral resistance and heart rate, but is achieved with a lower incidence of sedation compared to clonidine (van Zwieten, 1996; Yu & Frishman, 1996). The latter feature has been interpreted as evidence that rilmenidine lacks significant agonist activity at central α_2-adrenoceptors at concentrations that exert an antihypertensive effect, and supports numerous other studies suggesting that part, or all, of the antihypertensive activity involves a non-α_2-adrenoceptor mechanism (Feldman *et al*., 1990; Yu & Frishman, 1996). To date, much evidence favours

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an action on putative imidazoline I_1 binding sites in the rostral ventrolateral medulla (Chan *et al*., 1994; Chan & Head, 1996), but the significance of the α_2-adrenoceptor agonist activity in man remains unclear.

In the present study we have re-evaluated the agonist activity of rilmenidine, in light of the recognized pharmacological differences between pre-junctional α_{2A}- and α_{2D}-adrenoceptors and the evidence that only one of these subtypes is thought to be present in man. The selective α_2-adrenoceptor agonists, UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) and clonidine, have been compared with rilmenidine, against electrically-evoked contractions in five isolated preparations from the rat, guinea-pig and the pig. The rat isolated vas deferens and guinea-pig isolated ileum have been used as preparations known to possess pre-junctional α_{2D}-adrenoceptors capable of modulating the motor response (Smith & Docherty 1992; Funk *et al*., 1995), while the rat isolated tail artery was included in this study because of an earlier report of pre-junctional α_2-adrenoceptors capable of modulating noradrenaline release (Msghina *et al*., 1992). The porcine isolated tail artery and porcine isolated urinary bladder, previously uncharacterised preparations, were used because of preliminary evidence suggesting the presence of pre-junctional α_2-adrenoceptors (Cheng *et al*., 1997; Thongsaard *et al*., 1997), and the pig, like man, is thought to express only the α_{2A}-subtype (Bockman *et al*., 1993; O'Rourke *et al*., 1994; Wright *et al*., 1995; Trendelenburg *et al*., 1996a).

Methods

The isolated tissues

Male Wistar rats (200–300 g) were killed by carbon dioxide asphyxiation. The prostatic end of the vas deferens was removed and placed in oxygenated, ice-cold modified Krebs-Henseleit (K-H) solution. The lower end of a 2 cm segment of the vas deferens was secured to a plastic holder between parallel platinum wire electrodes, while the upper end was attached by cotton to a Grass FT-03 isometric transducer connected to a Grass Polygraph. The holder was placed in an isolated organ bath containing 20 ml modified K-H solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. After an equilibration period of 30 min, 1 g wt. tension was slowly applied and the preparation allowed to relax to a final resting tension of 0.3–0.4 g wt. over 60 min. Contractions of the tissues were elicited by (single pulse) transmural stimulation via electrodes with single supramaximal voltage (0.5 ms duration, 0.05 Hz, 25 volts) with a SRI 6030 stimulator (Edenbridge, U.K.) until the responses were stable.

The ventral artery of the tail was dissected and placed on a dissecting disc immersed in gassed modified K-H solution. The blood vessel was carefully cleaned of fat and connective tissues with the aid of a dissecting microscope (Nikon SMZ-2B, Japan) and the proximal portion (1 cm from the end) divided into ring segments 2 mm in length. Each segment was suspended by 25 μm thick wire between two supporting jaws with platinum plate electrodes, one connected to an isometric transducer, in a stainless steel chamber of a Mulvany-Halpern wire-myograph unit. Each chamber contained modified K-H solution maintained at 34±1°C and gassed with 95% O_2/5% CO_2. After an equilibration period of 40 min, each segment was placed under an initial resting tension of 0.5 g wt. and allowed to relax for a further 40 min. Changes in isometric tension were recorded by a MacLab 4e and displayed on a Macintosh LC475 computer. Vessels were contracted with KCl (60 mM) to assess tissue viability and provide a reference contracture for subsequent data analysis. A D330-Multisystem stimulator (Digitimer Ltd., U.K.) was used to deliver 5 s trains of electrical pulses (100 mA; 0.3 ms pulse width) at a frequency of 1–3 Hz every 4–5 min. The voltage and frequency of the electrical field stimulation were modified in each set of experiment to obtain stable, neurogenic contractions of between 0.15–0.3 g wt (approximately 40% of the response to 60 mM KCl).

Male Dunkin-Hartley guinea-pig (500–1000 g) were killed by cervical dislocation and the intestines exteriorized. The ileo-caecal junction was located and 5 cm of the terminal ileum discarded. Approximately 30 cm of the terminal ileum was removed and the lumen flushed with modified K-H solution. Four 2 cm long segments of the ileum were secured on to a perspex holder supporting two parallel wire electrodes and placed in a 20 ml isolated organ bath containing modified K-H solution maintained at 37°C and gassed with 95% O_2/5% CO_2. The upper end of the ileum was attached to a Grass FT03 isometric transducer and the signal amplified by a WPI TBM4 amplifier and displayed an a Gould BS 272 flatbed recorder. After 40 min equilibration, the segments were placed under 2 g wt. resting tension and allowed to relax for a further 40 min. Contractions of the tissue were elicited by transmural stimulation using single pulses (0.1 Hz, 0.3 ms, 200 mA) delivered by a Digitimer multisystem D330 stimulator.

Porcine bladders and tails were obtained from a local abattoir and transported to the laboratory, in ice-cold modified K-H solution, within 1 h. The bladder, and a 5 cm segment of the proximal end of the ventral tail artery, were cleaned of fat and connective tissue and placed in modified K-H solution containing 2% ficoll. The solutions were gassed with 95% O_2/5% CO_2 and stored overnight at 4°C. The following day strips of the urinary bladder (3 cm long, 5 mm wide) were prepared and the lower end secured to a perspex holder between parallel platinum wire electrodes, while the upper end was attached by cotton to Grass FT-03 isometric transducer. Changes in isometric tension were recorded by a MacLab 4e and displayed on a Macintosh LC475 computer. The holder was placed in an isolated organ bath containing 20 ml modified K-H solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. After a 40 min equilibration period, each segment was placed under 10 g wt. tension and allowed to relax over the following 40 min. Preparations were stimulated twice with 60 mM KCl, each exposure separated by 30 min, and retensioned to achieve a final resting tension of 4 g wt. A D330-Multisystem stimulator (Digitimer Ltd., U.K.) was used to deliver 10 s trains of electrical pulses (200 mA; 0.3 ms pulse width) at a frequency of 1–2 Hz every 90 s until the responses had stabilised (usually 90 min).

Two wire supports (0.2 mm thick) were inserted in the lumen of the tail artery ring segments and placed in a 20 ml isolated organ bath containing modified K-H solution maintained at 37°C and gassed with 95% O_2/5% CO_2. The lower support was part of a perspex holder with two small platinum plate electrodes placed either side of the preparation, while the upper support was connected by cotton to a Grass FT03 isometric transducer. After a 40 min equilibration period, each segment was placed under 6 g wt. tension and allowed to relax over the following 40 min. Preparations were stimulated twice with 60 mM KCl, each exposure separated by 30 min, and retensioned to achieve a final resting tension of 3 g wt. A D330-Multisystem stimulator (Digitimer Ltd., U.K.) was used to deliver 5 s trains of electrical pulses (100 mA; 0.3 ms pulse width) at a frequency of 6 Hz every 5 min until the associated contractile responses had stabilised (usually 60 min).

The protocol

Once the electrically-evoked responses had stabilised in each preparation, UK-14304, clonidine or rilmenidine were added cumulatively in increasing concentrations. The period of exposure to each concentration varied from 5 min in rat isolated vas deferens and guinea-pig ileum, usually the time required for a maximum effect to be noted, to 10 min in the rat isolated tail artery, porcine isolated tail artery and the urinary bladder. At the end of the concentration response curve, each preparation was exposed to 1 μM RX-811059, with the exception of the rat isolated tail artery (0.1 μM RX-811059) and porcine isolated urinary bladder (3 μM RX-811059). Only a single agonist concentration response curve was generated in each preparation.

In the guinea-pig isolated ileum, the effect of clonidine against the electrically-evoked contractions was also determined after 40 min exposure to 0.1 μM rauwolscine and 0.1 μM phentolamine. Similarly, in the porcine isolated urinary bladder the effect of UK-14304 against electrically-evoked contractions was determined after 40 min exposure to either 1 μM prazosin, 0.01–0.1 μM rauwolscine and 0.03–0.1 μM phentolamine. In the case of the porcine preparations, the effect of UK-14304 was also examined in the presence of 10 μM (tail) or 30 μM (urinary bladder) rilmenidine.

Data analysis

Neurogenic contractions have been expressed as a percentage of either the response to 60 mM KCl or, when examining the effect of putative agonists, as a percentage of the pre-agonist responses. In the case of the porcine isolated detrusor muscle, the final three neurogenic contractions in the presence of the agonist were averaged. For the porcine isolated tail artery, where high concentrations of UK-14304 and clonidine produced direct vasoconstriction, the neurogenic contractions were measured as the response above the existing tone in the vessel. All data has been given as the mean$\pm$s.e.mean. Differences between mean values were considered statistically significant if $P<0.05$ for unpaired or paired observations (Student's *t*-test). The potency of the agonists in the absence and presence of the antagonists was assessed as the negative logarithm of the concentration required to cause 50% of the maximum response (pD_2) using the logistic equation described by DeLean *et al.* (1978) with Kaleidagraph software (Synergy) on a Macintosh LC II computer. In addition, the intrinsic activity (E_{max}) for each agonist was determined as the ratio of the maximum inhibition observed and complete inhibition of the electrically-evoked contraction. The agonist concentration-ratio (CR) in the presence and absence of the antagonist was determined at the level of 50% of the maximum response. Using the agonist concentration ratio produced by the lowest effective concentration of the antagonist (one producing a 3–5 fold rightward displacement of the agonist concentration response curve), an estimate of the negative logarithm of the dissociation constant ($\log K_B$) was determined by the method of Furchgott (1972).

$$\log K_B \quad \log \; CR \quad 1 \quad \log \; Antagonist$$

Drugs

The following compounds were used: prazosin HCl (Pfizer); rauwolscine HCl (Roth); phentolamine mesylate (Rogitine, Ciba Geigy); RX-811059 (2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline, Reckitt and Coleman); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); clonidine HCl (Sigma); rilmenidine HCl (Institut de Recherches Internationales Servier); α-β-methylene ATP (Sigma) Ficoll 70,000 (Sigma). Prazosin (1 mM) was dissolved in 0.1 M lactic acid and dilutions made in distilled water. All other drugs were dissolved in distilled water and added to the organ baths in a volume of 0.1 ml or less. The composition of the modified Krebs-Henseleit solution was (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1.

Results

Pharmacological characteristics of electrically-evoked responses

In preliminary experiments, electrically-evoked contractions of the rat isolated vas deferens (0.68$\pm$0.08 g wt., $n=5$), guinea-pig isolated ileum (2.10$\pm$0.27 g wt., $n=5$), porcine isolated tail artery (2.98$\pm$0.20 g wt, $n=6$) and porcine isolated urinary bladder (8.58$\pm$0.81 g wt, $n=4$) were abolished by 0.1 μM tetrodotoxin. In a further series of experiments, a combination of 0.1 μM prazosin and 300 μM suramin abolished neurogenic responses of the rat vas deferens ($n=4$), 0.1 μM atropine abolished neurogenic responses of the guinea-pig isolated ileum ($n=4$) and 0.1 μM prazosin reduced responses of the tail

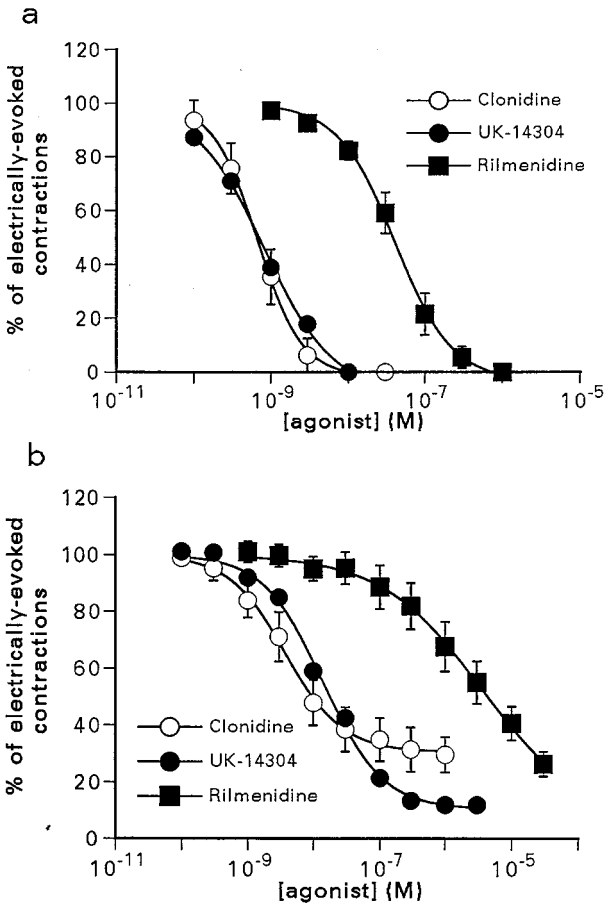

Figure 1 The effects of UK-14304, clonidine and rilmenidine against electrically-evoked contractions of (a) the rat isolated vas deferens and (b) the guinea-pig isolated ileum. The electrically-evoked responses in the presence of the agonists have been expressed as a percentage of the control response and are shown as the mean$\pm$s.e.mean of four to six observations.

artery of the rat ($n=4$) and pig ($n=6$) by more than 85%. In contrast, neurogenic contractions of the porcine isolated urinary bladder (52.8±8.7%) of the response to 60 mM KCl, $n=6$) were not significantly altered by a combination of either 0.1 μM atropine and 300 μM suramin (40.0±9.0% of the response to 60 mM KCl, $n=6$) or 0.1 μM atropine and 1 μM phentolamine (54.4±4.4% of the response to 60 mM KCl, $n=4$). In the presence of 0.1 μM atropine, α, β-methylene ATP (10 μM) produced large transient contractions of the porcine detrusor muscle (87.4±9.2% of 60 mM KCl, $n=4$) but failed to significantly modify neurogenic contraction in three out of four preparations (100.2±14.8% of the pre- α, β-methylene ATP response); in the remaining preparation large, spontaneous contractions developed after the α, β-methylene ATP-induced tone had declined and these masked the neurogenic responses.

The effect of UK-14304, clonidine and rilmenidine against neurogenic responses

Figure 1a shows that UK-14304, rilmenidine and clonidine produced concentration-dependent inhibition of neurogenic contractions of the rat isolated vas deferens, with clonidine and UK-14304 approximately 300 fold more potent than rilmenidine (Table 1). Subsequent addition of the selective α_2-adrenoceptor antagonist, RX-811059 (1 μM), reversed fully the effect of the highest concentration of the agonist (Table 2). Qualitatively similar effects were observed against neurogenic contractions in the guinea-pig isolated ileum (Figure 1b), but each agonist was approximately 1/10th to 1/30th as potent as observed in the rat isolated vas deferens and none was able to abolish the response (Table 1). RX-811059 (1 μM) significantly reversed the inhibitory effect of all three agonists, but not to the same degree as observed in the rat isolated vas deferens (Table 2). UK-14304, clonidine and rilmenidine also produced a concentration-dependent inhibition of neurogenic contractions in the rat isolated tail artery (Table 1), with only a 2 fold difference between the least (rilmenidine) and most potent (UK-14304) agonist. Each agonist produced a maximal inhibition of approximately 70–90% (Table 1). Subsequent addition of even 0.1 μM RX-811059 abolished the residual neurogenic responses in all cases (Table 2). In a separate series of experiments, prazosin and RX-811059 produced a concentration-dependent inhibition of the electrically-evoked contractions in the absence of the agonist; based on the negative logarithm of concentration of the antagonist causing 50% inhibition ($-\log IC_{50}$) prazosin (9.75±0.38, $n=3$) was 300 fold more potent than RX-811059 (7.29±0.28, $n=3$).

Figure 2 shows that UK-14304 and clonidine produced concentration-dependent inhibition of neurogenic contractions of the porcine isolated tail artery and isolated urinary bladder. Although clonidine was only slightly less potent than UK-14304 in the detrusor muscle it was markedly less efficacious,

Table 1 The mean pD_2 and E_{max} values for UK-14304, clonidine and rilmenidine against neurogenic contractions of several isolated smooth muscle preparations from the guinea-pig, rat and pig

Agonist	*Rat vas deferens*	*Rat tail artery*	*Guinea-pig ileum*	*Porcine tail artery*	*Pig urinary bladder*
UK-14304	9.25±0.21	7.97±0.17	7.66±0.16	7.14±0.04	7.32±0.26
pD_2	($n=4$)	($n=7$)	($n=6$)	($n=5$)	($n=9$)
E_{max}	1	0.91±0.05	0.89±0.06	0.77±0.02	0.53±0.04
Clonidine	9.29±0.20	7.98±0.27	8.43±0.10	<7	7.28±0.36
pD_2	($n=4$)	($n=7$)	($n=6$)	($n=5$)	($n=7$)
E_{max}	1	0.73±0.06	0.71±0.08	>0.2	0.30±0.12
Rilmenidine	7.45±0.09	7.63±0.27	5.86±0.23	–	–
pD_2	($n=9$)	($n=5$)	($n=6$)	($n=9$)	($n=9$)
E_{max}	1	0.71±0.08	0.78±0.04	<0.1	<0.1

Table 2 The effect of RX-811059 against UK-14304-, clonidine- and rilmenidine-induced inhibition of neurogenic responses in isolated smooth muscle preparations from the guinea-pig, rat and pig

Agonist	*Rat vas deferens*	*Rat tail artery*	*Guinea-pig ileum*	*Porcine tail artery*	*Pig detrusor muscle*
UK-14304	0	9.9±5.5	11.9±7.0	27.1±2.4	47.3±4.2
Control	($n=4$)*	($n=7$)*	($n=6$)*	($n=5$)*	($n=7$)*
RX811059	89.1±7.6	0	59.8±12.0	89.1±7.6	142.8±10
Clonidine	0	29.8±5.9	21.3±5.1	72.9±5.4	77.9±9.3
Control	($n=4$)*	($n=7$)**	($n=6$)*	($n=4$)*	($n=6$)*
RX811059	123.7±20	0	73.0±11.2	123±20.8	109.5±8.2
Rilmenidine	0	29.6±7.1	26.8±4.3	112.1±12	110.4±12
Control	($n=11$)*	($n=5$)**	($n=6$)*	($n=9$)	($n=7$)*
RX811059	98.9±9.7	0	48.8±7.5	115±11.2	148.1±21

Responses have been expressed as a percentage of the neurogenic contraction prior to exposure to agonist and are shown as the mean±s.e.m. of 'n' observations. *Denotes a statistically significant ($P<0.05$) increase in the neurogenic response following addition of 1–3 μM RX-811059. Please note that RX-811059 (0.1 μM) abolished neurogenic contractions of the rat tail artery in the presence of the agonists (**). With the exception of the rat isolated vas deferens, the highest concentrations of UK-14304 and clonidine employed were either 1 μM or 3 μM while the highest concentration of rilmenidine was 30 μM. For the vas deferens, 10 nM UK-14304, 10 nM clonidine and 1 μM rimenidine were used.

producing only 20 to 25% inhibition of the motor response (Table 1); in the tail artery no true maximum was obtained for the inhibitory effect of clonidine. In marked contrast, rilmenidine (1 nM – 30 μM) failed to inhibit neurogenic contractions in either preparation (Figures 2 and 3). In the case of the porcine isolated tail artery, UK-14304 (0.1 μM – 3 μM) and clonidine (0.01 μM – 3 μM) also produced concentration-related contractions; at the highest concentration the response was equivalent to 42.0 $\pm$ 3.3% ($n=5$) and 63.9 $\pm$ 9.2% ($n=5$) of the control electrically-evoked contraction, respectively. Rilmenidine did not elicit a contraction of the porcine isolated tail artery (Figure 3b). It is noteworthy that in some preparations of the porcine isolated urinary bladder high concentrations of the agonists (>1 μM) appeared to increase the magnitude and frequency of spontaneous contractions. In approximately 10% of preparations (particularly when antagonists were used) these contractions were so large the experiment was abandoned.

The addition of 1 μM RX-811059 reversed the effects of UK-14304 and clonidine on neurogenic contractions in both preparations (Table 2), but failed to significantly alter responses of the porcine tail artery in either the absence (to 109 $\pm$ 6.8% of control, $n=4$) or in the presence (Table 2) of 30 μM rilmenidine. RX-811059 (1 μM) also abolished UK-14304-induced contractions (see Figure 3) and reduced the response to clonidine (3 μM) by approximately 50%. In the porcine isolated urinary bladder, 3 μM RX-811059 produced a significant increase ($P<0.05$) in the neurogenic responses in the absence (to 119.7 $\pm$ 3.9% of control, $n=4$) and the presence (Table 2) of 30 μM rilmenidine. In the case of the latter combination of agents, both the frequency and magnitude of spontaneous contractions were increased markedly.

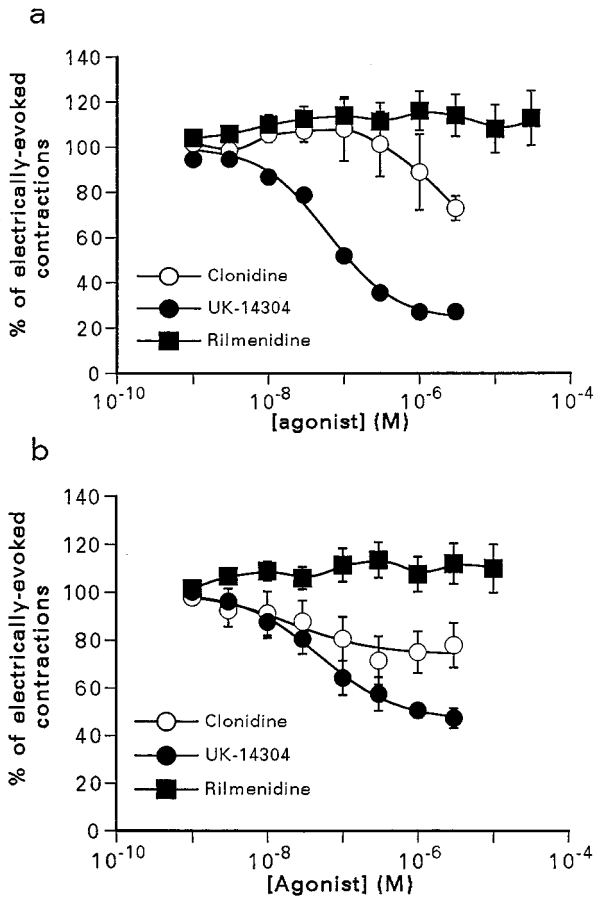

Figure 2 The effects of UK-14304, clonidine and rilmenidine against electrically-evoked contractions of (a) the porcine isolated tail artery and (b) the porcine isolated urinary bladder. The electrically-evoked responses in the presence of the agonists have been expressed as a percentage of the control response and are shown as the mean $\pm$ s.e.mean of 5 – 11 observations.

Pharmacological characteristics of the pre-junctional α_2-adrenoceptors

The failure of rilmenidine to cause a significant inhibition of neurogenic responses of either the porcine isolated tail artery or urinary bladder prompted us to examine whether this agent recognizes α_2-adrenoceptors in these preparations. Figure 4a shows that 30 μM rilmenidine caused a 20 fold rightward displacement of the UK-14304 concentration response curve in the porcine isolated urinary bladder. The apparent pK_B value for rilmenidine in this preparation was 5.82 $\pm$ 0.09 ($n=6$). In the porcine isolated tail artery, the addition of 10 μM rilmenidine (as a single concentration) was associated with a small but significant reduction (19.0 $\pm$ 4.0%, $n=5$) in the electrically-evoked responses and caused a 5 – 10 fold rightward displacement of the UK-14304 concentration response curve (Figure 4b) and practically abolished UK-14304-induced vasoconstriction. Significantly, the maximum response to UK-14304 was not altered by rilmenidine in spite of the finding that UK-14304 failed to elicit a contraction; taken together these observations also indicate that UK-14304-induced tone does not significantly modify neurogenic responses *per se*. The apparent pK_B value for rilmenidine in the porcine tail artery was estimated at 5.93 $\pm$ 0.18 ($n=5$).

Figures 5a, b and c show that 1 μM prazosin failed to alter the inhibitory effect of UK-14304 against neurogenic contractions of the porcine isolated urinary bladder, while rauwolscine (0.01 – 0.1 μM) and phentolamine (0.03 μM and 0.1 μM) caused a concentration-dependent rightward displacement of the UK-

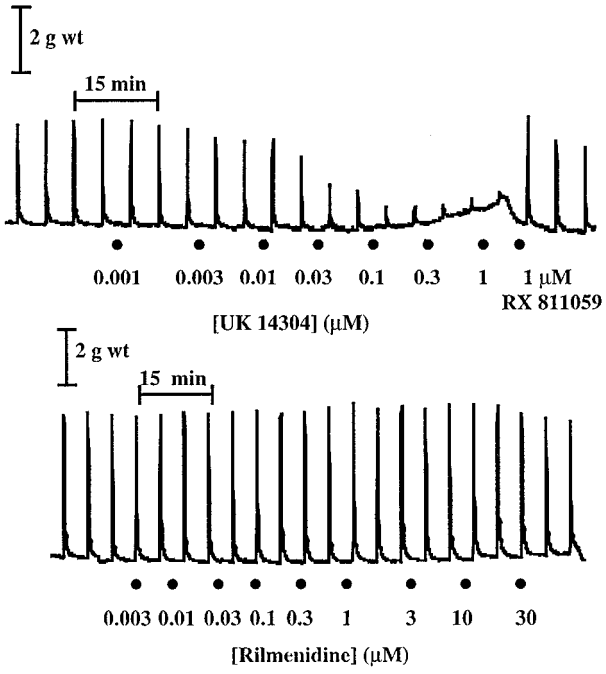

Figure 3 Representative trace recordings of the effect of (upper) UK-14304 and (lower) rilmenidine against electrically-evoked contractions (6 Hz, 5 s trains every 5 min) of porcine isolated tail artery. The addition of 1 μM RX-811059 reversed the effect of UK-14304 (upper) on the electrically-evoked contractions and inhibited the agonist-induced contractile response. Please note the different time base for each trace.

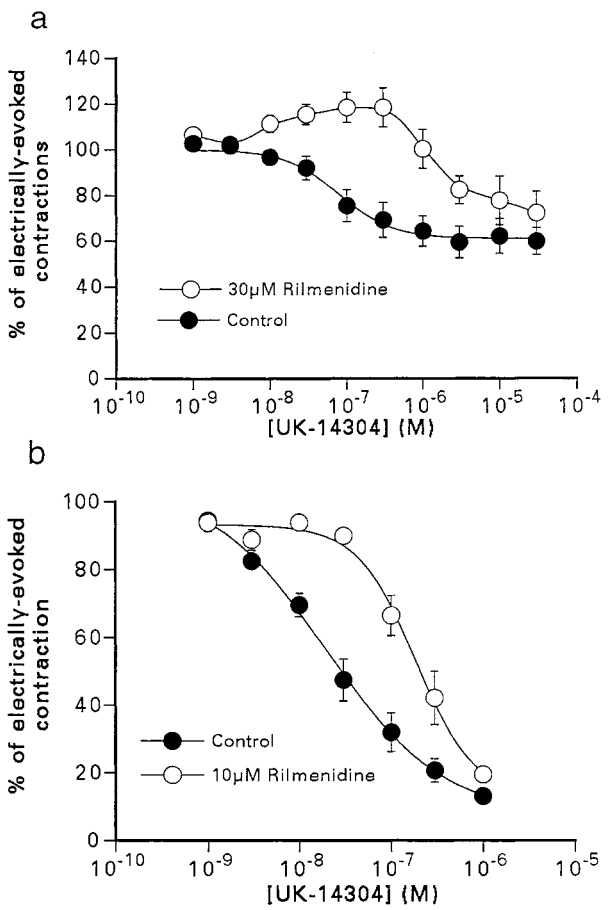

Figure 4 The effect of UK-14304 against electrically-evoked contractions of (a) the porcine isolated urinary bladder in the presence and the absence of 30 μM rilmenidine and (b) the porcine isolated tail artery in the presence and absence of 10 μM rilmenidine. The electrically-evoked responses in the presence of the agonists have been expressed as a percentage of the control response and are shown as the mean$\pm$s.e.mean of five to six observations.

14304 concentration response curve. In the presence of 0.1 μM rauwolscine, low concentrations of UK-14304 appeared to increase neurogenic contractions (Figure 5b). Based on the displacement of the UK-14304 concentration response curve produced by 0.01 μM rauwolscine and 0.1 μM phentolamine (approximately 10 fold), the apparent pK_B values were 8.87 ± 0.19 ($n=7$) and 7.56 ± 0.42 ($n=5$), respectively. Phentolamine (0.1 μM) and rauwolscine (0.1 μM) also produced a rightward displacement of the clonidine-induced inhibition of neurogenic contractions in the guinea-pig isolated ileum (Figure 5d), but the rank order of potency was reversed; phentolamine (pK_B 8.42 ± 0.31, $n=7$) was 2 fold more potent than rauwolscine (pK_B 8.05 ± 0.32, $n=7$).

Discussion

In this study we have compared the effects of three agonists, UK-14304, clonidine and rilmenidine, at pre-junctional α_2-adrenoceptor regulating transmitter release by assessing their ability to inhibit electrically-evoked contractions in five isolated preparations. Pharmacological examination of the responses with tetrodotoxin, which abolished all contractions, and a combination of receptor antagonists, revealed the role of a number of neurotransmitter substances. In the rat isolated vas deferens, the sensitivity of the responses to a combination of the selective α_1-adrenoceptor antagonist, prazosin (Cambridge, 1981), and P_{2X} receptor blocking agent, suramin (Dunn & Blakeley, 1988), suggest the involvement of noradrenaline and ATP. Based upon the effect of prazosin, noradrenaline appears to be the principal neurotransmitter in the tail arteries of the pig and the rat. However, in the latter preparation the sensitivity of the response to the selective α_2-adrenoceptor antagonist, RX-811059 (Mallard *et al.*, 1992), indicates a role for post-junctional α_2-adrenoceptors in addition to α_1-adrenoceptors. These findings agree with earlier reports by several other groups (Medgett *et al.*, 1983; Rajanayagam *et al.*, 1990; Bao *et al.*, 1993). In the guinea-pig isolated ileum, a well recognized model of cholinergic neurotransmission, the electrically-evoked responses were abolished by the muscarinic receptor blocker atropine. Finally, Sibley (1984) and Fujii (1988) have reported a role for acetylcholine (particularly at high frequencies of stimulation) and ATP in the mechanical and electrophysiological response to electrical field stimulation, respectively, in the detrusor muscle of the porcine isolated urinary bladder. However, in the present study neither muscarinic (atropine), purinergic (suramin and α, β-methylene ATP) and noradrenergic (phentolamine) receptor blocking agents affected the mechanical response. Thus, the nature of the excitatory neurotransmitter in this preparation at low stimulation frequencies is unclear, although we have recently confirmed the involvement of acetylcholine in mechanical responses to high frequencies of stimulation (Cheng *et al.*, 1997).

Preliminary evidence for pre-junctional α_2-adrenoceptors regulating transmitter release in each preparation was provided by the observation that UK-14304 and clonidine inhibited neurogenic contractions. Although the potency of clonidine and UK-14304 in the rat isolated vas deferens was 5 fold greater than that reported by Lattimer & Rhodes (1985), the values obtained in the guinea-pig isolated ileum were similar to those from earlier studies (Drew, 1978; Galligan 1993). Also, the potency of clonidine against neurogenic contractions in the rat isolated tail artery was similar to that observed against electrochemically detected release of noradrenaline elicited by electrical field stimulation (Msghina *et al.*, 1992). Overall, clonidine exhibited similar potency to UK-14304 in each preparation but, with the exception of the rat isolated vas deferens, clonidine behaved as a partial agonist (see Table 1). Confirmation of the involvement of α_2-adrenoceptors was provided by the observation that the effects of both agonists in the rat isolated vas deferens, guinea-pig isolated ileum, porcine isolated tail artery and urinary bladder were reversed, or partially reversed, by the selective α_2-adrenoceptor antagonist, RX-811059 (Mallard *et al.*, 1992); the presence of post-junctional α_2-adrenoceptors activated by neuronally-released noradrenaline in the rat isolated tail artery militates against detecting pre-junctional α_2-adrenoceptors.

Rilmenidine revealed some interesting differences between pre-junctional α_2-adrenoceptors in the five preparations. In the rat isolated vas deferens and guinea-pig isolated ileum, it behaved as an agonist but with 1/300th the potency of UK-14304 and clonidine; a potency ratio similar to that observed at both prejunctional and post-junctional α_2-adrenoceptors in other preparations (Verbeuren *et al.*, 1986, 1989; Marsault *et al.*, 1996). In contrast, rilmenidine was only slightly less potent than UK-14304 in the rat isolated tail artery, but behaved as a partial agonist. To our knowledge this is the first report of rilmenidine possessing high potency at pre-junctional α_2-adrenoceptors in the rat, but is similar to that reported in guinea-pig airways, where rilmenidine and UK-14304 were

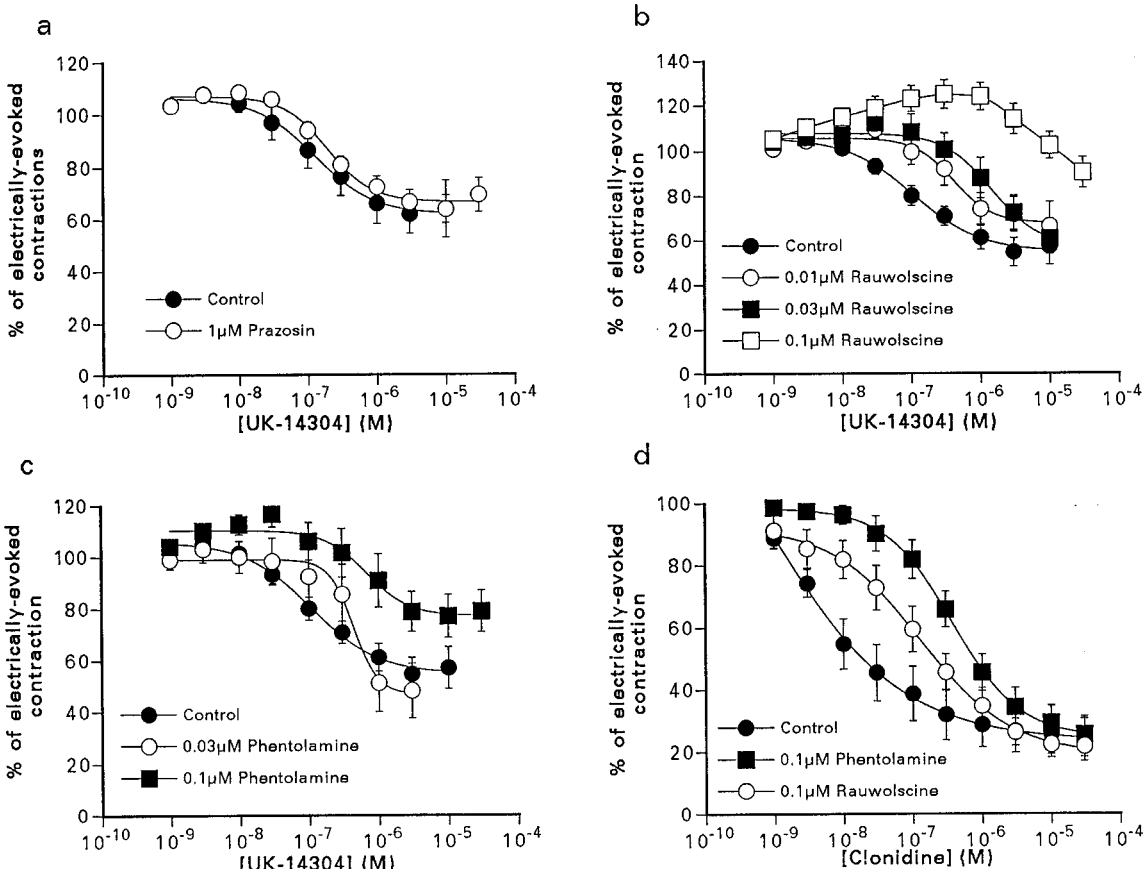

Figure 5 The effect of α-adrenoceptors antagonists, (a) prazosin, (b) rauwolscine, (c) phentolamine against UK-14034-induced inhibition of electrically evoked contractions of the porcine isolated urinary bladder and (d) clonidine-induced inhibition of electrically evoked contraction of the guinea-pig isolated ileum. The electrically-evoked responses in the presence of the agonists have been expressed as a percentage of the control response and are shown as the mean $\pm$ s.e.mean of six to eight observations.

equipotent at α_2-adrenoceptors on sensory nerves (Biyah & Advenier, 1995).

The most striking aspect of the pharmacology of rilmenidine, however, was the lack of significant agonist activity in the two isolated preparations from the pig, the tail artery and detrusor muscle. Although unable to elicit a detectable response in these preparations, evidence that rilmenidine recognized the pre-junctional α_2-adrenoceptors in these preparations was provided by the observation that it antagonized competitively the inhibitory effect of UK-14304 on electrically-evoked contractions. The estimated dissociation constant (pK_B 5.8) is comparable to that reported in other species (see Verbeuren *et al.*, 1989). It is also noteworthy that rilmenidine failed to elicit RX-811059-sensitive contractions of the tail artery, unlike UK-14304 and clonidine, suggesting that it is also devoid of agonist activity at postjunctional α_2-adrenoceptors in the pig under these conditions. In addition, rilmenidine reduced UK-14304-induced contractions in this preparation. However, two observations raise the possibility that rilmenidine may be a very weak agonist at pre-junctional α_2-adrenoceptors in the pig, producing less than a 10% reduction in the neurogenic contractions. First, although 30 μM rilmenidine did not produce a significant inhibition of electrically-evoked contractions in the detrusor muscle, subsequent exposure to 3 μM RX-811059 caused a larger increase (reversal?) in the responses than that observed in the absence of rilmenidine. Second, exposure to a single concentration of 10 μM rilmenidine (rather than following cumulative addition) caused a 20% reduction in the electrically-evoked contractions of the porcine isolated tail artery; it was not possible to examine the effect of 3 μM RX-811059 against this 'response' since this concentration of the antagonist also reduced the electrically-evoked contractions. Irrespective of whether rilmenidine is eventually shown to possess agonist activity at pre-junctional α_2-adrenoceptor in the pig, the results of the present study clearly highlight pronounced differences in the effect of this agonist at peripheral, pre-junctional α_2-adrenoceptors in the rat and guinea-pig, on the one hand, and the pig.

The α_2-adrenoceptors on the rat vas deferens have been classified previously as belonging to the α_{2D}- subtype (Smith & Docherty, 1992) which, based on the 3 fold greater potency of phentolamine compared to rauwolscine, is similar to that present in the guinea-pig isolated ileum. Although it was not possible to classify pharmacologically the pre-junctional α_2-adrenoceptor subtype in the porcine isolated tail artery, as phentolamine does not discriminate between α_1- and α_2-adrenoceptors, the non-adrenergic nature of the motor response of porcine isolated detrusor muscle did permit a comparison of the potency of rauwolscine, phentolamine and prazosin against the effect of UK-14304. The experiments revealed that prazosin was inactive (up to 1 μM) and rauwolscine was 5 fold more potent than phentolamine, which is a similar profile to that reported for α_2-adrenoceptor regulating noradrenaline release in isolated slices of the porcine cerebral cortex (Trendelenburg *et al.*, 1996a), and is evidence for the presence of the α_{2A} subtype. Thus, the pharmacological data from this study supports a simple α_{2A}/α_{2D} split between α_2-

adrenoceptors in the pig, on the one hand, and the rat and guinea-pig. It is possible, therefore, that the ability of rilmenidine to discriminate between α_2-adrenoceptors in these species on the basis of agonism may simply reflect differences in the efficiency of coupling and/or receptor density for each subtype. Interestingly, Duzic *et al*. (1992) reported major differences between α_{2D}- and α_{2C}-adrenoceptors expressed in NIH-3T3 fibroblasts (at similar densities) in the regulation of forskolin-stimulated cyclic AMP. It should be noted, however, that a clear α_{2D}/α_{2A}-adrenoceptor split for the agonist/antagonist action of rilmenidine may not be true of all species. For example, rilmenidine was originally reported as an agonist at pre-junctional α_2-adrenoceptors regulating noradrenaline release in the rabbit isolated saphenous vein (Verbeuren *et al*., 1989), a species reported to possess the α_{2A}-adrenoceptor subtype (Bylund *et al*., 1994; Trendelenburg *et al*., 1996b). More recently, Urban *et al*. (1995) reported that rilmenidine reduced plasma noradrenaline levels in pithed rabbit stimulated electrically to elevate blood pressure, an effect presumably mediated by activation of pre-junctional α_2-adrenoceptors. Thus, it seems unlikely that agonists, and rilmenidine in particular, can be used to discriminate between α_{2A} and α_{2D} receptor subypes. Nonetheless, further experiments are warranted to establish whether there are any pre-junctional or post-junctional α_{2A}-adrenoceptors in the pig at which rilmenidine possesses appreciable agonist activity.

The preliminary observation that rilmenidine is either devoid of agonist activity, or a very weak agonist, at pre-junctional α_{2A}-adrenoceptors in the pig raises the question as whether it possesses a similar profile at the α_2-adrenoceptors in man. As far as we are aware, however, there are no reported studies on the effect of rilmenidine at either pre- or post-junctional α_2-adrenoceptors in isolated preparations from man, a situation that has implications for the perceived mechanism(s) of action underlying its clinical use as an antihypertensive agent. Specifically, since the α_{2A}-adrenoceptor is the predominant central subtype in man (De Vos *et al*., 1992; Sastre & Garcia-Sevilla, 1994), the absence of significant agonist activity would place the locus of action of rilmenidine at a non-adrenoceptor site, perhaps imidazoline-preferring sites in the rostral lateral ventrolateral medulla (Chan *et al*., 1994; Chan & Head, 1996). In addition, this would also provide an explanation for the reported lack of sedative side effects in man compared to clonidine (van Zwieten 1996; Yu & Frishmann, 1996); the latter action is thought to be mediated by α_2-adrenoceptors in the locus coeruleus (Drew *et al*., 1979; DeSarro *et al*., 1987).

In summary, we have demonstrated that rilmenidine differs from other non-catecholamine derivatives that interact with α_2-adrenoceptors, in that it possesses agonist activity at peripheral prejunctional α_{2D}-adrenoceptors in the rat and guinea-pig but fails to activate prejunctional α_{2A}-adrenoceptors in the pig. It would appear that rilmenidine, a partial agonist in many preparations, may be particularly sensitive to differences in the efficiency of coupling of pre-junctional α_2-adrenoceptors between species. While the above data does not provide support for agonists being used to discriminate subtypes of α_2-adrenoceptors, it does, however, highlight the possibility that differences may exist between species in the cellular basis of the antihypertensive action of rilmenidine.

The authors express their gratitude to Institut de Recherche Internationales Servier (Courbevoie, France) for their support and are indebted to Dr. E. Scalbert and Professor S.J. Hill for their comments during preparation of the manuscript. H.-Y. Cheng was a Wellcome Trust Vacation Student.

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(Received January 6, 1998
Revised April 13, 1998
Accepted May 29, 1998)