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The pharmacology of RS-15385-197, a potent and selective α_2-adrenoceptor antagonist

[1]C.M. Brown, A.C. MacKinnon, W.S. Redfern, *P.E. Hicks, A.T. Kilpatrick, C. Small, M. Ramcharan, R.U. Clague, †R.D. Clark, C.B. MacFarlane & M. Spedding

Syntex Research Centre, Research Park, Riccarton, Edinburgh EH14 4AP, *Recherche Syntex France, Leuville-sur-Orge, 91310 Montlhery, France and †Institute of Organic Chemistry, Syntex Research, Palo Alto, California, USA

1 RS-15385-197 ((8a**R**, 12a**S**, 13a**S**)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(methylsulphonyl)-6H-isoquino [2,1-g][1,6]-naphthyridine) was evaluated in a series of *in vitro* and *in vivo* tests as an antagonist at α_2-adrenoceptors.

2 RS-15385-197 had a pK_i of 9.45 for α_2-adrenoceptors in the rat cortex (pA_2 in the guinea-pig ileum of 9.72), whereas the 8aS, 12aR, 13aR enantiomer, RS-15385-198, had a pK_i of only 6.32 (pA_2 6.47) indicating a high degree of stereoselectivity. The racemate RS-15385-196 had a pK_i of 9.18.

3 RS-15385-197 showed unprecedented α_2 vs. α_1 adrenoceptor selectivity *in vitro*. In the rat cortex, RS-15385-197 had a pK_i of 9.45 in displacing [^{3}H]-yohimbine and 5.29 in displacing [^{3}H]-prazosin (α_2/α_1 selectivity ratio in binding experiments >14000). The compound had a pA_2 of 9.72 as a competitive antagonist of the inhibitory effects of UK-14,304 in transmurally-stimulated guinea-pig ileum and 10.0 against BHT-920-induced contractions in dog saphenous vein (DSV); this latter value was unaltered by phenoxybenzamine. An apparent pK_B of 5.9 was obtained against cirazoline-induced contractions in DSV, whilst a pA_2 of 6.05 was obtained against phenylephrine-induced contractions in the rabbit aorta (α_2/α_1 selectivity ratio in functional experiments >4000).

4 RS-15385-197 was highly selective for α_2-adrenoceptors over other receptors: the compound showed low affinity for 5-HT_{1A} (pK_i 6.50) and 5-HT_{1D} (pK_i 7.00) receptor subtypes, and even lower affinity ($pK_i \leqslant 5$) for other 5-HT receptor subtypes, dopamine receptors, muscarinic cholinoceptors, β-adrenoceptors and dihydropyridine binding sites. RS-15385-197 was devoid of affinity for the non-adrenoceptor imidazoline binding site, labelled by [^{3}H]-idazoxan, which provides further evidence that these sites are not related to α_2-adrenoceptors. In the DSV, contractile responses to 5-hydroxytryptamine (5-HT) were unaffected by a concentration of 1 μM RS-15385-197.

5 RS-15385-197 was non-selective for the α_{2A}- and α_{2B}-adrenoceptor subtypes in that the pK_i for the α_{2A}-adrenoceptor in human platelets was 9.90 and the pK_i for the α_{2B}-adrenoceptor in rat neonate lung was 9.70. However, RS-15385-197 showed lower affinity for the α_2-adrenoceptor subtype in hamster adipocytes (pK_i 8.38).

6 In anaesthetized rats, RS-15385-197 was a potent antagonist of the mydriasis response induced by UK-14,304 or clonidine (AD_{50} 5 and 7 μg kg^{-1}, i.v., respectively; 96 μg kg^{-1}, p.o.) and of UK-14,304-induced pressor responses in pithed rats (AD_{50} 7 μg kg^{-1}, i.v.); the compound therefore is both centrally and orally active. Even at a high dose (10 mg kg^{-1}, i.v.), RS-15385-197 did not antagonize pressor responses to cirazoline in pithed rats, indicating that the selectivity for α_2 vs. α_1-adrenoceptors was maintained *in vivo*.

8 RS-15385-197 is therefore a very potent, selective, competitive α_2-adrenoceptor antagonist, both *in vitro* and *in vivo*, is orally active and readily penetrates the brain. It will thus be a powerful pharmacological tool for exploring the various physiological roles of α_2-adrenoceptors.

Keywords: RS-15385-197; α_2-adrenoceptors; yohimbine; idazoxan

Introduction

The role of α_2-adrenoceptors in mediating or modulating pre- and postjunctional events has previously been explored using poorly selective antagonists such as yohimbine, its diastereoisomer rauwolscine, and idazoxan (Langer, 1974; Ruffolo *et al.*, 1991). Using these relatively imprecise pharmacological tools, the distribution and function of α_2-adrenoceptors in a variety of species has been inferred (Goldberg & Robertson, 1983) and therapeutic applications of antagonists at these receptors proposed (Clark *et al.*, 1986; MacDonald *et al.*, 1988). However, yohimbine and rauwolscine have high affinity for subtypes of 5-hydroxytryptamine (5-HT) receptors (Convents *et al.*, 1988; Brown *et al.*, 1990c) and idazoxan has equivalent affinity for an imidazoline-preferring site compared with α_2-adrenoceptors (Hamilton *et al.*, 1988; Yablonsky *et al.*, 1988; MacKinnon *et al.*, 1989; Michel *et al.*, 1989; Brown *et al.*, 1990a). Yohimbine has been shown to cause a variety of effects in man, such as anxiety and increases in blood pressure and heart rate (Goldberg *et al.*, 1983), but it is not clear whether all of these effects arise from antagonism of ongoing activation of α_2-adrenoceptors. In order to investigate more definitively the role of α_2-adrenoceptor activation in different physiological systems we have synthesized a novel α_2-adrenoceptor antagonist, RS-15385-197 ((8a**R**, 12a**S**, 13a**S**)-5-8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(methylsulphonyl)-6H-isoquino [2,1-g][1,6]-naphthyridine; Figure 1), and the present paper describes its affinity and selectivity for α_2-adrenoceptors *in vitro* and *in vivo*. RS-15385-197 is the active enantiomer and the affinity of the racemate (RS-15385-196) and the inactive enantiomer (RS-15385-198) are listed. From our data it would appear that RS-15385-197 is the most potent and selective α_2-adrenoceptor antagonist thus far described. Preliminary reports of its pharmacology

[1] Author for correspondence.

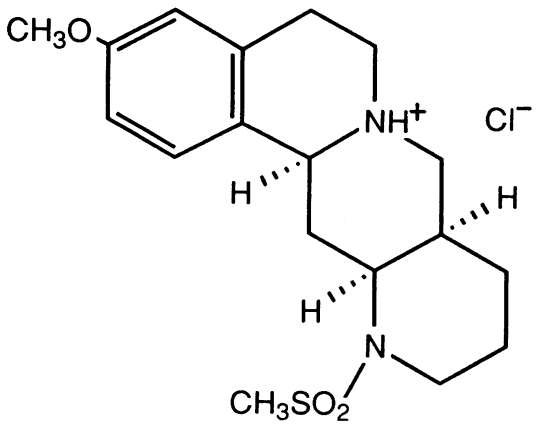

Figure 1 Structure of RS-15385-197.

have been published (Clark *et al.*, 1989a; 1990; Brown *et al.*, 1990b).

Methods

In vitro *binding studies*

Membrane preparation A washed, total membrane fraction was used in the majority of binding studies. Species and tissues are shown in Table 1. Tissues were rapidly removed and carefully dissected on ice. Tissue was homogenized in 25 vol ice cold Tris HCl buffer (50 mM, pH 7.4) in a polytron PT10 tissue disrupter (at setting 10; 2 × 10 s). The homogenate was centrifuged at 48,000 *g* in a refrigerated centrifuge (Sorvall RC-RB) at 4°C for 15 min. The resultant pellet was washed a further three times by resuspension and centrifugation and the final pellet was resuspended in 3 ml buffer and stored under liquid nitrogen until required.

Hamster adipocyte membranes were prepared as previously described (MacKinnon *et al.*, 1989). White adipose tissue taken from male Syrian hamsters (100–150 g) was finely chopped and suspended in Krebs-Ringer bicarbonate buffer (3 ml g^{-1} tissue) containing 1 mg ml^{-1} collagenase (Sigma type 1) and 3.5% w/v fatty acid free bovine serum albumin. The suspension was incubated at 37°C for 20 min and shaken vigorously every 5 min. The suspension was filtered through muslin and the cells washed three times by suspension in collagenase-free buffer. Isolated adipocytes were lysed by suspension in 10 vol lysing buffer (5 mM Tris HCl pH 7.4, 0.5 mM EDTA) at room temperature and centrifuged at 400 *g* for 1 min. The membrane fraction was collected and the unbroken cells lysed by a further two washes in lysing buffer. The membrane fractions were pooled and centrifuged at 30,000 *g* for 15 min at 4°C. The final pellet was suspended in 50 mM Tris HCl pH 7.4 containing 10 mM $MgCl_2$ and stored under liquid nitrogen until required.

Binding assays The membrane fraction (0.05–0.5 mg protein) was incubated to equilibrium with radiolabelled drug in the presence of 8–15 concentrations of RS-15385-197 or a saturating concentration of a specific drug to define non-specific binding. Conditions for the various assays are summarized in Table 1. Separation of bound from free ligand was achieved with a Brandel M24 cell harvester at a constant vacuum pressure of 22 mmHg. Bound radioactivity was determined by liquid scintillation spectrometry. α_2-Adrenoceptor saturation assays were performed with [^{3}H]-yohimbine and [^{3}H]-idazoxan (0.25–12 nM). Non-specific binding was determined with 10 μM phentolamine. Membrane protein was determined by use of Pierce BCA protein assay reagent (Sorensen & Brodbeck, 1986).

Saturation analysis Fitting of data to the appropriate models was achieved by use of the Scafit non-linear regression analysis programme LIGAND (Munson & Rodbard, 1980). Comparisons between different binding models were made using the extra sum of the squares principle as outlined by Munson & Rodbard (1980), and shown in equation 1.

$$F = \frac{(SS_1 - SS_2)/(d.f._1 - d.f._2)}{(SS_2/d.f._2)} \quad (1)$$

SS and d.f. refer to the residual sum of the squares and degrees of freedom associated with the two fits being compared. The subscript 1 is designated to the fit with the highest number of degrees of freedom (i.e. the least complicated model of analysis). Statistical significance of the F value was determined from a table of the percentage of the F distribution using $d.f._1 - d.f._2$ and $d.f._1$ degrees of freedom as the numerator and denominator respectively.

Competition analysis The inhibition of the radioligands by competing ligands was analysed graphically to estimate the IC_{50} (concentration of competitor displacing 50% of specifi-

Table 1 Binding assays

Receptor	*Tissue*	*Ligand*	*Incubation*	*Non-specific drug* (M)	*Standard*
α_1	Rat cerebral cortex	0.5 nM [^{3}H]-prazosin	30 min 25°C	10^{-5} phentolamine	prazosin
α_2	Rat cerebral cortex	2.0 nM [^{3}H]-yohimbine	30 min 25°C	10^{-5} phentolamine	yohimbine
α_2	Rat cerebral cortex	1.0 nM [^{3}H]-idazoxan	15 min 25°C	3×10^{-6} phentolamine	yohimbine
α_2	Baboon cortex	2.0 nM [^{3}H]-yohimbine	30 min 25°C	10^{-6} phentolamine	yohimbine
α_2	Baboon cortex	1.0 nM [^{3}H]-idazoxan	30 min 25°C	10^{-6} phentolamine	yohimbine
α_2	Hamster adipocytes	1.0 nM [^{3}H]-idazoxan	30 min 25°C	10^{-6} phentolamine	yohimbine
α_{2A}	Human platelets	1.0 nM [^{3}H]-yohimbine	30 min 25°C	10^{-6} phentolamine	yohimbine
α_{2B}	Rat neonate lung	1.0 nM [^{3}H]-yohimbine	45 min 25°C	10^{-5} phentolamine	yohimbine
β_1	Guinea-pig left ventricle	1.0 nM [^{3}H]-dihydroalprenolol	30 min 25°C	10^{-4} isoprenaline	propranolol
β_2	Rat lung	0.9 pM [^{125}I]-cyanopindolol	30 min 25°C	10^{-4} isoprenaline	propranaol
D_1	Rat striatum	0.3 nM [^{3}H]-SCH23390	30 min 25°C	10^{-7} SCH23390	SCH23390
D_2	Rat striatum	0.1 nM [^{3}H]-spiperone	30 min 37°C	10^{-6} (+)-butaclamol	haloperidol
5-HT_{1A}	Rat cerebral cortex	1.0 nM [^{3}H]-8-OH-DPAT	15 min 37°C	3×10^{-6} buspirone	buspirone
5-HT_{1B}	Rat striatum	30 pM [^{125}I]-cyanopindolol	40 min 37°C	10^{-5} 5-HT	RU 24969
5-HT_{1C}	Pig choroidal plexus	1.0 nM [^{3}H]-mesulergine	15 min 37°C	10^{-5} 5-HT	mesulergine
5-HT_{1D}	Baboon cortex	2.0 nM [^{3}H]-5-HT	15 min 37°C	10^{-5} 5-HT	rauwolscine
5-HT_2	Rat frontal cortex	1.0 nM [^{3}H]-ketanserin	10 min 37°C	2×10^{-6} methysergide	ritanserin
M_1	Rat cerebral cortex	0.2 nM [^{3}H]-methylscopolamine	3 h 32°C	10^{-6} atropine	atropine
M_2	Rat heart	0.2 nM [^{3}H]-methylscopolamine	3 h 32°C	10^{-6} atropine	atropine
DHP	Rat striatum	0.2 nM [^{3}H]-PN200-110	3 h 32°C	10^{-6} nifedipine	nifedipine

cally bound radioligand), by a non-linear least squares programme specially designed for the interpretation of sigmoidal concentration curves in terms of total and non-specific binding as well as inhibition constants and curve steepness. When Hill coefficients were not significantly different from unity the concentration of competitor displaying 50% of specific binding (IC_{50}) was converted to an affinity constant (K_i) using the expression derived by Cheng & Prusoff (1973), as shown in equation 2.

$$K_i = \frac{IC_{50}}{1 + ([L]/K_d)} \quad (2)$$

In this expression [L] and K_d represent the radioligand concentration and dissociation constant respectively. All data were initially analysed assuming a one site model of radioligand binding. The data with Hill coefficients less than unity were then analysed assuming a two site model, and the results of the curve fitting were statistically compared with those of the one site fit by an F test. The two site model was accepted if the observed fit was significantly better ($P < 0.05$) than the one site fit.

In vitro *functional studies*

Composition of physiological salt solution (PSS) Apart from the guinea-pig ileum preparation, the composition of the PSS in each of the *in vitro* assays was Krebs bicarbonate solution, as follows (mM): NaCl 118.93, KCl 4.69, $MgSO_4.7H_2O$ 1.01, KH_2PO_4 1.18, glucose 11.1, $NaHCO_3$ 25.0 and $CaCl_2.6H_2O$ 2.5, gassed with 95% O_2/5% CO_2 and maintained at 37°C. Inhibition of neuronal or extraneuronal uptake were not included.

Guinea-pig ileum preparation Ileum preparations taken from female Dunkin Hartley guinea-pigs (200–400 g), were set up in 30 ml isolated organ baths containing physiological Tyrode solution of the following composition (mM): NaCl 136.89, KCl 2.68, $MgCl_2.6H_2O$ 1.05, $NaH_2PO_4.2H_2O$ 0.42, glucose 5.55, $NaHCO_3$ 11.9 and $CaCl_2.6H_2O$ 1.8, gassed with 100% O_2 and maintained at 37°C. An initial tension of 1 g was applied. The preparations were stimulated co-axially at 0.1 Hz (1 ms pulse durations, supramaximal voltage) via parallel stainless steel electrodes with the anode passing through the lumen. The resulting contractions were recorded isometrically on a LectroMed oscillograph. After a period of 1 h an initial cumulative concentration-response curve (CCRC) to the agonist (UK-14,304 or clonidine) was obtained. The preparations were then washed three times and left to equilibrate for a further 40 min, after which a second agonist CCRC was obtained and measured as the control. The antagonist was added to the bath for a period of 40 min before repeating the agonist CCRC. In washout experiments, following the agonist CCRC in the presence of the antagonist, the preparations were washed by emptying and filling the bath 5 times and left to equilibrate for 40 min before repeating the agonist CCRC.

Vas deferens preparation Prostatic portions of vas deferens were taken from Sprague-Dawley rats (250–350 g) and set up in 30 ml organ baths. An initial tension of 0.5 g was applied. The preparations were field stimulated at 0.01 Hz (1 ms pulse durations, supramaximal voltage) via stainless steel electrodes running parallel to the tissue. The protocol was similar to that used for the ileum.

Dog saphenous vein Saphenous veins (DSV) were obtained from mongrel dogs of either sex under pentobarbitone (35 mg kg^{-1}, i.v.) anaesthesia. Tissues were studied after 24 h storage at 4°C in PSS. Veins were cleared of connective tissue, cut into rings of approximately 5 mm length and denuded of endothelium by carefully rubbing with forceps. The success of this technique was shown in certain tissues by demonstrating the failure of acetylcholine to relax a phenylephrine-mediated contraction. Preparations were mounted in 10 ml organ baths on steel hooks, under 2 g resting tension in PSS containing propranolol (1 μM). Isometric contractile concentration-response curves were obtained in separate preparations with BHT-920, cirazoline or 5-HT and responses displayed on a Gould BS-274 chart recorder.

Two consecutive CCRCs were obtained to BHT-920 before incubating tissues with RS-15385-197 for 30 min at varying concentrations, using only one concentration in each preparation ($n = 4-7$ tissues/group); the CCRC to BHT-920 was then repeated. In a further series of experiments the antagonist effects of RS-15385-197 were evaluated in DSVs treated with phenoxybenzamine (10 nM; 30 min followed by 30 min washout) in order to eliminate any α_1-adrenoceptor stimulation by BHT-920 at high concentrations. The effects of cirazoline washed out slowly, so only one concentration-response curve per preparation was obtained to this agonist in vehicle or antagonist-treated DSV rings after an initial contraction to KCl (80 mM) which served as a 100% reference response. Response curves to 5-HT, mediated by stimulation of 5-HT_1-'like' receptors, were studied in the presence of ketanserin (0.1 μM) in order to antagonize any 5-HT_2 receptor effects of the agonist (Humphrey *et al.*, 1988).

Rabbit aorta Aortic ring preparations were taken from male New Zealand white rabbits (2 kg). The endothelium was removed by rubbing and the rings set up in 30 ml organ baths. An initial tension of 1.5 g was applied to the tissue. After 30 min equilibration time the tissues were contracted by phenylephrine (10 nM–10 μM) in the presence or absence of one concentration of RS-15385-197.

Calculation of results from in vitro *studies* CCRCs to agonists were obtained in the presence of various concentrations of antagonists. Antagonist concentration ratios (CR) were then calculated at the EC_{50} level of the agonist in the presence of each concentration of antagonist [B]. Where possible, pA_2 values were calculated by the method of Arunlakshana & Schild (1959) by plotting $\log_{10}(CR - 1)$ vs. $-\log_{10}[B]$. The slope of this plot should theoretically be equal to 1 for a competitive antagonist and the pA_2 should then equal the antagonist dissociation constant pK_B. For the experiments on the DSV with cirazoline and 5-HT, an apparent pK_B was calculated from a single concentration of antagonist by the method of Furchgott (1972): EC_{50} values calculated for control tissues were used for the calculation of K_B, where

$$K_B = \frac{[B]}{CR - 1} \quad (3)$$

and thus pK_B is $-\log_{10}K_B$.

Pithed rat studies

Male Sprague-Dawley rats (250–350 g) were anaesthetized with halothane (5% in oxygen) or diethyl ether, pithed through the orbit, and then artificially ventilated with room air (1 ml kg^{-1}) at a rate of 50–60 strokes min^{-1} using a Harvard small animal ventilator. Body core temperature was maintained at 37°C with a heating blanket. A carotid artery and jugular (or femoral) vein were cannulated for arterial blood pressure measurement and injection of drugs respectively. Blood pressure was registered and displayed by use of either an Elcomatic 524 pressure transducer connected to a Lectromed Multitrace 4 chart recorder, or a Gould pressure transducer connected to a Gould 8000 S chart recorder.

Reversal of agonist response UK-14,304 (30 μg kg^{-1}, i.v.), was administered at 10 min intervals, following administration of prazosin (100 μg kg^{-1}, i.v.) to exclude any involvement of α_1-adrenoceptors. Increases in diastolic pressure were measured; the pressor responses to the second and third

UK-14,304 challenge doses were averaged and taken as the control response. Beginning 5 min after the third injection of UK-14,304, the antagonist was injected at 10 min intervals in ascending doses, preceding each agonist challenge dose by 5 min. %Original control response was plotted against dose of antagonist; from this, the dose of antagonist which reduced the response to UK-14,304 by 50% (AD_{50}; Berridge *et al.*, 1983) was calculated. In early studies clonidine was used as the agonist, but without prior administration of prazosin.

Displacement of agonist dose-response curves Following injection of prazosin (100 $\mu g\,kg^{-1}$, i.v.), RS-15385-197 (9, 45, 225 or 1000 $\mu g\,kg^{-1}$, i.v.) or saline was injected; separate groups of rats were used for each dose of antagonist. UK-14,304 was administered in ascending doses (1–1000 $\mu g\,kg^{-1}$, i.v.) at 5 min intervals, beginning 5 min after injection of the antagonist. Increase in diastolic blood pressure was plotted against cumulative dose of UK-14,304. To assess α_2- vs. α_1-adrenoceptor selectivity, pressor responses were evoked either by UK-14,304 or by cirazoline, respectively. Cumulative dose-response curves to these agonists were determined in groups of rats given 10 min i.v. infusions of each antagonist at varying doses and in a control group given an i.v. vehicle infusion of 0.5 $ml\,kg^{-1}$ for 10 min. Increases in diastolic blood pressure were plotted vs. dose of agonist. Separate groups of rats were used for each dose of antagonist. From these curves, '*in vivo* Schild plots' (i.e. plots of log (dose ratio-1) vs. log dose of antagonist) were constructed to estimate the i.v. dose of antagonist giving a dose-ratio of 2 (DR_2; Clineschmidt *et al.*, 1988).

Prejunctional α_2-adrenoceptor effects All rats were bilaterally vagotomized and given (+)-tubocurarine (1 $mg\,kg^{-1}$, i.v.) and atropine (0.5 $mg\,kg^{-1}$, i.v.). Heart rate was raised by about 100 beats min^{-1} by continuous electrical stimulation of the thoracic sympathetic outflow (50 V; 0.3–0.5 ms; 0.3–0.5 Hz) via an uninsulated portion of the pithing rod. After a period of 20 min stabilization under electrical stimulation, cardioinhibitory response curves to UK-14,304 were constructed in vehicle- or antagonist-treated groups of rats. Separate groups of rats were used for each dose of antagonist.

Mydriasis studies

The methods used were based on those described by Berridge *et al.* (1983). Male Sprague-Dawley rats (200–400 g) were anaesthetized with sodium pentobarbitone (60 $mg\,kg^{-1}$, i.p.). Body core temperature was maintained at 37°C with a heating blanket. A jugular vein was cannulated for drug injections. Pupil diameter was measured with an illuminated inspection glass with × 7 magnification (RS Components Ltd).

Reversal of agonist response In early studies, mydriasis was produced by injection of clonidine (300 $\mu g\,kg^{-1}$, s.c.), and the antagonist was administered intravenously at 10 min intervals, the pupil diameter being measured 5 min after each dose. Alternatively, the antagonist was administered orally in increasing doses every 20 min, the pupil diameter being measured 15 min after each dose. In subsequent studies, mydriasis was produced by injection of UK-14,304 (100 $\mu g\,kg^{-1}$, i.v.), and the antagonist was administered at 5 min intervals, beginning 5 min after injection of the agonist, and pupil diameter was measured 4.5 min after each dose of antagonist. A graph of % original increase in pupil diameter vs. dose of antagonist was plotted, from which the AD_{50} (see above) was calculated.

Displacement of agonist dose-response curves Rats received a single dose of antagonist 5 min before performing a cumulative dose-response curve to UK-14,304 (1–1000 $\mu g\,kg^{-1}$, i.v.), administered at 5 min intervals. Increase in pupil diameter was plotted vs. dose of UK-14,304. In order to assess the time course of antagonism following oral administration, conscious rats were treated orally with antagonist or vehicle. They were prepared as described above at appropriate intervals following gavage, then a dose-response curve to clonidine (injected i.v. at 5 min intervals) was constructed 1, 2, 4 or 6 h after the oral dose. Pupil diameter was measured 4.5 min after each dose of agonist.

Drugs

Reagents used were of the highest analytical grade. Compounds were kindly donated by their manufacturers, synthesized at the Institute of Organic Chemistry, Syntex (Palo Alto) or purchased. The following compounds were used: [^{3}H]-idazoxan (40 Ci $mmol^{-1}$), [^{3}H]-5-HT (40 Ci $mmol^{-1}$), [^{3}H]-mesulergine (80 Ci $mmol^{-1}$), [^{3}H]-8-OH-DPAT (220 Ci $mmol^{-1}$) and [^{3}H]-prazosin (86 Ci $mmol^{-1}$) from Amersham, U.K., and [^{3}H]-dihydroalprenolol (90 Ci $mmol^{-1}$), [^{125}I]-cyanopindolol (2200 Ci $mmol^{-1}$), [^{3}H]-yohimbine (89 Ci $mmol^{-1}$), [^{3}H]-ketanserin (76 Ci $mmol^{-1}$), PN200110 (80 Ci $mmol^{-1}$), [^{3}H]-spiperone (80 Ci $mmol^{-1}$), [^{3}H]-SCH23390 (80.4 Ci $mmol^{-1}$) and [^{3}H]-n-methylscopolamine (71.3 Ci $mmol^{-1}$) from DuPont, U.K.; atropine sulphate (BDH); BHT-920 HCl (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-ol]-azepine; Boehringer Ingleheim); phentolamine mesylate (Ciba); L-659,066 (MSD); prazosin HCl (Pfizer); (+)-butaclamol, ketanserin tartrate and phenoxybenzamine HCl (RBI); methysergide maleate (Sandoz); SCH 23390 maleate (Schering); (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate; clonidine HCl, oxymetazoline HCl, yohimbine HCl, L-phenylephrine HCl, 5-HT creatinine sulphate and (±)-propranolol HCl (Sigma); cirazoline HCl (Synthelabo); buspirone HCl, idazoxan HCl, piperoxan HCl, ritanserin, UK-14,304 HCl (5-bromo-6-[2-imidazolin-2-ylamino] quinoxaline), RS-15385-197, RS-15385-198 and RS-15385-196 (synthesized by Dr R. Clark, Syntex, Palo Alto, CA, U.S.A.).

Results

Binding studies

The affinities of RS-15385-197 for a wide range of receptor binding sites are listed in Table 2. The highest affinity of RS-15385-197 was for α_2-adrenoceptors in a range of different tissues from different species (pK_i 9.18–10.15).

Saturation analysis of [^{3}H]-yohimbine and [^{3}H]-idazoxan binding indicated that it was specific and both ligands bound to a single population of high affinity sites in the baboon cortex (K_d 3 and 2.1 nM; B_{max} 80 and 93 fmol mg^{-1} protein, respectively). The affinities of a number of compounds were determined and showed the site to be characteristic of an α_2-adrenoceptor with high affinity for yohimbine, idazoxan, phentolamine and noradrenaline. Both radioligands labelled a site characteristic of the α_{2A}-like subtype with high affinity for oxymetazoline (pK_i 7.87 ± 0.20 and 8.04 ± 0.21 respectively) and low affinity for prazosin (pK_i 6.23 ± 0.26 and 6.28 ± 0.11 respectively), which has been reported to have higher affinity for both α_{2B}- and α_{2C}-adrenoceptor subtypes (Bylund, 1988).

RS-15385-197 revealed markedly lower affinity for various other receptor binding sites (Table 2). The selectivity ratio for α_2-adrenoceptors was > 14,000 over α_1-adrenoceptors in the rat cortex. The compound showed low affinity for 5-HT_{1A} (pK_i 6.50) and 5-HT_{1D} (pK_i 7.00) and even lower affinity for other 5-HT subtypes, dopamine receptors, muscarinic cholinoceptors, β-adrenoceptors and dihydropyridine binding sites (pK_i ≤ 5). Inhibition of [^{3}H]-yohimbine binding to α_2-adrenoceptors by RS-15385-197 was monophasic (Figure 2), consistent either with an interaction at one site or with equal affinity for subtypes labelled by 2 nM [^{3}H]-yohimbine in the rat cortex. RS-15385-196 was slightly less potent than the

Table 2 Affinity values of RS-15385-197 (pK_i)

Receptor	*Ligand*	*Tissue*	*Affinity*
α_2	[^{3}H]-yohimbine	Rat cortex	9.45 ± 0.12
α_2	[^{3}H]-idazoxan	Rat cortex	9.18 ± 0.13
α_{2A}	[^{3}H]-yohimbine	Human platelets	9.90 ± 0.10
α_{2B}	[^{3}H]-yohimbine	Rat neonate lung	9.70 ± 0.10
α_2	[^{3}H]-yohimbine	Baboon cortex	10.12 ± 0.3
α_2	[^{3}H]-idazoxan	Baboon cortex	10.15 ± 0.31
α_2	[^{3}H]-idazoxan	Hamster adipocyte	8.38 ± 0.01
α_1	[^{3}H]-prazosin	Rat cortex	5.29
I_2	[^{3}H]-idazoxan	Hamster adipocyte	<4
5-HT$_{1A}$	[^{3}H]-8-OH-DPAT	Rat cortex	6.50
5-HT$_{1B}$	[^{125}I]-CYP	Rat striatum	<5
5-HT$_{1C}$	[^{3}H]-mesulergine	Pig choroidal plexus	<5
5-HT$_{1D}$	[^{3}H]-5-HT	Baboon cortex	7.0 ± 0.2
5-HT$_2$	[^{3}H]-ketanserin	Rat frontal cortex	5.10
β_1	[^{3}H]-dihydroalprenolol	Guinea-pig left ventricle	5.27
β_2	[^{125}I]-CYP	Rat lung	<5
D_1	[^{3}H]-SCH23390	Rat striatum	<5
D_2	[^{3}H]-spiperone	Rat striatum	<5
M_1	[^{3}H]-N-methylscopolamine	Rat cortex	<5
M_2	[^{3}H]-N-methylscopolamine	Rat heart	<5
DHP	[^{3}H]-PN 200-110	Rat striatum	<5

Assays were performed as described in methods and Table 1. The results are expressed as the pK_i mean ± s.e.mean of 3 experiments performed in duplicate. DHP = dihydropyridine Ca^{2+} binding sites.

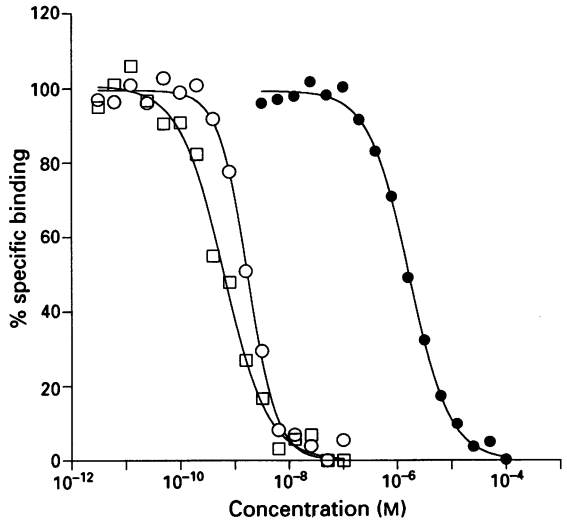

Figure 2 Displacement of [^{3}H]-yohimbine binding from rat cerebral cortex membranes by RS-15385-197 (□, pK_i 9.4), RS-15385-196 (○, pK_i 8.9) or RS-15385-198 (●, pK_i 5.9).

Table 3 Displacement of [^{3}H]-idazoxan, 1.5 nM, from hamster adipocytes

	pK_i		
	Site 1 20–30%	*Site 2* 70–80%	*Selectivity*
Idazoxan	8.34 ± 0.06	–	
Yohimbine	7.28 ± 0.02	4.48 ± 0.16	631
Phentolamine	7.92 ± 0.14	5.21 ± 0.02	513
(−)-Noradrenaline	6.21 ± 0.10	< 4.00	> 162
(−)-Adrenaline	6.92 ± 0.12	< 4.00	> 832
RS-15385-197	8.38 ± 0.01	< 4.00	> 23988

Adipocyte membranes were incubated with 1.5 nM [^{3}H]-idazoxan and 13 concentrations of competing ligand to equilibrium in a final volume of 0.5 ml 50 mM Tris HCl pH 7.4, 10 mM $MgCl_2$ buffer. Non-specific binding was defined using 1 mM phentolamine. The data represent 3–5 experiments performed in duplicate. The fit for a 1 or 2 site model were compared using the differential F value. A 2 site fit was assumed to be better than a 1 site fit if the F value achieved significance of $P<0.05$.

−197 isomer, and the −198 isomer was more than a thousand fold less potent (Figure 2), thus indicating stereoselectivity.

RS-15385-197 was non-selective for the α_{2A} (human platelet pK_i 9.90) and α_{2B} (rat neonate lung pK_i 9.70) subtype of the α_2-adrenoceptor. However, RS-15385-197 showed lower affinity for the α_2 subtype in hamster adipocytes (pK_i 8.38); this subtype has previously been described as α_{2A}-like (MacKinnon *et al.*, 1989) but shows lower affinity for tetracyclic yohimbine-like structures. Yohimbine, phentolamine and noradrenaline inhibited 1.5 nM [^{3}H]-idazoxan binding to hamster adipocytes with Hill slopes less than unity, which were better fitted to a 2 site model (Table 3). RS-15385-197 inhibited only 20–30% specific binding with an affinity constant, pK_i = 8.38, but did not displace the imidazoline specific component at concentrations up to 1 mM. Idazoxan exhibited a monophasic displacement curve on [^{3}H]-idazoxan binding, consistent with this compound having equal affinity for both sites, *viz.* the α_2-adrenoceptor and the non-adrenoceptor imidazoline binding site.

Guinea-pig ileum and rat vas deferens

UK-14,304 concentration-response curves for inhibition of twitch height in the transmurally-stimulated guinea-pig ileum were shifted to the right by RS-15385-197 (0.1–3 nM), RS-15385-196 (0.1–3 nM) and RS-15385-198 (300–3000 nM; Figure 3a–c). The Schild plots derived from these data are shown in Figure 3d; the slopes for all three compounds were not significantly different from unity, which is compatible with a competitive interaction at the α_2-adrenoceptor. The pA_2 for RS-15385-197 was 9.72 ± 0.05 (slope 1.10 ± 0.02, n = 11), for RS-15385-196 was 9.69 ± 0.06 (slope 1.10 ± 0.05, n = 5) and for RS-15385-198 was 6.47 ± 0.06 (slope 0.96 ± 0.04, n = 4) indicating a highly stereoselective interaction of RS-15385-197 with the α_2-adrenoceptor. The effects of RS-

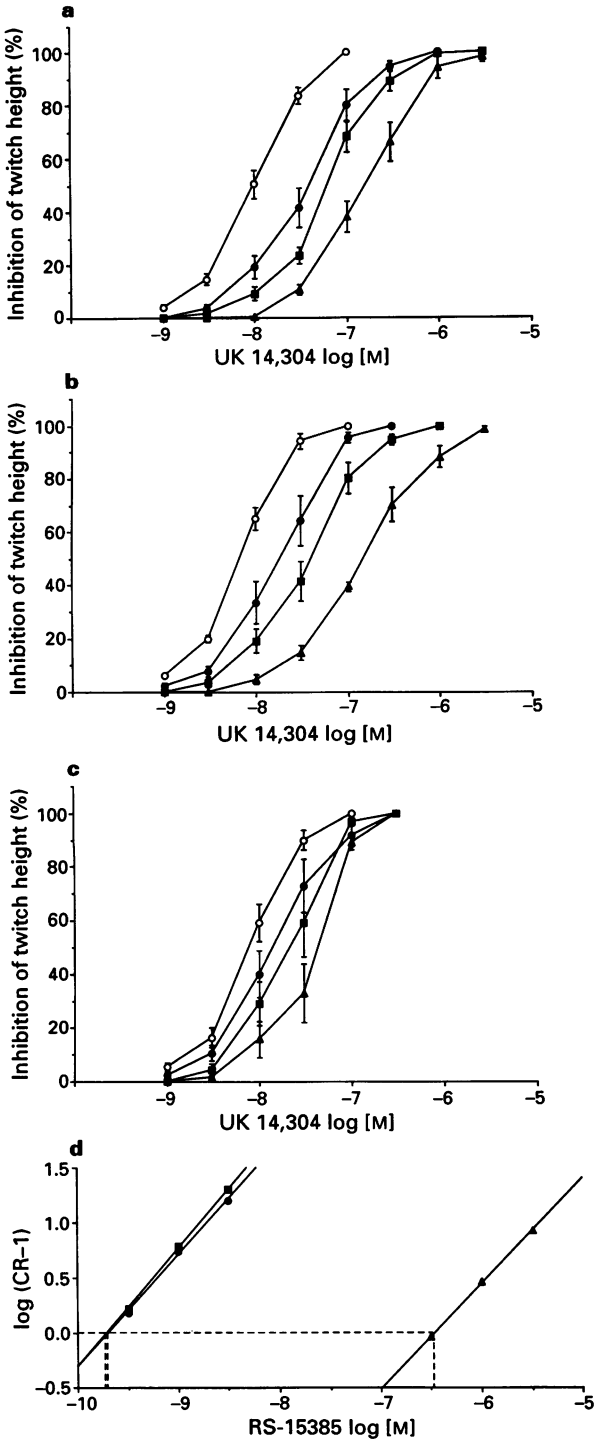

Figure 3 Antagonism of the inhibitory responses to UK-14,304 (controls O) on the contractile responses of guinea-pig ileum prepaations to field stimulation by RS-15385-197 (a: ●, 0.1 nM; ■, 1 nM; ▲, 3 nM); RS-15385-196 (b: ●, 0.1 nM; ■, 1 nM; ▲, 3 nM) or RS-15385-198 (c: ●, 0.3 μM; ■, 1 μM; ▲, 3 μM). The antagonistic effects of RS-15385-197 (■), RS-15385-196 (●) and RS-15385-198 (▲) are quantified by the method of Arunlakshana & Schild (1959) in (d). Vertical bars represent s.e.mean, $n = 5-6$.

15385-197 (3 nM) were reversible as the concentration-ratio for UK-14,304 was <2 after 30 min washout. RS-15385-197 (0.1 to 3 nM) did not modify the responses to co-axial stimulation directly and showed no partial agonist effects. With clonidine as the agonist, RS-15385-197 had a pA_2 of 9.46 ± 0.26 (slope 0.75 ± 0.10, $n = 4$). RS-15385-197 was selective for α_2-adrenoceptors in that high concentrations (1 and 10 μM) did not modify the inhibitory responses to morphine in the ileum (concentration-ratio <2; data not shown).

RS-15385-197 was also a potent antagonist of the response to UK-14,304 in the rat vas deferens preparation (pA_2 9.28 ± 0.10, slope 0.70 ± 0.04, $n = 6$). RS-15385-198 was slightly more potent in the rat vas deferens as an antagonist of UK-14,304 (pA_2 7.42 ± 0.07, slope 1.70 ± 0.09, $n = 4$) than in the guinea-pig ileum.

Dog saphenous vein

Concentration-dependent contractile response curves obtained to BHT-920 were progressively displaced to the right of controls by RS-15385-197 (1.0–100 nM) with little change in the maximum responses to the agonist (Figure 4). In control tissues three subsequent response curves to BHT-920 were superimposable. Schild analysis of these data gave a pA_2 of 10.00 with a slope of 0.85. Pretreatment of the tissues with phenoxybenzamine at a concentration (10 nM) which irreversibly inactivates the α_1-adrenoceptors in this preparation (Ruffolo & Zeid, 1985) did not modify the antagonist effects of RS-15385-197 (pA_2 9.95, slope 0.80; Figure 5).

A high concentration of RS-15385-197 (10 μM) was required to displace the contractile response curves to cirazoline to the right of controls (apparent pK_B 5.9 ± 0.2) and RS-15385-197 (1.0 μM) failed to antagonize the contractile responses to 5-HT in tissues treated with ketanserin (Figure 4).

Rabbit aorta

RS-15385-197 was a weak antagonist of phenylephrine-induced contractions in the rabbit aorta (pA_2 6.05 ± 0.16, slope 0.90 ± 0.06, $n = 4$). This estimate of α_1-adrenoceptor antagonist affinity was similar to that determined on cirazoline-induced contractions in dog saphenous vein. These data indicate a very high α_2 vs. α_1-adrenoceptor selectivity ratio in *in vitro* functional assays of >4000.

Pithed rat studies

UK-14,304 (1–1000 μg kg^{-1}, i.v.) produced a dose-related increase in diastolic blood pressure. The challenge dose used in the agonist reversal study (30 μg kg^{-1}, i.v.) was at approximately the ED_{80} level.

Reversal of agonist response In the presence of prazosin (100 μg kg^{-1}, i.v.), UK-14,304 (30 μg kg^{-1}, i.v.) evoked reproducible increases in diastolic blood pressure of around 30 mmHg. RS-15385-197, idazoxan and L-659,066 reduced this response in a dose-related manner, although in each case there was a small residual response of around 10% of the original pressure response which was resistant to the α_2-adrenoceptor antagonists (Figure 6). When this residual response was corrected for, RS-15385-197 gave an AD_{50} of 7 μg kg^{-1}, i.v. ($n = 6$), which was the same as the value obtained (7 μg kg^{-1}) for reversal of the pressor response to clonidine, and far more potent than the other four antagonists tested (Table 4).

Displacement of agonist dose-response curves The dose-response curve to UK-14,304 was displaced to the right in parallel by RS-15385-197 (45, 225 and 1000 μg kg^{-1}, i.v.) in a dose-related manner, without reduction in the maximum response (Figure 7a). Construction of '*in vivo*' Schild plots' from these data indicated that RS-15385-197 gave a dose-ratio of 2 (DR_2) at a dose of 45 μg kg^{-1}, i.v. (slope = 1.0). In comparison, yohimbine (DR_2 = 180 μg kg^{-1}, i.v.; slope = 0.9) and idazoxan (DR_2 = 200 μg kg^{-1}, i.v.; slope = 0.9) were approximately 4 fold less potent.

α_2- vs. α_1-Adrenoceptor selectivity RS-15385-197 (10 mg kg^{-1}, i.v.) caused only a small rightward shift (dose-ratio =

1.6) in the pressor response curve to cirazoline. The maximal vasoconstrictor to cirazoline was, however, slightly decreased after this dose of RS-15385-197 (Figure 7b). Prazosin (100 μg kg^{-1}, i.v.) caused a rightward displacement of the vasoconstrictor responses to cirazoline with a dose-ratio of 7 (data not shown).

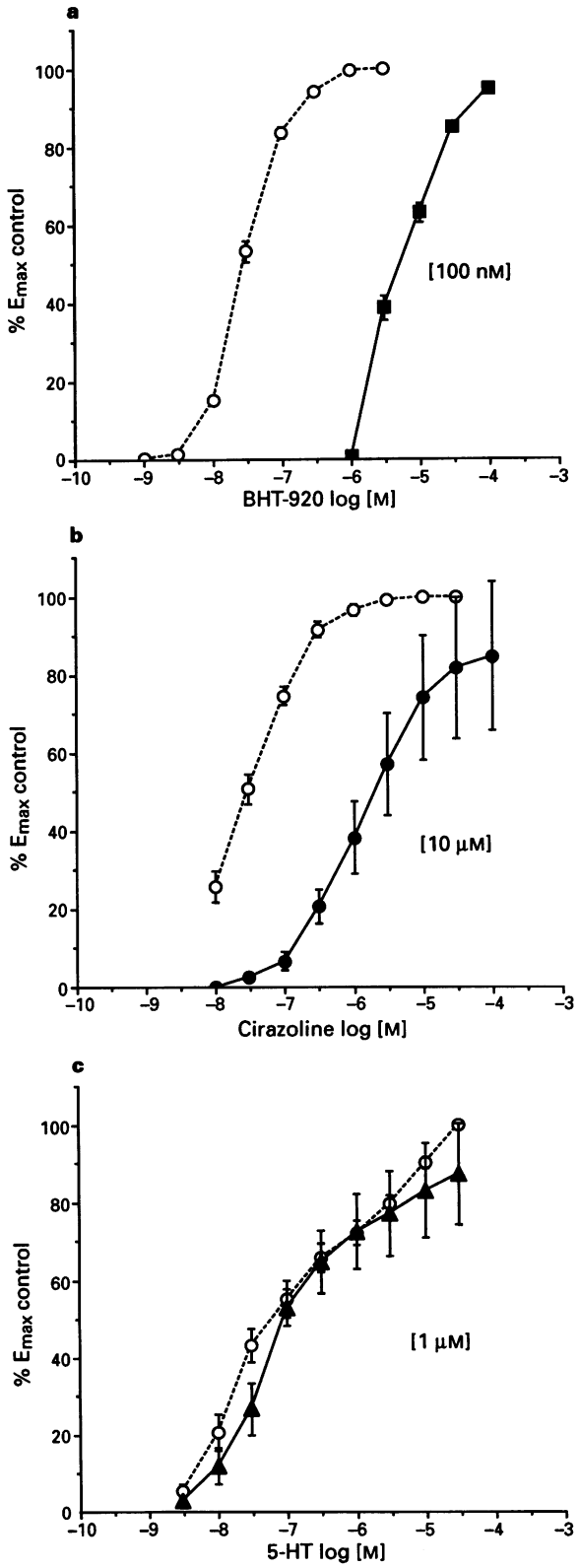

Figure 4 Contractile concentration-response curves for (a) BHT-920, (b) cirazoline and (c) 5-HT in dog saphenous vein rings in the absence (○) or presence of different concentrations of RS-15385-197 (■ 100 nM; ● 10 μM; ▲ 1 μM). Results are expressed as % maximum response to each agonist ± s.e. mean ($n = 4-7$ preparations per curve).

Prejunctional α_2-adrenoceptor effects UK-14,304 produced a dose-related decrease in the tachycardia evoked by continuous electrical stimulation of the thoracic spinal sympathetic outflow. RS-15385-197 progressively displaced the UK-14,304 dose-response curve to the right in a dose-related manner, without reduction in the maximum response (Figure 7c). The displacements were apparently greater than comparable doses tested against the pressor responses to UK-14,304 (cf. Figure 7a and 7c).

Mydriasis studies

Before drug administration, pupil diameter in the anaesthetized rat was around 0.2–0.3 mm. UK-14,304 (1–1000 μg kg^{-1}, i.v.) produced a dose-related increase in pupil diameter, reaching a maximum of about 4 mm. The dose used for the reversal studies (100 μg kg^{-1}, i.v.) was at approximately the

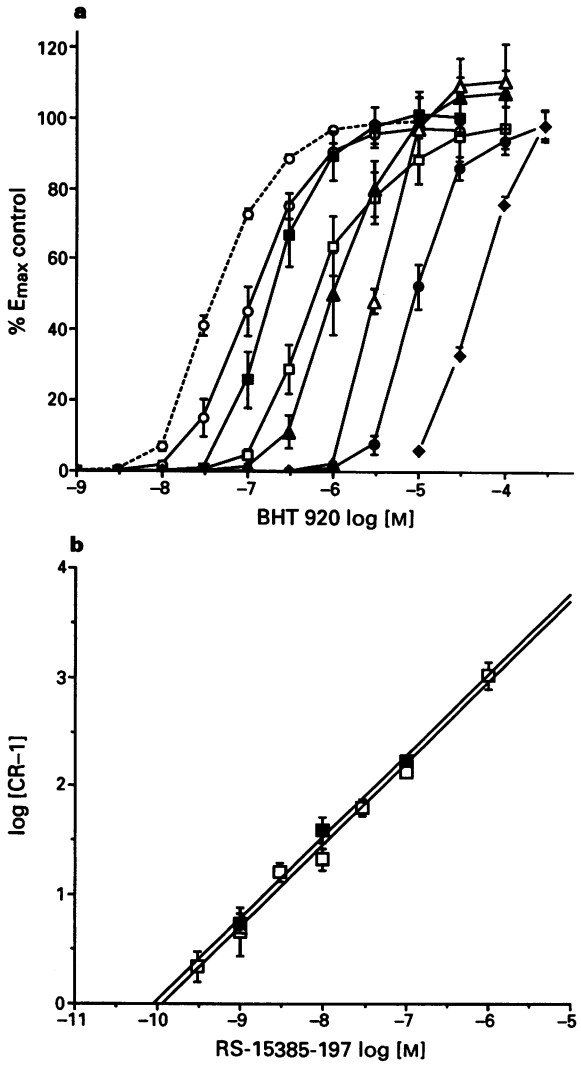

Figure 5 Contractile concentration-response curves for BHT-920 in dog isolated saphenous vein rings treated with phenoxybenzoamine (10 nM) in the absence (○, dashed line), or presence of different concentrations of RS-15385-197: (○) 0.3 nM; (■) 1 nM; (□) 3 nM; (▲) 10 nM; (△) 30 nM; (●) 100 nM; and (◆) 1000 nM. Results are expressed as % max response to BHT ± s.e.mean; n = 4–7 preparations per curve. (b) Schild analysis of the log [CR-1] versus −log [M] concentration of RS-15385-197 obtained in phenoxybenzamine-treated veins (■) and the superimposed Schild plot obtained in non-treated preparations (□).

ED_{90} level, and produced a sustained mydriasis for the duration of the antagonist dose-response curve.

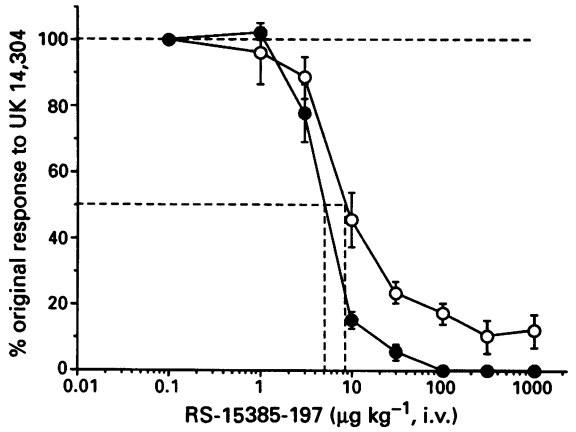

Figure 6 Reversal of response to UK-14,304 by cumulative doses of RS-15385-197: (○) reversal of diastolic pressor response to a standard challenge dos (30 μg kg^{-1}, i.v.) of UK-14,304 in rats pretreated with prazosin (100 μg kg^{-1}, i.v.; $n = 6$). Note the residual pressor response of about 10% of the original control response. (●) Reversal of mydriasis induced by UK-14,304 (100 μg kg^{-1}, i.v.) in anaesthetized rats ($n = 6$).

Reversal of agonist response/oral activity RS-15385-197 reversed the mydriatic response to UK-14,304 (100 μg kg^{-1}, i.v.) with an AD_{50} of 5 μg kg^{-1}, i.v. ($n = 6$), achieving complete reversal at 100 μg kg^{-1}, i.v. (Figure 6). This was far more potent than the other antagonists tested (Table 4). RS-15385-197 reversed the response to clonidine (300 μg kg^{-1}, s.c.) with an i.v. AD_{50} of 7 μg kg^{-1} ($n = 6$) and an oral AD_{50} of 95 μg kg^{-1} ($n = 6$). Idazoxan was less potent by either route (AD_{50} value of 17 and 1200 μg kg^{-1} respectively). Yohimbine and piperoxan were also weaker antagonists than RS-15385-197 when tested intravenously against clonidine (Table 4).

Table 4 Antagonism of responses to UK-14,304 and clonidine *in vivo*

	Peripheral AD_{50} (μg kg^{-1}, i.v.)		Central AD_{50} (μg kg^{-1}, i.v.)	
	UK-14,304	*Clonidine*	*UK-14,304*	*Clonidine*
RS-15385-197	7	7	5	7
L-649,066	650	–	>10000	–
Yohimbine	900	130	80	185
Idazoxan	>1000	79	20	17
Piperoxan	–	2000	–	2400

The AD_{50} value is the antagonist dose which reduced the agonist response by 50%. Data are the mean from at least 5 rats.

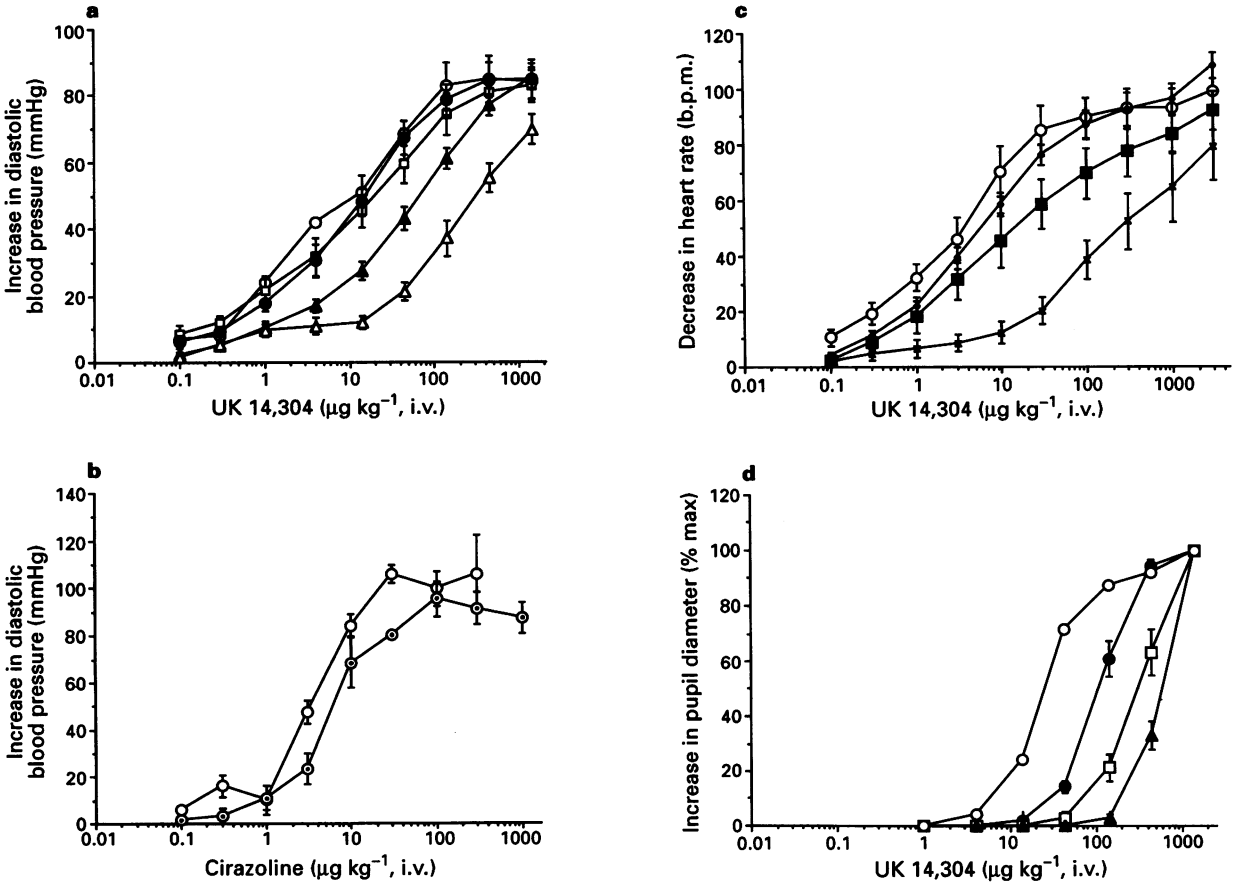

Figure 7 Displacement of agonist dose-response curves by RS-15385-197 *in vivo*. (a) Diastolic pressor response curves to cumulative doses of UK-14,304 in pithed rats pretreated with prazosin (100 μg kg^{-1}, i.v.); (b) diastolic pressor response curves to cirazoline in pithed rats; (c) bradycardiac response curves to UK-14,304 in pithed rats during stimulation of the cardiac sympathetic outflow; (d) mydriatic response curves to UK-14,304 in anaesthetized rats. (○) Control ($n = 6$–10); (●) RS-15385-197 9 μg kg^{-1}, i.v. ($n = 6$); (◆) 10 μg kg^{-1}, i.v. ($n = 6$); (■) 30 μg kg^{-1}, i.v. ($n = 6$); (□) 45 μg kg^{-1}, i.v. ($n = 6$); (×) 100 μg kg^{-1}, i.v. ($n = 6$); (▲) 225 μg kg^{-1}, i.v. ($n = 6$); (△) 1000 μg kg^{-1}, i.v. ($n = 6$); (○) 10000 μg kg^{-1}, i.v. ($n = 6$).

Displacement of agonist dose-response curves RS-15385-197 (9, 45 or 225 $\mu g\,kg^{-1}$, i.v.) caused a dose-related rightward displacement of the dose-response curve to UK-14,304 (Figure 7d). Idazoxan and yohimbine were less potent (data not shown). The dose-response curves in this assay were steeper than in the pithed rat pressor response assay and all three antagonists produced greater displacement of the agonist dose-response curve in the mydriasis assay than in the pithed rat pressor response tests.

Time course of antagonism following an oral dose At a dose of 1 $mg\,kg^{-1}$, p.o., RS-15385-197 had a dose-ratio of 46 after 1 h. This diminished over time, with an apparent effective half-life of around 2.5 h. Idazoxan, although given at a larger dose (30 $mg\,kg^{-1}$, p.o.), was less effective (dose-ratio 1 h after dosing was 40) and had a shorter duration of action (apparent effective half life $\sim$1.5 h).

Discussion

The present results demonstrate that RS-15385-197 is a highly potent α_2-adrenoceptor antagonist in a variety of test systems. The compound has exceptional affinity for the α_2-adrenoceptor in a range of tissues from different species with a $pK_i \geqslant 10$ in baboon cortex. The compound had essentially similar affinity in displacing [^{3}H]-yohimbine and [^{3}H]-idazoxan from α_2-adrenoceptors. RS-15385-197 has no affinity for imidazoline binding sites in hamster adipocytes, which provides further evidence that the α_2-adrenoceptor and the imidazoline binding site are very different recognition sites and probably represent different proteins. Furthermore, the structure-activity relationships for imidazolines at α_2-adrenoceptors and imidazoline binding sites are very different (Clark *et al.*, 1989b; 1991). RS-15385-197 did not differentiate between α_{2A} and α_{2B} sites, as represented by affinity for the human platelet and rat neonatal lung, consistent with previous data on [^{3}H]-RS-15385-197 binding in these preparations (MacKinnon *et al.*, 1992). These sites apparently have different genes (α_2 C10 represents α_{2A} whereas α_2 C2 represents α_{2B}; Lomasney *et al.*, 1990), yet RS-15385-197 had very similar affinity (pK_i 9.7 to 9.9) indicating that the recognition sites on these two subtypes show some similarities. However, RS-15385-197 did have significantly lower affinity (pK_i 8.38) for the α_{2A}-'like' subtype in hamster adipocytes, which provides further evidence that this site is different from the 'classical' α_{2A} subtype on human platelets (see also Brown *et al.*, 1990a). Yohimbine also shows lower affinity for this site (MacKinnon *et al.*, 1989). In this respect, high doses of RS-15385-197 (30 $\mu g\,kg^{-1}$, i.v.) are required to increase non-esterified fatty acid levels in dog plasma (unpublished observations).

The particular subtypes of α_2-adrenoceptors which mediate responses to α_2-adrenoceptor agonists in the guinea-pig ileum, rat vas deferens and dog saphenous vein are as yet unclear, and species differences may exist (Ruffolo *et al.*, 1991; Limberger *et al.*, 1992; Smith *et al.*, 1992). RS-15385-197 exhibited no selectivity between prejunctional (pA_2 in guinea-pig ileum 9.7) and postjunctional (pA_2 in dog saphenous vein 10.0) α_2-adrenoceptors *in vitro*. However, a slightly lower pA_2 value (9.3) with a slope less than unity (0.7) was obtained in the rat vas deferens. The reason for this is unknown, but it could be due to the presence of a mixed population of subtypes of prejunctional α_2-adrenoceptors, as has been suggested recently for this tissue (Harsing & Vizi, 1992; Smith *et al.*, 1992).

In contrast, RS-15385-197 had very low affinity for the α_1-adrenoceptor in rat cortex ([^{3}H]-prazosin binding) and in functional assays, with approximately 4000–14000 fold selectivity for α_2- over α_1-adrenoceptors. The α_1-adrenoceptor antagonist affinity against phenylephrine in rabbit aorta, and against cirazoline in dog saphenous vein were virtually identical; the α_1-adrenoceptor stimulated by cirazoline in this latter tissue has been considered to represent the α_{1A}-adrenoceptor subtype (Hicks *et al.*, 1991). In both binding and functional studies the −198 enantiomer was essentially devoid of α_2-adrenoceptor affinity, indicating a highly stereoselective interaction at the α_2-adrenoceptor. The structure-activity relationship for the interaction of various analogues of RS-15385 at the α_2-adrenoceptor has been described by Clark *et al.* (1991). It is quite possible that the residual activity of RS-15385-198 at the α_2-adrenoceptor may be due to contamination with the −197 isomer. The high performance liquid chromatography assay for RS-15385-198 only assures 99.9% purity and a small contamination with the active isomer could account for the activity observed with RS-15385-198. RS-15385-197 is therefore a potent α_2-adrenoceptor antagonist with greater selectivity over other receptors than previously available agents, having lower affinity for 5-HT receptors than yohimbine and rauwolscine and negligible affinity for imidazoline-preferring sites, unlike idazoxan. [^{3}H]-RS-15385-197 has recently been shown to be an excellent radioligand for the study of α_2-adrenoceptor subtypes (MacKinnon *et al.*, 1992).

In pithed rats, when a protocol of reversal of responses evoked by the selective α_2-adrenoceptor agonist, UK-14,304 was used, RS-15385-196 was a more potent antagonist than yohimbine, idazoxan, piperoxan or the peripherally-selective α_2-adrenoceptor antagonist, L-659,066. Using this protocol, we observed a small residual ($\sim$10%) pressor response to UK-14,304 which was still present after exclusion of both α_1-adrenoceptors (with prazosin) and α_2-adrenoceptors (with RS-15385-197, idazoxan, yohimbine or L-659,066). The cause of this small residual response to UK-14,304 is unknown; it is clearly not mediated by α-adrenoceptors and is therefore due to some unknown effect of UK-14,304. The residual response was excluded from the data analysis accordingly. When a range of doses of RS-15385-197 was tested for displacement of the full dose-response curve to UK-14,304 in pithed rats, it was estimated to give a dose-ratio of 2 at a dose of 45 $\mu g\,kg^{-1}$, i.v., indicating that it was 4 fold more potent than either idazoxan or yohimbine, and >5 fold more potent than L-659,066, which has previously reported to have a DR_2 of 264 $\mu g\,kg^{-1}$, i.v., in this test (Clineschmidt *et al.*, 1988). RS-15385-197 was highly selective *in vivo* for α_2-adrenoceptors compared with α_1-adrenoceptors over the dose-range tested, since even a very large dose (10 $mg\,kg^{-1}$, i.v.) showed minimal antagonism of the pressor responses induced by the selective α_1-adrenoceptor agonist, cirazoline.

In anaesthetized rats, RS-15385-197 was a potent antagonist of the mydriasis response induced by UK-14,304 or clonidine. The i.v. AD_{50} values obtained (5 and 7 $\mu g\,kg^{-1}$ respectively) corresponded to that obtained in the pithed rat experiments (7 $\mu g\,kg^{-1}$, i.v.), indicating that RS-15385-197 readily penetrates into the CNS. However, this equipotency was not confirmed by more detailed studies on displacement of the full dose-response curve to UK-14,304: RS-15385-197 was apparently more potent as an antagonist of central and of prejunctional respones to UK-14,304, than of the postjunctional pressor response. This is unlikely to be due to differences in receptor subtypes for the three responses as, in our binding studies, RS-15385-197 did not differentiate between the two α_2-adrenoceptor subtypes tested, i.e. α_{2A} (in human platelets) and α_{2B} (in rat neonate lung), and both idazoxan and yohimbine, which have different selectivities for these α_2-adrenoceptor subtypes, were also apparently more potent in displacing agonist dose-response curves in the mydriasis assay than in the pithed rat pressor response test. It is possible that RS-15385-197, yohimbine and idazoxan may be preferentially distributed in the brain, but a more likely explanation is that there is a non-adrenoceptor component in the pressor response to UK-14,304, as observed in the agonist reversal studies. Alternatively, the differences may reflect non-equilibrium states (Kenakin, 1987) and indicate the limitations of comparing different tests *in vivo*. This issue can only be resolved by further studies.

RS-15385-197 was also a potent α_2-adrenoceptor antagonist following oral administration, which was apparent both in experiments on reversal of agonist-evoked mydriasis and in the displacement of the agonist dose-response curves. These latter studies also showed that RS-15385-197 was relatively long-acting, compared to idazoxan, with an apparent biological half-life of around 2.5 h following oral administration.

In summary, RS-15385-197 is a potent, selective, competitive α_2-adrenoceptor antagonist *in vitro* and *in vivo*, which is orally active and readily penetrates the brain. It should prove to be a powerful tool with which to explore the various physiological roles of α_2-adrenoceptors in animals and man.

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