British Journal of Pharmacology (1996) 119, 1093-1100

The effects of SB 216469, an antagonist which discriminates between the α_{1A}-adrenoceptor and the human prostatic α_1-adrenoceptor

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1 The affinity of the α_1-adrenoceptor antagonist SB 216469 (also known as REC 15/2739) has been determined at native and cloned α_1-adrenoceptor subtypes by radioligand binding and at functional α_1-adrenoceptor subtypes in isolated tissues.

2 In radioligand binding studies with [^{3}H]-prazosin, SB 216469 had a high affinity at the α_{1A}-adrenoceptors of the rat cerebral cortex and kidney (9.5-9.8) but a lower affinity at the α_{1B}-adrenoceptors of the rat spleen and liver (7.7-8.2).

3 At cloned rat α_1-adrenoceptor subtypes transiently expressed in COS-1 cells and also at cloned human α_1-adrenoceptor subtypes stably transfected in Rat-1 cells, SB 216469 exhibited a high affinity at the α_{1a}-adrenoceptors (9.6-10.4) with a significantly lower affinity at the α_{1b}-adrenoceptor (8.0-8.4) and an intermediate affinity at the α_{1d}-adrenoceptor (8.7-9.2).

4 At functional α_1-adrenoceptors, SB 216469 had a similar pharmacological profile, with a high affinity at the α_{1A}-adrenoceptors of the rat vas deferens and anococcygeus muscle ($pA_2 = 9.5-10.0$), a low affinity at the α_{1B}-adrenoceptors of the rat spleen (6.7) and guinea-pig aorta (8.0), and an intermediate affinity at the α_{1D}-adrenoceptors of the rat aorta (8.8).

5 Several recent studies have concluded that the α_1-adrenoceptor present in the human prostate has the pharmacological characteristics of the α_{1A}-adrenoceptor subtype. However, the affinity of SB 216469 at human prostatic α_1-adrenoceptors ($pA_2 = 8.1$) determined in isolated tissue strips, was significantly lower than the values obtained at either the cloned α_{1a}-adrenoceptors (human, rat, bovine) or the native α_{1A}-adrenoceptors in radioligand binding and functional studies in the rat.

6 Our results with SB 216469, therefore, suggest that the α_1-adrenoceptor mediating contractile responses of the human prostate has properties which distinguish it from the cloned α_{1a}-adrenoceptor or native α_{1A}-adrenoceptor. Since it has previously been shown that the receptor is not the α_{1B}- or α_{1D}-adrenoceptor, the functional α_1-adrenoceptor of the human prostate may represent a novel receptor with properties which differ from any of the α_1-adrenoceptors currently defined by pharmacological means.

Keywords: SB 216469; REC15/2739; α_1-adrenoceptors; prostate; [^{3}H]-prazosin; native receptors; recombinant receptors

Introduction

The human prostate receives a dense sympathetic innervation (Chapple *et al.*, 1991) and stimulation of sympathetic nerves (Guh *et al.*, 1995) or the exogenous administration of the sympathetic neurotransmitter noradrenaline causes contraction of human prostatic smooth muscle via α_1-adrenoceptors (Hieble *et al.*, 1985). It is believed that this stimulation of prostatic α_1-adrenoceptors gives rise to the 'dynamic' component of bladder outlet obstruction observed in benign prostatic hyperplasia (BPH) and α_1-adrenoceptor antagonists have proven useful in the symptomatic relief of patients with benign prostatic hyperplasia. While α_1-adrenoceptor antagonists generally are safe drugs, their use can be limited by side effects such as orthostasis, which result from the antagonism of vascular α_1-adrenoceptors (Oesterling, 1995). Therefore, an α_1-adrenoceptor antagonist which antagonizes α-adrenoceptors in the prostate, at doses which have little effect on other tissues such as the vasculature, might be therapeutically useful. This goal may be achieved if selectivity for a receptor subtype which is present in human prostate, but which is less important in other tissues, can be developed.

In recent years three subtypes of α_1-adrenoceptors, now designated α_{1a}, α_{1b} and α_{1d}, have been cloned and pharmacologically characterized (for review see Michel *et al.*, 1995). Previously the cloned α_{1a}-adrenoceptor has also been designated α_{1c} and the cloned α_{1d}-adrenoceptor has also been designated $\alpha_{1a/d}$ or even α_{1a}, but the use of this nomenclature is no longer recommended (Hieble *et al.*, 1995). While each of these receptors has a unique tissue distribution, it remains unclear which is involved in the regulation of human resistance vessels. On the other hand, several investigators have studied the presence of these subtypes in the human prostate. Thus, all three subtypes have been detected in the human prostate but the α_{1a}-adrenoceptor dominates at the mRNA (Price *et al.*, 1993; Faure *et al.*, 1994; Tseng-Crank *et al.*, 1995) and protein level (Goetz *et al.*, 1994; Testa *et al.*, 1995; Tseng-Crank *et al.*, 1995). To determine which α_1-adrenoceptor subtype is functionally dominant in human prostate, the potencies of antagonists for inhibition of human prostatic strip contraction *in vitro* have been compared with the affinities of these drugs at cloned α_1-adrenoceptor subtypes (Forray *et al.*, 1994; Marshall *et al.*, 1995). These data have revealed that neither the α_{1B}- nor the α_{1D}-adrenoceptor appear to be important for the contraction of human prostate. It has been concluded therefore that the α_{1A}-adrenoceptor is functionally the most important receptor in this tissue (Forray *et al.*, 1994; Marshall *et al.*, 1995).

Based on this information, attempts are being made to develop α_{1A}-adrenoceptor-selective antagonists which may be

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effective in patients with benign prostatic hyperplasia, but which may have fewer side-effects than non-selective α_1-adrenoceptor antagonists. Among the contemporary α_1-adrenoceptor antagonists in clinical use, only tamsulosin is selective for α_{1A}-adrenoceptors (Hanft *et al.*, 1989). Recently, SB 216469 (also known as REC 15/2739) has been described in radioligand binding studies as highly selective for α_{1A}-adrenoceptors (Testa *et al.*, 1995). Since radioligand binding data may not always correctly predict functional relevance, the present study investigates further the properties of this compound. Radioligand binding studies were performed in rat tissues (spleen, liver, cerebral cortex and kidney) and with cloned α_1-adrenoceptor subtypes, the actions of SB 216469 being compared with the known α_{1A}-adrenoceptor-selective reference compound (+)-niguldipine (Boer *et al.*, 1989). To investigate subtype-selectivity at functional receptors, studies were performed on isolated tissues: rat vas deferens and anococcygeus muscle which posses α_{1A}-adrenoceptors (Mir & Fozard, 1988; Burt *et al.*, 1995), rat spleen and guinea-pig aorta where responses are mediated via α_{1B}-adrenoceptors (Mir & Fozard, 1988; Burt *et al.*, 1995) and rat aorta where α_{1D}-adrenoceptors mediate contraction (Ko *et al.*, 1994; Kenny *et al.*, 1995). The potency of SB 216469 at these receptors has been compared with that obtained at the α_1-adrenoceptors of human isolated prostatic strips.

Methods

Radioligand binding studies

Membrane preparations from rat liver, spleen, cerebral cortex and kidney were prepared from male Wistar rats (200–300 g) as previously described in detail (Michel *et al.*, 1993). The expression vector plasmids pCMVα_{1a} containing the EcoR1Pst1 2520 bp fragment of the rat α_{1d}-adrenoceptor cDNA and pcDV1Rα_{1b} containing a 2573 bp fragment including the entire coding region of the rat α_{1b}-adrenoceptor cDNA (Lomasney *et al.*, 1991) were obtained from Dr R. J. Lefkowitz (Durham, NC). The plasmid pMT2'α_{1c} which contains the entire coding region of the rat α_{1a}-adrenoceptor (Perez *et al.*, 1994) was obtained from Dr R. M. Graham (Sydney, Australia). The pBCα_{1c} plasmid which contains the entire coding region of the bovine α_{1a}-adrenoceptor (Schwinn *et al.*, 1990) was obtained from Dr S. Cotecchia (Lausanne, Switzerland). All four constructs were transfected into COS-1 cells for transient expression using the DEAE dextran method with addition of chloroquine and dimethylsulphoxide steps as described previously (Suryanarayana & Kobilka, 1991; Michel & Insel, 1994). Four days after transfection, cells were harvested, resuspended into ice-cold 20 mM $NaHCO_3$ solution and homogenized by a Tissuemizer for 10 s at full speed followed twice for 20 s at 2/3 speed. The homogenate was centrifuged for 20 min at 50,000 *g* and the resulting pellet was resuspended in binding buffer (50 mM Tris, 0.5 mM EDTA, pH 7.5) at a concentration of 0.6–2 mg ml^{-1}.

For an examination of human recombinant receptors, rat-1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4.5 g l^{-1} glucose, 1 mM L-glutamine and 5% heat-inactivated foetal calf serum. These cells had been stably transfected with expression vectors encoding the human α_{1a}-, α_{1b}- or α_{1d}-adrenoceptor as described previously (Schwinn *et al.*, 1995).

[^{3}H]-prazosin binding to membrane preparations from rat tissues, COS-1 cells or Rat-1 cells was performed in binding buffer (see above) as previously described (Michel *et al.*, 1993). Briefly, 100 μl of membrane suspension were incubated with the indicated [^{3}H]-prazosin concentrations for 45 min at 25°C. The incubations were terminated by rapid vacuum filtration through Whatman GF/C filters. Non-specific binding was defined as binding in the presence of 10 μM phentolamine. In competition experiments a single [^{3}H]-prazosin concentration ($\approx$200 pM) was used.

Isolated tissue studies

Male Wistar rats (250–300 g) were killed by a blow to the head and epididymal vas deferens, hemi-spleen cut longitudinally, and anococcygeus muscles set up in 30 ml organ baths. Segments (5 mm) of aorta from rats (250 g, male Wistar) and guinea-pigs (300 g, male Dunkin-Hartley) were also isolated, cut longitudinally, the endothelium removed by gentle rubbing and the vessels set up for recording contractions of the circular muscle. Strips of human prostate were obtained with informed consent from patients undergoing transurethral resection of the prostate. Ethical approval was obtained from the South Sheffield Research Ethics Committee. All tissues were set up in a Krebs-bicarbonate solution (composition in mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, KH_2PO_4 1.2 and glucose 11.7) gassed with 5% CO_2 in O_2 and maintained at 37°C. Tissues were set up under 1 g resting tension and the tension developed following the addition of phenylephrine or noradrenaline was measured by means of isometric force transducers (Lectromed UF1, 57 g sensitivity) connected to a Tandon PCA-sl computer via an analogue to digital converter (Cambridge Electronic Design). Developed tension was recorded by use of 'CHART' and analysed with 'SPIKE 2' software.

Drug administration

Tissues were equilibrated for 120 min with several changes of bathing medium. Control concentration-response curves were initially obtained to phenylephrine or noradrenaline before the addition of antagonist. Following washout of the agonist, tissues were incubated with SB 216469 for 30 min before obtaining a second concentration-response curve to the agonist in the presence of antagonist. Cumulative concentration-response curves using three fold increments in agonist concentration were obtained on all the tissues except the rat vas deferens, where responses to single concentrations of phenylephrine were obtained, washing between each drug addition.

Two concentration-response curves were obtained on each tissue (except spleen) the second curve being obtained either in the absence or presence of SB 216469. Identical control experiments were performed but without the addition of antagonist and these were used to correct for any time-dependent changes in sensitivity occurring during the course of the experiment. Due to significant tachyphylaxis with the rat spleen, only one concentration-response curve was constructed on each tissue. All experiments in the functional studies were performed in the presence of cocaine (10 μM) and corticosterone (10 μM) to inhibit amine uptake, and propranolol (1 μM) to antagonise β-adrenoceptors.

Data analysis

Radioligand binding data are mean $\pm$ s.e.mean of n experiments. Competition binding experiments were analyzed by fitting mono and biphasic sigmoidal curves to the experimental data; a biphasic fit was accepted only if it resulted in a significant improvement of the fit as judged by an F test with a $P<0.05$. All curve fitting procedures were performed by use of the InPlot program (GraphPAD Software, San Diego, CA, U.S.A.).

In functional studies, increases in developed tension induced by agonist were plotted as a percentage of the maximum increase for each concentration-response curve. Individual EC_{50} values (concentration for a half-maximal response) were determined and geometric mean EC_{50} values with 95% confidence limits calculated. Differences in mean EC_{50} values were analysed by Student's t test applied to individual logarithmic EC_{50} values. Dissociation constants (pK_B values) were determined from the equation:

$$pK_B = \log(CR - 1) - \log[SB]$$

where CR is the concentration-ratio (ratio of the EC_{50} values in the presence and absence of the antagonist) obtained with a concentration [SB] of SB 216469. Schild plots were also constructed and pA_2 values determined from the intercept on the abscissa scale as defined by Arunlakshana & Schild (1959). Data given in the text represent the mean with s.e.mean for pK_B values and maximal responses, whilst EC_{50} values are given as the geometric mean with 95% confidence limits.

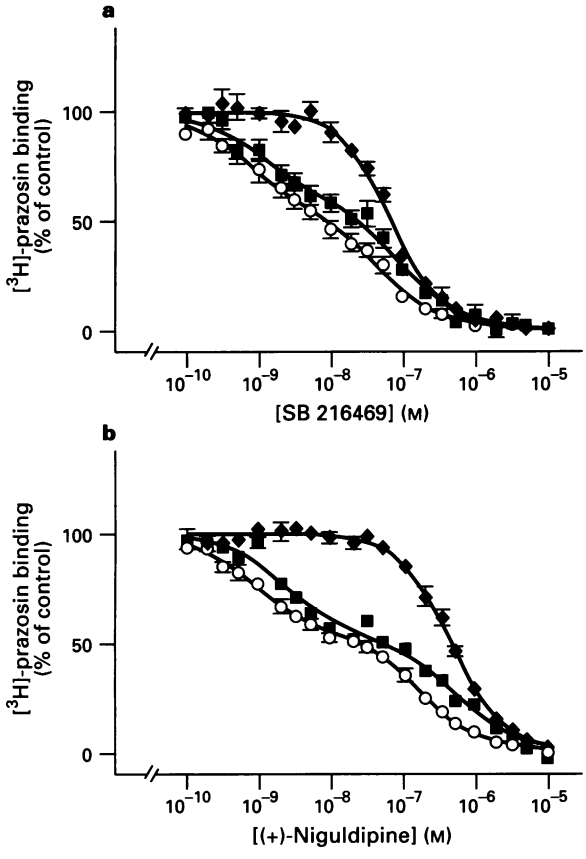

Figure 1 Competition of SB 216469 (a) and (+)-niguldipine (b) for [^{3}H]-prazosin binding to membranes from rat liver (◆), cerebral cortex (○) and kidney (■). Data are mean of 3–4 experiments; vertical lines show s.e.mean.

Drugs

[^{3}H]-prazosin (specific activity 70–80 Ci mmol^{-1}) was obtained from New England Nuclear. (+)-Niguldipine HCl was purchased from RBI (Natick, MA, U.S.A.). SB 216469 (N-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4-H-1-benzopropyran-8-carboxamide monoymethanesulfonate) (REC 15/2739) was kindly supplied as a gift by Smithkline Beecham. (L)-Phenylephrine hydrochloride, noradrenaline hydrochloride, cocaine hydrochloride, corticosterone-21-acetate and DL-propranolol hydrochloride were obtained commercially from Sigma. All reagents were of Analar grade.

Results

Radioligand binding data

SB 216469 and (+)-niguldipine competed for [^{3}H]-prazosin binding in rat liver and spleen with steep and monophasic competition curves (Figure 1, Table 1). In contrast both compounds competed for [^{3}H]-prazosin binding to rat cerebral cortex and kidney membranes with shallow curves which were significantly better explained by a two-site model (Figure 1, Table 1). The percentage of high affinity sites (45% in cortex, 38%–47% in kidney) was similar for both compounds.

Affinities of SB 216469 and (+)-niguldipine were slightly higher at bovine than at rat or human α_{1a}-adrenoceptors. At rat and human cloned receptors both compounds discriminated between the α_1-adrenoceptor subtypes and had highest affinity for the α_{1a}-adrenoceptor (Table 2). While SB 216469 had lowest affinity at the α_{1b}-adrenoceptor and intermediate affinity at the α_{1d}-adrenoceptor, (+)-niguldipine had lowest affinity at the α_{1d}- and intermediate affinity at the α_{1b}-adrenoceptor.

Isolated tissue results

At the α_{1A}-adrenoceptors of the rat vas deferens (Figure 2), SB 216469 caused rightward shifts of concentration-response curves to phenylephrine without affecting maximum responses (Table 3). The shifts were used to calculate mean pK_B values (−log dissociation constant, ±s.e.mean) and to construct Schild plots. The antagonist had a high affinity for the α_{1A}-adrenoceptors of these tissues and acted as a competitive antagonist yielding Schild plots with slopes not significantly different from unity (Table 3).

SB 216469 also acted as a potent antagonist of the contractile responses of the rat anococcygeus muscle (Figure 3) without altering maximum responses. The Schild plot had an intercept of 9.7 but the slope was significantly less than unity (Table 3).

Table 1 Parameters of competition binding in rat tissues

	Hill-slope	$-log\,K_{i\ high}$	$-log\,K_{i\ low}$	*% high*
Spleen				
SB 216469	1.12 ± 0.06	–	8.22 ± 0.04	*
(+)-Niguldipine	1.04 ± 0.03	–	7.49 ± 0.07	*
Liver				
SB 216469	1.16 ± 0.07	–	7.74 ± 0.04	*
(+)-Niguldipine	1.20 ± 0.07	–	6.88 ± 0.04	*
Cerebral cortex				
SB 216469	0.53 ± 0.03	9.84 ± 0.13	8.15 ± 0.08	45 ± 4
(+)-Niguldipine	0.39 ± 0.02	9.72 ± 0.10	7.42 ± 0.02	45 ± 2
Kidney				
SB 216469	0.59 ± 0.05	9.54 ± 0.03	7.73 ± 0.06	38 ± 10
(+)-Niguldipine	0.39 ± 0.02	9.23 ± 0.05	6.87 ± 0.03	47 ± 1

Data are mean ± s.e.mean of 3–4 experiments. *Competition curves were not significantly better explained by a two- compared to a one-site fit in an F test. Hill-slopes refer to monophasic fits; affinity values were taken from monophasic fits with unrestrained Hill-slopes in these cases. Where two-site fits were significantly better than one-site fits, affinity values are calculated from two-site fits where Hill-slopes for each component were restrained to unity. Calculation of K_i values was based on the following K_d values for [^{3}H]-prazosin: spleen 45 ± 14 pM (n = 4), liver 132 ± 36 pM (n = 3), cerebral cortex 104 ± 10 pM (n = 3) and kidney 110 ± 15 pM (n = 3).

Results obtained for SB 216469 at the α_{1B}-adrenoceptors of the rat spleen (Figure 4) and guinea-pig aorta (Figure 5) and at the α_{1D}-adrenoceptors of the rat aorta (Figure 6) were similar to those obtained for the vas deferens, the antagonist causing rightward shifts of concentration-response curves to phenylephrine without altering maximum responses (Table 3). Schild plots were again linear and had slopes similar to unity indicating competitive antagonism. However, pK_B values at these receptors were significantly ($P<0.05$) lower than those obtained on the rat vas deferens and anococcygeus muscle, the order of potency of SB 216469 at these functional receptors being the same as that obtained in the radioligand binding studies, $\alpha_{1A}>\alpha_{1D}>\alpha_{1B}$.

On the human prostate, SB 216469 again acted as a competitive antagonist, producing a Schild plot with a slope of unity and without altering maximum responses to noradrenaline (Figure 7, Table 3). The pK_B value obtained for SB 216469 on the prostate was significantly lower than those values obtained at the functional α_{1A}-adrenoceptors of the vas deferens and anococcygeus muscle, the native α_{1A}-adrenoceptors of the rat cerebral cortex and kidney and the cloned rat and bovine α_{1a}-adrenoceptors ($P<0.0001$ for all comparisons).

Discussion

The present study has investigated the properties of SB 216469 at α_1-adrenoceptor subtypes in radioligand binding and functional studies. Initially the binding properties of SB 216469 were characterized in comparison to the α_{1A}-adrenoceptor- selective reference compound, (+)-niguldipine, in rat tissues which are known to express homogeneous populations of α_{1B}-adrenoceptors (liver and spleen, Michel *et al.*, 1993) or possess a mixed α_1-adrenoceptor population (cerebral cortex and kidney, Michel *et al.*, 1993). The data demonstrate that SB 216469 and (+)-niguldipine have steep and monophasic competition curves in rat spleen and kidney with dissociation constants of 6–18 nM and 32–132 nM, respectively. This is in good agreement with a previously obtained affiity of 11 nM for SB 216469 in rat liver (Testa *et al.*, 1995) and with values found for (+)-niguldipine at α_{1B}-adrenoceptors by many investigators (for review see Michel *et al.*, 1995). In the tissues expressing multiple α_1-adrenoceptor subtypes, rat cerebral cortex and kidney, SB 216469 and (+)-niguldipine had shallow and biphasic competition curves. Their affinities at the low affinity site in both tissues corresponded to that at the α_{1B}-adrenoceptors in rat spleen and liver, and the percentage of high affinity sites was similar to that previously described as the percentage of α_{1A}-adrenoceptors in these tissues (Michel *et al.*, 1993). Thus, in rat cerebral cortex and kidney SB 216469 appeared to be approximately 50 fold selective for α_{1A}- over α_{1B}-adreno-

Table 2 Drug affinities at cloned α_1-adrenoceptor subtypes

Recombinant receptor	*SB 216469*	*(+)-Niguldipine*
Human α_{1a}	9.75±0.09	9.65±0.12
Rat α_{1a}	9.63±0.08	9.79±0.10
Bovine α_{1a}	10.38±0.22	10.25±0.06
Human α_{1b}	8.41±0.07	8.13±0.16
Rat α_{1b}	8.00±0.02	7.78±0.02
Human α_{1d}	9.17±0.15	7.89±0.04
Rat α_{1d}	8.70±0.14	7.39±0.25

Data are mean±s.e.mean of $-\log K_i$ of 3–4 experiments. Calculations of K_i values were based on the following K_d values for [^{3}H]-prazosin: α_{1a} 263±36 pM ($n=3$), α_{1b} 176±22 pM ($n=3$) and α_{1d} 137±51 pM ($n=3$).

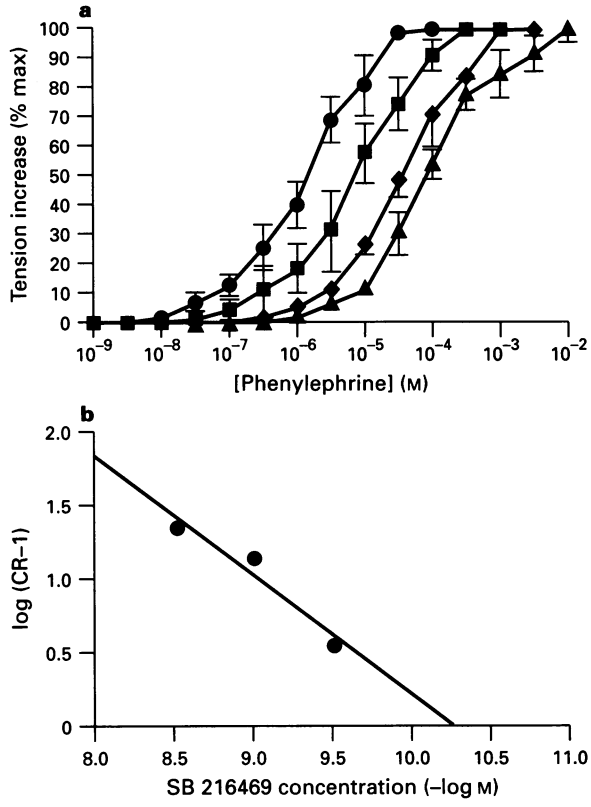

Figure 2 Concentration-response curves of rat vas deferens to phenylephrine in the absence (●) and presence of SB 216469 at concentrations of 0.3 nM (■) 1.0 nM (◆) and 3.0 nM (▲). (b) Schild plot for the antagonism of responses of the rat vas deferens to phenylephrine by SB 216469.

Table 3 Dissociation constants for SB 216469 obtained at α_1-adrenoceptors in various tissues

Tissue	*pK_B value*	n	*pA_2*	*Schild slope*	*Maximum 1* (g)	*Maximum 2* (g)
Rat vas deferens	10.0±0.1	12	10.3	0.80±0.21	2.53±0.24	2.34±0.15
Rat anococcygeus muscle	9.5±0.1	5	9.7	0.70±0.08*	6.94±0.32	7.60±0.32
Rat spleen	6.5±0.1	14	6.8	0.80±0.14	0.37±0.05	0.44±0.01
Guinea-pig aorta	8.0±0.2	10	7.9	1.10±0.16	1.08±0.05	1.12±0.08
Rat aorta	8.8±0.1	15	8.4	1.48±0.25	0.49±0.08	0.54±0.10
Human prostrate	8.4±0.1	15	8.1	0.96±0.19	1.49±0.73	1.13±0.59

Dissociation constants (pK_B) values are the mean (±s.e.mean) values of n experiments. The pK_B value for anococcygeus muscles is an apparent value obtained with the lowest concentration of SB 216469 (1 nM), since the slope of the Schild plot was significantly less than unity. Maximum 1 is the control maximum response, maximum 2 is the maximum response in the presence of the highest concentration of SB 216469. Only one concentration-response curve was obtained on each tissue for rat spleen with a total of 14 tissues being used to examine 3 different concentrations of antagonist (1–10 μM) and 6 tissues for controls. *$P<0.05$.

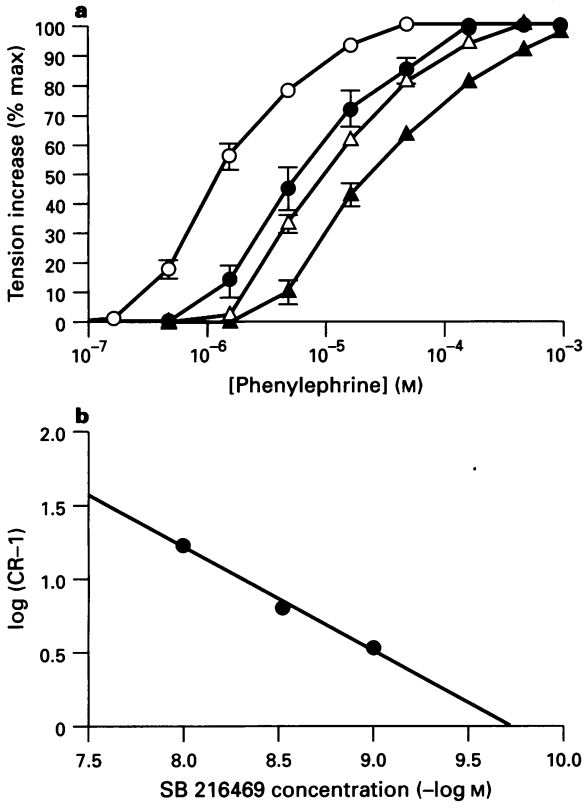

Figure 3 (a) Concentration-response curves of rat anococcygeus muscles to phenylephrine in the absence (○) and presence of SB 216469 at concentrations of 1.0 nM (●), 3.0 nM (△) 10 nM. (b) Schild plot for the antagonism of responses of the rat anococcygeus muscle to phenylephrine by SB 216469.

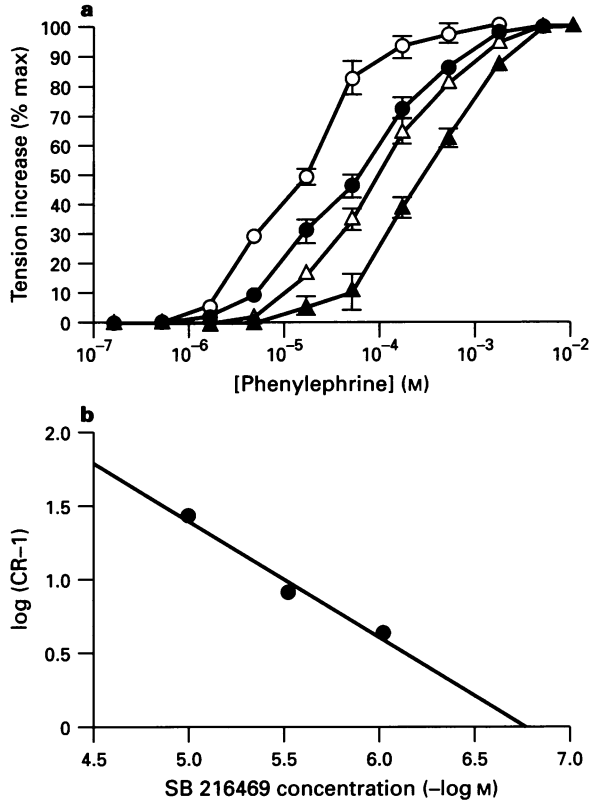

Figure 4 (a) Concentration-response curves of rat spleen to phenylephrine in the absence (○) and presence of SB 216469 at concentrations of 1 μM (●), 3 μM (△) and 10 μM (▲). (b) Schild plot for the antagonism of responses of the rat spleen to phenylephrine by SB 216469.

ceptors while the selectivity factor for (+)-niguldipine was approximately 160 fold.

To obtain a more definitive selectivity profile, both compounds were studied with bovine α_{1a}-adrenoceptors and also human and rat α_{1a}-, α_{1b} α_{1d}-adrenoceptors expressed in rat-1 or COS cells. The data with the human and rat cloned α_1-adrenoceptor subtypes indicate that (+)-niguldipine has an order of potency $\alpha_{1A} > \alpha_{1B} \geq \alpha_{1D}$ which is in agreement with most previous studies (Michel *et al.*, 1995). On the other hand, SB 216469 had an order of potency $\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$; a similar order of potency with slightly lower absolute affinities has recently been described at human α_1-adrenoceptor subtypes (Testa *et al.*, 1995). In the present study SB 216469 and (+)-niguldipine had somewhat higher affinities for bovine compared to human or rat α_{1a}-adrenoceptors, and a higher affinity of SB 216469 for bovine (9.54) compared to human α_{1A}-adrenoceptors (9.15) has also been described by others (Testa *et al.*, 1995). Taken together the present data indicate that SB 216469 is almost as selective for α_{1A}- over α_{1B}-adrenoceptors as (+)-niguldipine, but in contrast to (+)-niguldipine has intermediate affinity for α_{1D}-adrenoceptors.

Previously, the actions of SB 216469 at functional α_1-adrenoceptors have only been characterized in a very limited way (Testa *et al.*, 1995). Therefore, the second aim of this study was to investigate whether the α_{1A}-adrenoceptor selectivity of SB 216469 also translates into selectivity in functional tests. For this purpose we have used well established models of α_{1A}-adrenoceptors (rat vas deferens, Burt *et al.*, 1995; anococcygeus muscle, Mir & Fozard, 1988), α_{1B}-adrenoceptors (rat spleen, Burt *et al.*, 1995; guinea-pig aorta, Mir & Fozard, 1988, and α_{1D}-adrenoceptors (rat aorta, Ko *et al.*, 1994; Kenny *et al.*, 1995). The affinities obtained in these functional systems were in excellent agreement with those obtained at the cloned rat α_1-adrenoceptor subtypes, and the value obtained at the rat aortic α_{1D}-adrenoceptor is in close agreement with that found by Testa *et al.* (1994). Thus, SB 216469 is highly selective for α_{1A}-adrenoceptors in both radioligand and functional tests.

Finally the potency of SB 216469 has been examined at the functional α_1-adrenoceptors of the human prostate. In these experiments with four concentrations of SB 216469 ranging between 10 and 300 nM, the Schild regression line has a slope which was not significantly different from unity, indicating the absence of disequilibrium conditions and suggesting an interaction with a homogeneous population of receptors. Maximum responses were not depressed by even the highest concentration of SB 216469 which together with the slope of the Schild plot indicate a competitive interaction in this tissue. Surprisingly, the potency of SB 216469 on the human prostate was considerably lower than on the rat vas deferens or rat anococcygeus, and also lower than its binding affinity at native or recombinant rat $\alpha_{1A/a}$-adrenoceptors. A slightly higher affinity ($pK_B = 8.57$) for SB 216469 at the prostatic α_1-adrenoceptor has recently been obtained by Testa *et al.* (1996), but this value is also well below that obtained at the native or recombinant $\alpha_{1A/a}$-adrenoceptors in the present study.

As a comparison with SB 216469, we have also determined the affinity of WB4101 for these α_1-adrenoceptors under identical conditions. For this antagonist, pK_B values ranged from 9.4 for the vas deferens and 9.2 for the rat aorta, down to 8.4 for both the rat spleen and the guinea-pig aorta, thus demonstrating the selectivity of this antagonist for α_{1A}- and α_{1D}-adrenoceptors over α_{1B}-adrenoceptors. A value of 8.8 was obtained for both the human prostate and the rat anococcygeus muscle, a value that is similar to those previously found for WB4101 on the human prostate (8.9, Hatano *et al.*, 1994; 9.0, Chapple *et al.*, 1994). It is interesting to note that when the relative affinities of SB 216469 and WB4101 are compared (SB

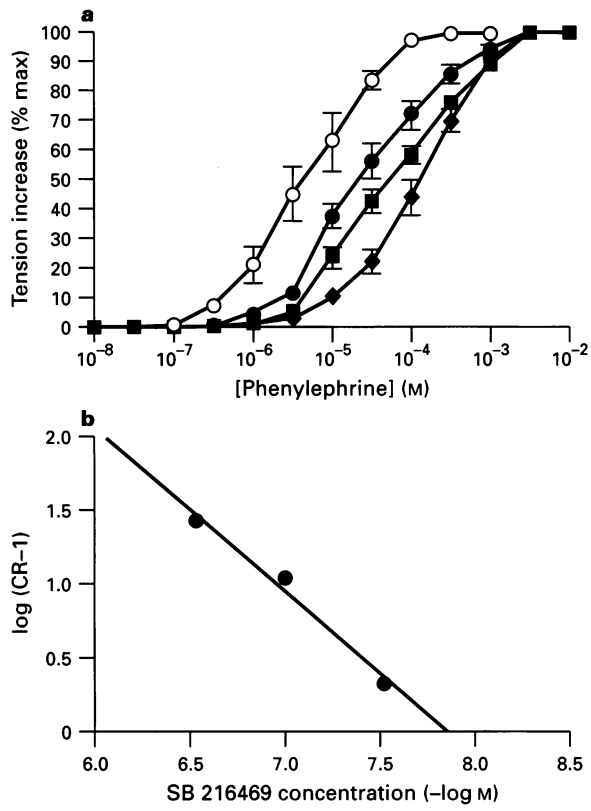

Figure 5 (a) Concentration-response curves of guinea-pig aorta to phenylephrine in the absence (○) and presence of SB 216469 at concentrations of 30 nM (●), 100 nM (■) and 300 nM (◆). (b) Schild plot for the antagonism of responses of the guinea-pig aorta to phenylephrine by SB 216469.

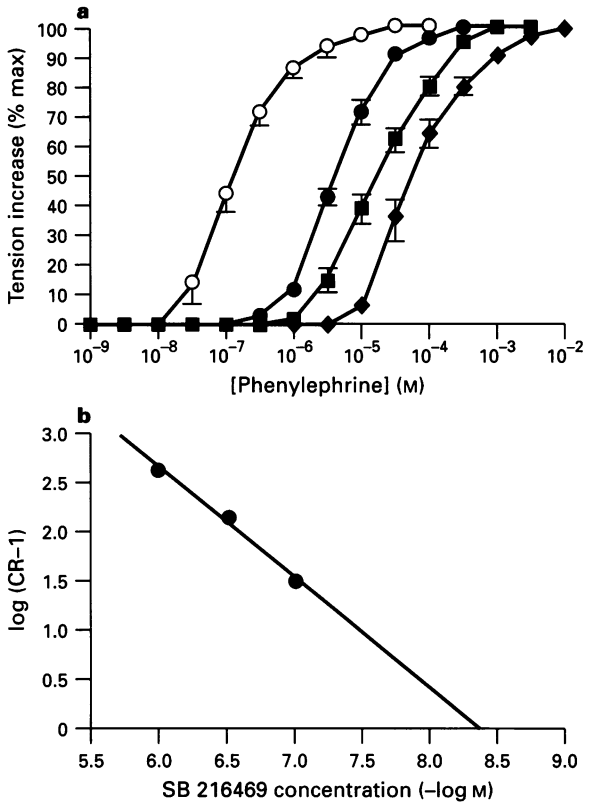

Figure 6 (a) Concentration-response curves of rat aorta to phenylephrine in the absence (○) and presence of SB 216469 at concentrations of 100 nM (●), 300 nM (■) and 1.0 μM (◆). (b) Schild plot for the antagonism of responses of the rat aorta to phenylephrine by SB 216469.

216469 K_D)/WB4101 K_D), the ratios are 0.3 for the vas deferens and 0.2 for the rat anococcygeus muscle but 2.0 for the human prostate. Thus, if the relative affinity of SB 216469 is compared to a 'standard' antagonist like WB4101, the results again support the hypothesis that the prostatic receptor is different from the α_{1A}-adrenoceptor.

Some of the differences may be species related, since the affinity of SB 216469 appears to be higher for bovine than rat or human recombinant α_{1a}-adrenoceptors. However, all these affinities are greater than the value obtained at the prostatic receptor in the present study, where the affinity of SB 216469 was between that obtained at α_{1B}-adrenoceptors and α_{1D}-adrenoceptors in both binding and functional assays. These data are not compatible with the idea that contraction of the human prostate is mediated via a typical α_{1A}-adrenoceptor.

Previous evidence for the mediation of human prostate contraction by an α_{1A}-adrenoceptor mainly rests on two studies (Forray *et al.*, 1994; Marshall *et al.*, 1995). In these two studies the potency to inhibit contraction of human prostate strips was compared with affinities at cloned α_1-adrenoceptor subtypes for four (Forray *et al.*, 1994) and seven (Marshall *et al.*, 1995) subtype-selective compounds. Both studies clearly demonstrated that α_{1B}- and α_{1D}-adrenoceptors are unlikely to be involved in mediating contraction of human prostate, while antagonist affinities at human prostatic receptors correlate well with those at α_{1A}-adrenoceptors.

Our present observations on SB 216469 are difficult to reconcile with the conclusions of Forray *et al.* (1994) and Marshall *et al.* (1995). On the other hand a recent study (Ford *et al.*, 1996) on another α_1-adrenoceptor antagonist, RS 17053, demonstrated that this compound is also selective for α_{1A}-relative to α_{1B}- or α_{1D}-adrenoceptors in radioligand binding and functional studies, but its affinity determined at the functional α_1-adrenoceptors of the human prostate is approximately 100 times lower than at α_{1A}-adrenoceptors. This supports our

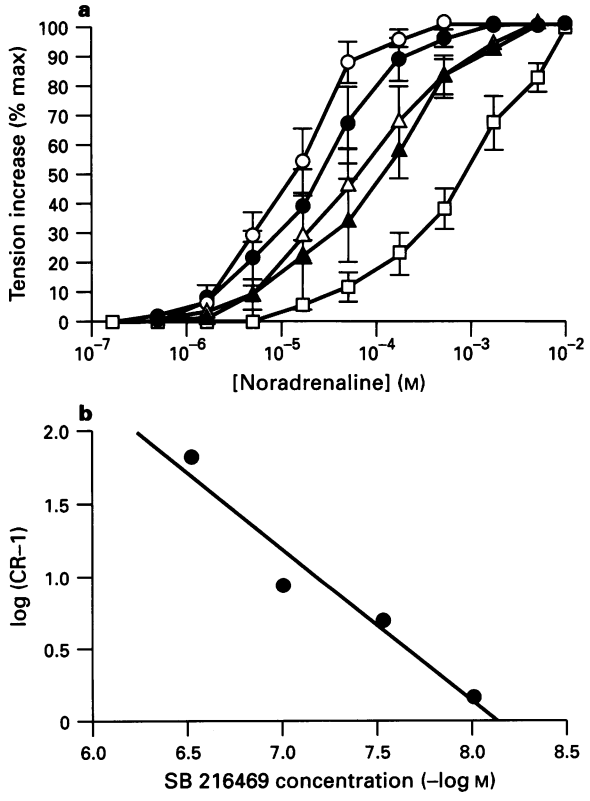

Figure 7 (a) Concentration-response curves of the human prostate to noradrenaline in the absence (○) and presence of SB 216469 at concentrations of 10 nM (●), 30 nM (△) and 100 nM (▲) and 300 nM (□). (b) Schild plot for the antagonism of responses of the human prostate to noradrenaline by SB 216469.

premise that a typical α_{1A}-adrenoceptor is not involved in mediating the contractions of the human prostate. Since previous studies provide conclusive evidence that neither α_{1B}- nor α_{1D}-adrenoceptors contribute to this response (Forray *et al.*, 1994; Marshall *et al.*, 1995), it is possible that a novel α_1-adrenoceptor is responsible. Although only three α_1-adrenoceptor subtypes have been unequivocally identified by cloning and in pharmacological studies (Hieble *et al.*, 1995; Michel *et al.*, 1995), several studies have suggested the possible existence of additional α_1-adrenoceptor subtypes, which are characterized by a relatively low affinity for prazosin (Flavahan & Vanhoutte, 1986; Murumatsu *et al.*, 1990). The recent demonstration of splice variants of the α_{1A}-adrenoceptor (Hirasawa *et al.*, 1995) also suggest the possibility of further α_{1A}-adrenoceptor heterogeneity. Since many α_{1A}-selective drugs also have a high potency in human prostate (Forray *et al.*, 1994; Marshall *et al.*, 1995), it is possible that one of these splice variants is present in human prostate and has low affinity for some α_{1A}-adrenoceptor-selective drugs such as SB 216469 and RS 17053. Thus, we speculate that a novel α_1-adrenoceptor subtype distinct from α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, but possibly a splice variant of the α_{1A}-adrenoceptor, may be involved in mediating catecholamine-induced contraction of human prostate.

While our findings support the view that the prostatic α_1-adrenoceptor may be distinguished from the α_{1A}-adrenoceptor, SB 216469 may still be useful for the treatment of benign prostatic hyperplasia. It is not yet clear which subtype mediates constriction in human blood vessels, but early indications suggest that the receptor has the pharmacological characteristics of an α_{1B}-adrenoceptor (Hatano *et al.*, 1994). In our functional studies, the affinity of SB 216469 differed considerably between the α_{1B}-adrenoceptors of the rat spleen and guinea-pig aorta, perhaps reflecting species or tissue differences in receptor characteristics. This could result from differential processing of a single α_{1B}-transcript which has been described (McGehee *et al.*, 1990) and which appears to be tissue-dependent. Affinity values for both tissues were significantly lower than any value obtained at α_{1A}- or α_{1D}-adrenoceptors which is consistent with the selectivity profile predicted by the binding affinity of SB 216469 to rat and human recombinant α_1-adrenoceptors (present data and that of Testa *et al.*, 1995). The results therefore confirm the greater selectivity of SB 216469 for cloned α_{1a}- over α_{1b}-adrenoceptors, the isolated tissues studies further demonstrating this selectivity at functional receptor subtypes. Thus, SB 216469 exhibits the greatest α_{1A}-adrenoceptor versus α_{1B}-adrenoceptor selectivity of any α_1-adrenoceptor antagonist currently being developed for the treatment of benign prostatic hyperplasia. In conclusion, our study demonstrates that SB 216469 is an α_1-adrenoceptor antagonist with selectivity for α_{1A}- relative to α_{1D}- and α_{1B}-adrenoceptors but with a relatively lower affinity for human prostatic α_1-adrenoceptors than predicted by binding affinities for recombinant α_{1a}-adrenoceptors or native α_{1A}-adrenoceptors. The relevance of this receptor selectivity to the treatment of benign prostatic hyperplasia remains to be determined in clinical studies. These data together with those of Ford *et al.* (1996) open the possibility that an α_1-adrenoceptor subtype distinct from the previously identified and cloned subtypes may be involved in contraction of the human prostate.

We thank Dr Geoff Johnston at Pfizer Central Research for supplying the stably transfected Rat-1 cells.

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(Received December 11, 1995
Revised June 10, 1996
Accepted July 25, 1996)