ELSEVIER

European Journal of Pharmacology 336 (1997) 29–35

ejp

The antipsychotic drug sertindole is a specific inhibitor of α_{1A}-adrenoceptors in rat mesenteric small arteries

Merete Ipsen [a], Youyi Zhang [c], Nils Dragsted [b], Chide Han [c], Michael J. Mulvany [a,*]

[a] *Department of Pharmacology, University of Aarhus, Universitetsparken 240, 8000 Aarhus C, Denmark*
[b] *H. Lundbeck A / S, Copenhagen, Denmark*
[c] *Institute of Vascular Medicine, Third Hospital Beijing Medical University, Beijing, China*

Received 18 July 1997; revised 4 August 1997; accepted 5 August 1997

Abstract

We have investigated the adrenergic antagonist effect of the antipsychotic sertindole in rat resistance vessels and in membranes of HEK293 cells transfected with α_1-adrenoceptors. Segments of rat mesenteric small arteries or rat aorta were mounted on a myograph for isometric tension recording. In mesenteric small arteries, specific α_{1A}-adrenoceptor antagonists (5-methyl urapidil and WB-4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane)) inhibited phenylephrine responses with high affinity (pA_2 9.1 and 9.5, respectively). Chlorethylclonidine (α_{1B}- and α_{1D}-adrenoceptor antagonist) and BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4,5] decane-7,9-dione dihydrochloride, α_{1D}-adrenoceptor antagonist) had little effect. This indicated that the adrenoceptor subtype in the mesenteric small arteries was of the α_{1A} subtype. Sertindole inhibited the phenylephrine response of mesenteric small arteries (pA_2 9.0), but had little effect on the phenylephrine response of aorta (which lacks α_{1A}-adrenoceptors). The specific action of sertindole on α_{1A}-adrenoceptors was supported by experiments with membranes of HEK293 (human embryonic kidney) cells transfected with the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors. Here, with concurrent incubation, sertindole showed selective competitive inhibition of BE2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) binding to α_{1A}-adrenoceptors (K_i 8.9), compared to α_{1B}-adrenoceptors (K_i 7.1) and α_{1D}-adrenoceptors (K_i 6.8). Despite the apparent competitive action sertindole, it was not possible to wash out its antagonist effect within 6 h in the functional phenylephrine concentration–response experiments. Furthermore, in the membranes of HEK293 cells transfected with the α_{1A}-adrenoceptors, binding of [^{125}I]BE2254 was reduced by 45% following preincubation with sertindole (1 nM). We conclude that the α-adrenoceptors of rat mesenteric small arteries are of the α_{1A}-type, and that sertindole is a specific pseudo-irreversible competitive antagonist of this adrenoceptor subtype. © 1997 Elsevier Science B.V.

Keywords: Adrenoceptor subtype; Antipsychotic; Small artery

1. Introduction

Sertindole (5-chloro-1-(4-fluorophenyl)-3-4-piperidyl]-1H-indole) is an antipsychotic with a high selectivity for dopamine neurones in the ventral tegmental area (Skarsfeldt and Perregaard, 1990; Sánchez et al., 1991). In patients, as with other antipsychotics (Olesen et al., 1995; Curtis and Kerwin, 1995), some indications of transient orthostatic hypotension to sertindole has been observed (Dunn and Fitton, 1996; Van Kammen et al., 1996). This has suggested that sertindole might be an antagonist for α-adrenoceptors. High affinity for α_1-adrenoceptors has been noted in vitro (Sánchez et al., 1991) and ex vivo (Hyttel et al., 1992), but the functional effects of sertindole on the blood vessels which determine peripheral resistance and thus blood pressure have not been investigated. We have therefore tested the hypothesis that sertindole acts on the α-adrenoceptors of resistance vessels. This has been done by studying the effects of sertindole on the phenylephrine responses of rat mesenteric small arteries in vitro, vessels which are small enough to participate in the control of resistance in this vascular bed (Fenger Gron et al., 1995).

Adrenoceptor classification, now well-defined (Graham et al., 1996), has indicated that the adrenoceptors in rat mesenteric small arteries are of the α_{1A}-type (Chen et al., 1996), in contrast to, for example, rat aorta where α_{1D}-adrenoceptors appear to predominate (Piascik and Alexander, 1995; Kenny et al., 1995; Saussy et al., 1996; Buckner et al., 1996; Deng et al., 1996). In the present investiga-

* Corresponding author. Tel.: (45) 8942-1726; Fax: (45) 8612-8804.

tion, the aim was to determine the specificity of sertindole for this adrenoceptor sub-type, using both functional studies on other tissues and binding studies on subclones of cells stably transfected with α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors.

Some of these results have been previously presented in brief (Ipsen et al., 1995).

2. Materials and methods

2.1. Animals and preparations

Male Wistar rats (250–350 g) were killed by cervical dislocation or by carbon dioxide gas. Thereafter, the mesentery or the aorta was removed immediately and placed in a cold physiological salt solution (PSS) of the following composition (mM): $CaCl_2$ 2.5, NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5. The arteries (length: 2 mm; diameters, normalized as described below: mesenteric small artery approx. 200 μm and aorta approx. 2.5 mm were then dissected free from fat tissue and mounted as ring preparations in a myograph (Mulvany and Halpern, 1977). Up to 2 vessels of each type were taken from each animal. In the bath, the PSS was heated to 37°C and bubbled with 5% CO_2 in O_2. The vessels were equilibrated for at least 30 min in PSS before they were set to a normalized internal circumference, estimated to be 0.9 times the relaxed circumference at 100 mmHg transmural pressure (Mulvany and Halpern, 1977). After activation of the vessel, as described below, the endothelium was removed by gently rubbing the lumen with a hair. The presence of endothelium, before and after removal, was tested using phenylephrine 3 μM (aorta 1 μM) to get a contraction and then acetylcholine 1 μM (aorta 3 μM). The endothelium was assumed to be removed totally if no relaxation was observed; in cases where relaxation was still present, the lumen was rubbed again, and the procedure repeated.

2.2. Drugs

The following drugs were used: phenylephrine hydrochloride, noradrenaline (arterenol hydrochloride), corticosterone 21-acetate, yohimbine hydrochloride, propranolol chloride and acetylcholine chloride (Sigma, Poole, UK); phentolamine, 5 methylurapidil, WB-4101 hydrochloride (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane), BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4,5] decane-7,9-dione dihydrochloride) and chlorethylclonidine dihydrochloride (Research Biochemicals International, Natick, MA, USA); cocaine hydrochloride (local hospital dispensary); BE2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) (Beisdorf, Hamburg, Germany); $Na^{125}I$ (Beijing Institute of Atomic Energy, Chinese Academy of Sciences, Beijing, China). Sertindole was kindly provided by H. Lundbeck A/S, Denmark.

All drugs except corticosterone 21-acetate and sertindole were dissolved in distilled H_2O, corticosterone 21-acetate was dissolved in 100% acetone and sertindole was dissolved in a few drops of HCl, heated to 40°C, and then diluted in distilled H_2O.

2.3. Experimental procedure

2.3.1. Mesenteric small arteries

The vessels were primed using five solutions in turn: (a) PSS in which NaCl had been replaced by KCl on an equimolar basis (K-PSS) plus 10 μM noradrenaline, (b) repeat (a), (c) 10 μM noradrenaline, (d) K-PSS, (e) repeat (a); 3 min per activation, 5 min washout in PSS. After removal of endothelium (see above), propranolol (1 μM), cocaine (1 μM) and corticosterone 21-acetate (1 μM) were added to the bath to block β-adrenoceptors, and neuronal and extraneuronal uptake, respectively. A number of phenylephrine concentration–response-curves were then obtained by adding phenylephrine in cumulative half log doses (3 nM–1 mM) to the bath every 3 min, with 30 min between concentration–response curves. Antagonists, as indicated, were added to the PSS before each concentration–response curve: BMY7378 and sertindole were added 30 min, and 5-methylurapidil was added 10 min, before the next concentration–response curve; chlorethylclonidine was added for 30 min, and was then washed out 4 times every 10 min for 40 min before the next concentration–response curve. For 5 methylurapidil, 5 concentration–response curves were performed (control, and in the presence of 1 nM, 3 nM, 10 nM, or 30 nM 5 methylurapidil). For the other antagonists, 2 concentration–response curves were performed (control, and in the presence of the concentration of drug indicated). With sertindole, some washout experiments were performed. Following the concentration–response curve with sertindole, sertindole was washed out (5 washes of the chamber with PSS), and phenylephrine concentration–response curves were repeated at hourly intervals for 6 h, with frequent washes of the chamber in between.

2.3.2. Aorta

The vessels were primed three times with noradrenaline 1 μM (3 min per activation, 5 min washout in PSS). The endothelium was removed as described above. Yohimbine (0.1 μM), propranolol (1 μM), cocaine (6 μM) and corticosterone acetate (1 μM) were added to the bath to block α_2- and β-adrenoceptors, and neuronal and extraneuronal uptake, respectively. Phenylephrine concentration–response curves were obtained as described above for two vessels at the same time, one with and one without antagonist added. Antagonists were added prior to the concentration–response curves as described above.

2.4. Cloned cell experiments

2.4.1. Cell culture

Subclones of human embryonic kidney 293 cells (HEK293) transfected with the bovine α_{1A}, hamster α_{1B} or rat α_{1D} cDNA were kindly provided by Dr. K.P. Minneman (Atlanta, GA). Subclones were maintained in Dulbecco's modified Eagle's medium of the following composition: calf serum (10%), glucose (4.5 g/l), streptomycin (100 mg/l), penicillin (100 000 U/l). The cells were propagated in the continued presence of selective antibiotics: α_{1A}, histidinol-pREP8; α_{1B}, 0.05 mg/ml hygromycin-pREP4; α_{1D}, 0.15 mg/ml geneticin-pREP9).

2.4.2. Membrane preparation

Cells were harvested by scraping confluent 75 cm^3 flasks and pelleted by centrifugation at $500 \times g$ for 5 min, washed with 10 ml physiological buffer solution (Na_2HPO_4, 20 mM; NaCl, 154 mM, pH 7.6, PBS) and centrifuged again. Cells were homogenized with a Polytron (speed 6, 10 s) in 10 ml PBS. Membranes were collected by centrifugation at $20\,000 \times g$ for 10 min, resuspended in PBS and centrifuged again. Membranes were then resuspended in PBS (one Flask HEK 293 cell transfected with α_{1A} or α_{1D} per 10 ml final suspension, or one Flask HEK 293 cell transfected with α_{1B} per 15 ml final suspension).

2.4.3. Radioligand binding assays

BE2254 was radioiodinated to theoretical radioactivity of 2200 Ci/mmol as described by Engel and Hoyer (1981) and stored at −20°C in methanol. Measurements of specific [^{125}I]BE2254 binding were performed by incubating tissue preparation with [^{125}I]BE2254 in PBS in a final volume of 250 μl for 20 min at 37°C in the presence or absence of competing drugs. After 20 min, the incubation was terminated by adding 10 ml of 10 mM Tris–HCl (pH 7.4) and the mixture was filtrated over a glass fibre filter in vacuum. Each filter was washed with 10 ml of 10 mM Tris–HCl (pH 7.4), dried and its radioactivity (counts per minute, cpm) was measured. Non-specific binding was determined in the presence of 10 μM phentolamine. In the experiments, the non-specific binding was less than 15%. Protein concentrations of the preparation were measured by the Coomassie brilliant blue method.

The saturation curves were determined by incubating tissues with increasing concentrations of [^{125}I]BE2254 (15–520 pM, 15 000–500 000 cpm) and the data were subjected to Scatchard analysis. To determine the affinity of 5 methylurapidil, BMY7378 and sertindole to α_1-adrenoceptors, the potencies of the drugs in competition for the specific [^{125}I]BE2254 binding sites were determined by 20 min incubation in PBS, 37°C, of a single concentration of [^{125}I]BE2254 (40–50 pM) in the presence or absence of 16 concentrations of drug. IC_{50} values were determined from a Hill plot, and K_i values calculated by the method of Cheng and Prusoff (1973).

To investigate if sertindole binds to the α_1-adrenoceptors competitively or non-competitively, Scatchard analyses of the binding of [^{125}I]BE2254 (50–55 pM, 20 min incubations) to membranes of HEK293 cells transfected with the α_{1A}-adrenoceptors was determined as above either (a) under control conditions, or (b) in the presence of 1 nM sertindole (i.e close to the determined K_i value of sertindole for α_{1A}-adrenoceptors), added at the same time as the [^{125}I]BE2254, or (c) in cells which had been incubated in the presence of 1 nM sertindole for 20 min followed by three-fold thorough washing.

2.5. Data analysis

Results are presented as mean ± S.E.M. Comparison of values was by unpaired two-tailed Student *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Antagonism of phenylephrine response of mesenteric small arteries

Phenylephrine caused a concentration-dependent contraction of mesenteric small arteries (pD_2 6.31 ± 0.10, $n = 16$) which was competitively antagonized by 5 methylurapidil (Table 1, Fig. 1). Similarly, WB-4101 competitively antagonized the phenylephrine concentration–response curve with a pA_2 of 9.52 and Schild slope 1.07 ± 0.23 ($r^2 = 0.52$, $n = 6–8$, data not shown). BMY7378 also caused competitive antagonism of the phenylephrine concentration–response curve (Table 1, Fig. 2). Chlorethyl-

Table 1
Schild analysis of the effects of 5 methylurapidil and BMY7378 on phenylephrine concentration–response relation

	5 methylurapidil				BMY7378			
	pA_2	slope	r^2	n	pA_2	slope	r^2	n
Mes. small art.	9.11	1.06 ± 0.14	0.81	16	6.35	1.48 ± 0.15	0.86	18
Aorta	[a]	[a]	[a]	15	7.58	1.20 ± 0.17	0.78	15

Shown are calculated pA_2 values, the slope of the Schild regression line (mean ± S.E.M.) and the regression coefficient (r^2). n shows number of vessels (one vessel per animal). Mes. small art.: mesenteric small arteries.
[a] Small size of effect of 5 methylurapidil on aorta prevented Schild analysis.

Table 2
Effect of chlorethylclonidine (chorethylclonidine dihydrochloride) on phenylephrine (PE) concentration–response relation

Artery	Control	Chlorethylclonidine dihydrochloride		*n*
	PE-pD_2	PE-pD_2	max. response (% control)	
Mes. small art.	6.61 ± 0.05	5.72 ± 0.10	88 ± 2	6
Aorta	6.11 ± 0.27	—	12 ± 5	6

Table shows (mean ± S.E.M.) PE-pD_2-values under control conditions and following 30 min exposure to chorethylclonidine dihydrochloride (50 μM), together with the maximum response following chlorethylclonidine dihydrochloride exposure, expressed with respect to the response under control conditions. *n* shows number of vessels (one vessel per animal). Mes. small art.: mesenteric small arteries.

clonidine (50 μM) caused a right-shift in the phenylephrine concentration–response curve of 7.8-fold, and a 12% reduction in the maximum response (Table 2).

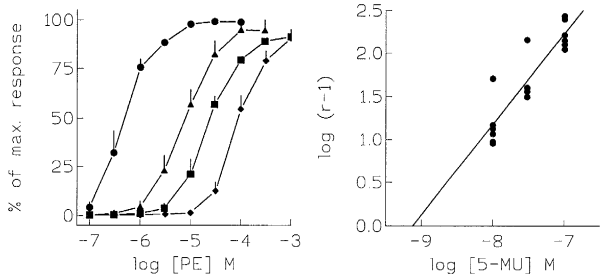

Fig. 1. Left, effect of 5 methylurapidil on phenylephrine concentration–response curve of rat mesenteric small arteries: circles, control; triangles, 10 nM 5 methylurapidil; squares, 30 nM 5 methylurapidil; diamonds, 100 nM 5 methylurapidil. Right, Schild plot; for characteristics, see Table 1. Points normalized to maximum response in control curves, and show mean ± S.E.M., 16 vessels.

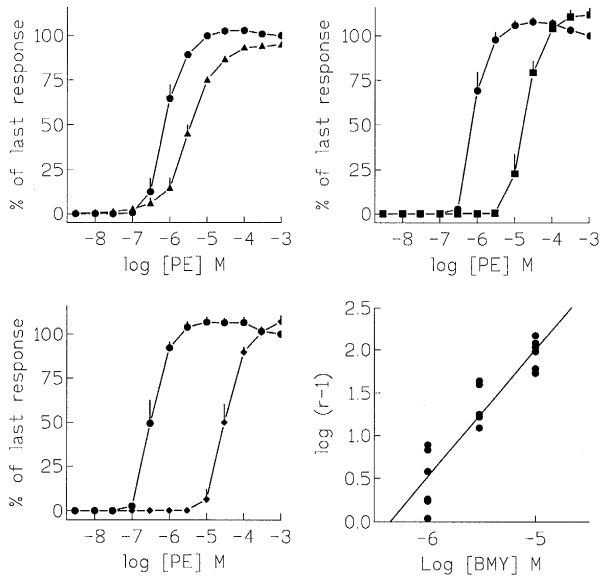

Fig. 2. Effect of BMY7378 on phenylephrine concentration–response curve of rat mesenteric small arteries. Experiments performed in pairs: one vessel (circles) control, one vessel exposed to: 1 μM BMY7378 (triangles, top left); 3 μM BMY7378 (squares, top right); 10 μM BMY7378 (diamonds, bottom left). Bottom right, Schild plot; for characteristics, see Table 1. Points normalized to maximum response in control curve, and show mean ± S.E.M., 6 vessels for each concentration.

3.2. Antagonism of phenylephrine response of aorta

The phenylephrine concentration–response curve of aorta (pD_2 6.33 ± 0.14, $n = 15$) was slightly right-shifted by 5 methylurapidil (0.1, 0.3 and 1 μM), but not concentration-dependently (shift for all concentrations approx. 3-fold), without depression of the maximum response (data not shown, and Table 1). By contrast, BMY7378 caused

Table 3
Schild analysis of the effects of sertindole on phenylephrine concentration–response curve

	pA_2	slope	r^2	*n*
Mes. small art.	8.96	1.09 ± 0.18	0.69	18
Aorta	6.14	1.24 ± 0.21	0.76	13

Shown are calculated pA_2 values, slope of the Schild regression line (mean ± S.E.M.) and the regression coefficient (r^2). *n* shows number of vessels (one vessel per animal). Mes. small art.: mesenteric small arteries.

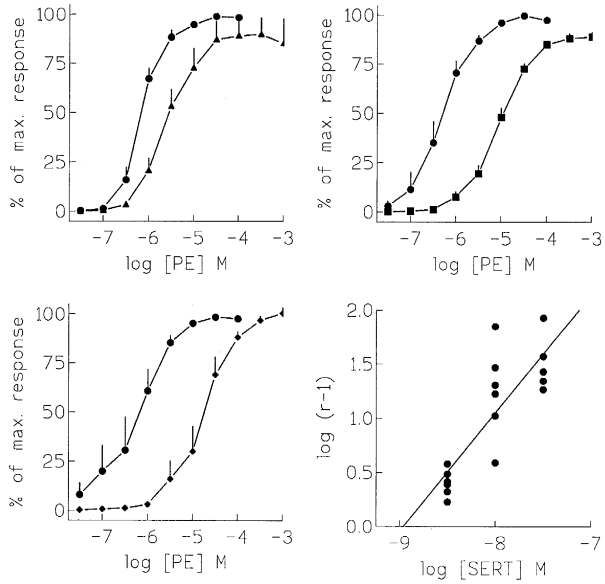

Fig. 3. Effect of sertindole on phenylephrine concentration–response curve of rat mesenteric small arteries. Experiments performed in pairs: one vessel (circles) control; other vessel exposed to either 3 nM sertindole (triangles, top left), or 10 nM sertindole (squares, top right), or 30 nM sertindole (diamonds, bottom left). Bottom right, Schild plot; for characteristics, see Table 3. Points normalized to maximum response in control curve, and show mean ± S.E.M., 4–5 vessels for each concentration.

Table 4
Inhibition of [^{125}I]BE2254 binding to cloned α_1-adrenoceptor subtypes expressed in membranes of HEK293 cell lines

	Cell line					
	α_{1A}		α_{1B}		α_{1D}	
	pK_i	n_H	pK_i	n_H	pK_i	n_H
Sertindole	8.90 ± 0.17	0.80 ± 0.10	7.06 ± 0.09	1.00 ± 0.10	6.78 ± 0.078	1.10 ± 0.10
5-Methylurapidil	8.24 ± 0.11	0.80 ± 0.10	6.40 ± 0.13	0.70 ± 0.10	6.76 ± 0.14	0.80 ± 0.10
BMY7378	6.11 ± 0.10	1.10 ± 0.10	6.40 ± 0.16	1.10 ± 0.10	8.29 ± 0.16	0.80 ± 0.10

Values show mean ± S.E.M. n_H is the Hill coefficient of binding displacement curve. 6 experiments.

competitive inhibition of the phenylephrine concentration–response curve (Table 1), with a pA_2 of 7.6. Chlorethylclonidine (50 μM) depressed the maximum response by 88% (Table 2).

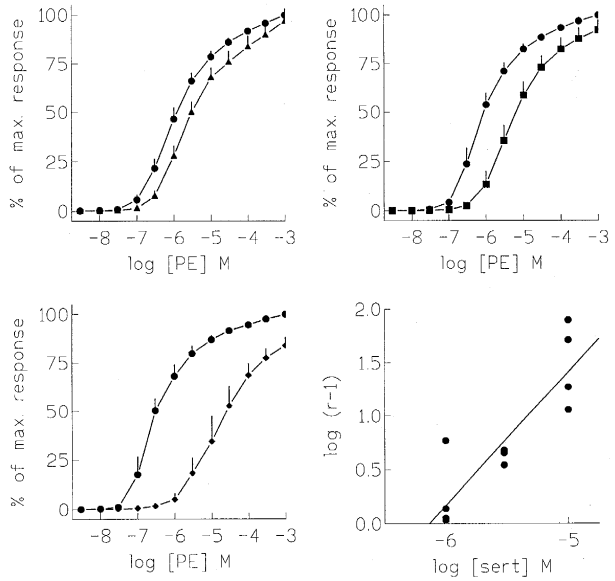

Fig. 4. Effect of sertindole on phenylephrine concentration–response curve of rat aorta. Experiments performed in pairs: one vessel (circles) control; other vessel exposed to 1 μM sertindole (triangles, top left), or 3 μM sertindole (squares, top right), or 10 μM sertindole (diamonds, bottom left). Bottom right, Schild plot; for characteristics, see Table 3. Points normalized to maximum response in control curve, and show mean ± S.E.M., 4–5 vessels for each concentration.

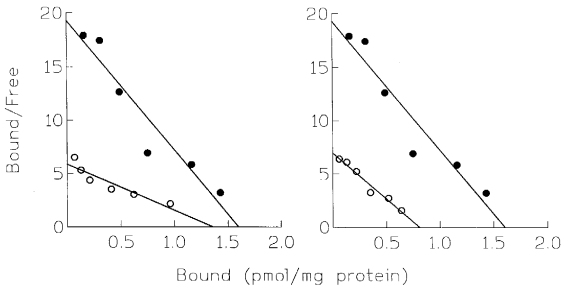

Fig. 5. Scatchard analyses of the binding of [^{125}I]BE2254 (50–55 pM) to membranes of HEK293 cells transfected with α_{1A}-adrenoceptors under control conditions (closed symbols, regression coefficient, r^2, 0.91) or in the presence of 1 nM sertindole (open symbols, left, $r^2 = 0.86$), or in membranes which had been incubated in the presence of 1 nM sertindole for 20 min followed by three-fold thorough washing (open symbols, right, $r^2 = 0.97$).

3.3. Effects of sertindole on phenylephrine responses

Sertindole caused potent competitive inhibition of the phenylephrine concentration–response curve of mesenteric small arteries (Table 3, Figs. 3 and 4). The reversibility of the inhibition of the phenylephrine concentration–response curve by sertindole was assessed by repeating further phenylephrine concentration–response curves at hourly intervals in the absence of sertindole. Following experiments where 3 nM sertindole had been used, the phenylephrine concentration–response curve did not change during washout, even after 5 h (pD_2 with sertindole, 5.63 ± 0.07; after 5 h washout, 5.83 ± 0.06; $n = 4$–6). Where 30 nM sertindole had been used, the phenylephrine concentration–response curve shifted slightly to the left, but did not regain the sensitivity seen under control conditions (pD_2 with sertindole, 4.85 ± 0.18; after 5 h washout, 5.21 ± 0.14; $n = 6$). Time control experiments ($n = 8$), where phenylephrine concentration–response curves were repeated at intervals for 4 h, showed no shift in the curves.

3.4. Binding studies

Table 4 shows the inhibition of [^{125}I]BE2254 binding to cloned α_1-adrenoceptor subtypes expressed in HEK293 cell lines by selective drugs. As indicated, sertindole showed selective inhibition of BE2254 binding to α_{1A}-adrenoceptors, compared to α_{1B}- and α_{1D}-adrenoceptors. As also indicated in Table 4, control experiments revealed that 5 methylurapidil showed specific binding to the expressed α_{1A}-adrenoceptors, and BMY7378 showed specific binding to the expressed α_{1D}-adrenoceptors.

In view of the difficulty in washing out sertindole in the functional experiments, and the apparent specificity of

Table 5
Binding of [^{125}I]BE2254 to membranes of HEK293 cells transfected with the α_{1A}-adrenoceptors either in the presence of 1 nM sertindole or after 20 min preincubation and washout of 1 nM sertindole

	K_d (pM)	B_{max} (pmol/mg protein)
Control	82 ± 2	1.72 ± 0.12
In presence of sertindole	270 ± 37 [a]	1.54 ± 0.11
After pre-incubation and washout of sertindole	136 ± 26	0.95 ± 0.09 [b]

Values show mean ± S.E.M. 6 experiments.
[a] $P < 0.05$, [b] $P < 0.01$, compared to control.

sertindole on α_{1A}-adrenoceptors, the binding characteristics of sertindole to these were investigated as follows. The binding of [^{125}I]BE2254 to membranes of HEK293 cells transfected with α_{1A}-adrenoceptors was determined (a) under control conditions, (b) in the presence of 1 nM sertindole, (c) in cells which were preincubated with 1 nM sertindole which were then washed thoroughly three times (the concentration of sertindole chosen was close to the K_i-value (Table 4). The results indicate (Table 5, Fig. 5) that binding in the presence of sertindole showed a significant increase in K_d, with little change in B_{max}, indicative of reversible binding. However, binding after preincubation with sertindole showed little change in K_d, but a decrease of 45% in B_{max}, suggesting irreversible behavior. It therefore appears that sertindole is a pseudo-irreversible competitive antagonist (Kenakin, 1993) against α_{1A}-adrenoceptors, with a slow off-rate, as discussed below.

4. Discussion

The main findings of this investigation are that the adrenoceptors of rat mesenteric small arteries are of the α_{1A}-type, and that sertindole is a specific antagonist of this adrenoceptor sub-type.

The data which indicate that the adrenoceptors in the rat mesenteric small arteries are of the α_{1A}-type, are the high potency of 5 methylurapidil and WB-4101 in antagonizing the responses of the vessels to phenylephrine (pA_2 9.1 and 9.5, respectively). This interpretation was supported by the Schild analysis of the 5 methylurapidil and WB-4101 data giving a slope close to 1. Furthermore, this finding is in agreement both with binding studies (Meier and Hyttel, 1992), and with functional studies on the pithed rat (Vargas et al., 1994), the rat mesenteric perfusion model (Kong et al., 1994), the rat perfused hindlimb (Zhu et al., 1997) and rat mesenteric small artery (Chen et al., 1996) (although the slope of the Schild plot of Chen et al. was only 0.46, a possible reason for the discrepancy being that Chen et al. used noradrenaline). Other indirect support for the presence of α_{1A}-adrenoceptors in the mesenteric small arteries is the limited effect of chlorethylclonidine dihydrochloride, which would have acted on α_{1B}- and α_{1D}-adrenoceptors. The mesenteric small arteries seem therefore to have α_{1A}-adrenoceptors as the dominant α-adrenoceptor sub-type, a finding which could be related to the orthostatic hypotension which, as mentioned in Section 1, is observed with this drug. Dominance of α-adrenoceptor sub-type is also seen in other tissues, such as human vas deferens (Furukawa et al., 1995) and to some extent human prostate (Teng et al., 1994; Marshall et al., 1995), but not in rat skeletal muscle arterioles, where α_{1D}-like adrenoceptors are seen (Leech and Faber, 1996).

The lack of effect of 5 methylurapidil in the aorta suggests a lack of α_{1A}-adrenoceptors in this vessel, and is thus consistent with the growing consensus that noradrenaline acts in this tissue predominantly (Kenny et al., 1995), if not exclusively (Testa et al., 1995; Van der Graaf et al., 1996), through α_{1D}-adrenoceptors. Further support comes from the comparatively high potency of BMY7378 (pA_2 7.6), even though with the protocol we used the potency was considerably less than that found by others (e.g., 9.0, Deng et al., 1996).

The finding that sertindole had a pharmacological profile rather similar to that of 5 methylurapidil, both in the functional experiments and with the binding experiments using the transfected HEK293 cells, suggests that this is a drug for which the α-adrenoceptor antagonist activity is primarily acting on the α_{1A}-subtype. This is in contrast to other antipsychotic drugs, such as risperidone, which are reported to have α_{1B}-specificity (Sleight et al., 1993) based on a high potency in rat spleen which has a high proportion of this subtype (Burt et al., 1995), even though such specificity has recently not been confirmed (Eltze, 1996). Whether the specificity of sertindole for the α_{1A}-subtype is related to some of the particular central effects of sertindole, e.g., antipsychotic action, remains to be determined.

The effects of sertindole on [^{125}I]BE2254 binding are unusual. On the one hand, as indicated in Fig. 5(left), the Scatchard analysis suggests that with concurrent incubation, sertindole acts as a competitive inhibitor of [^{125}I]BE2254 (reduced slope and no significant change in B_{max}). On the other hand, Fig. 5(right) suggests that pre-incubation with sertindole, followed by washout, reduces the number of [^{125}I]BE2254 binding sites irreversibly (no change in slope and reduced B_{max}). This suggests that although [^{125}I]BE2254 is able, in the short term, to inhibit sertindole binding, sertindole has a long off-rate so that once bound further [^{125}I]BE2254 binding is prevented within the time scale of the experiment. Such behavior can be termed 'pseudo-irreversible' (Kenakin, 1993). Further experiments are required to determine the time dependence of the phenomenon, and the mechanism of this apparently unusual behavior. Preliminary experiments by one of us (C.H., personal communication) indicate that similar irreversible behavior is also seen with the (low-affinity) binding of sertindole to α_{1B}- and α_{1D}-adrenoceptors, and it would also be interesting to investigate if this behavior is seen with the binding of sertindole to other classes of receptors.

The unusual binding behavior of sertindole correlated with the inability to reverse the mechanical effect of sertindole upon washout. The behavior contrasts, however, with the lack of reduction in the maximum response of the mesenteric small arteries to phenylephrine in the presence of sertindole (Fig. 3), although this may be accounted for by the presence of spare adrenoreceptors in these vessels (Nyborg and Bevan, 1988). The apparent irreversibility measured here under in vitro conditions is also in contrast to another series of experiments (Ipsen, 1995), where mesenteric small arteries taken from rats which had been treated with sertindole (0.31 mg/kg or 1.25 mg/kg) one

day previously, did not show any difference in the phenylephrine concentration–response curve characteristics. Therefore this suggests that in vivo, either the binding is reversible within 24 h, or that there is an upregulation of α_{1A}-adrenoceptors. Further experiments are required to elucidate the precise mechanism of adrenoceptor antagonism of sertindole.

In conclusion, we have demonstrated that the adrenoceptors of rat mesenteric small arteries are of the α_{1A}-type, and that sertindole is a specific pseudo-irreversible competitive antagonist of this adrenoceptor sub-type. The relation of this specific adrenoceptor antagonism to the antipsychotic properties of sertindole remains to be determined.

Acknowledgements

We thank Ms. Tina Benfeldt and Mr. Dong Zhao for technical assistance. This work has been supported by H. Lundbaek A/S (Copenhagen, Denmark) and by grants to M.J.M. by the Danish Medical Research Council and the Danish Heart Foundation.

References

Buckner, S.A., Oheim, K.W., Morse, P.A., Knepper, S.M., Hancock, A.A., 1996. Alpha 1-adrenoceptor-induced contractility in rat aorta is mediated by the alpha 1D subtype. Eur. J. Pharmacol. 297, 241–248.

Burt, R.P., Chapple, C.R., Marshall, I., 1995. Evidence for a functional alpha 1A- (alpha 1C-) adrenoceptor mediating contraction of the rat epididymal vas deferens and an alpha 1B-adrenoceptor mediating contraction of the rat spleen. Br. J. Pharmacol. 115, 467–475.

Chen, H., Fetscher, C., Schafers, R.F., Wambach, G., Philipp, T., Michel, M.C., 1996. Effects of noradrenaline and neuropeptide Y on rat mesenteric microvessel contraction. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 314–323.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor. Biochem. Pharmacol. 22, 3099–3108.

Curtis, V.A., Kerwin, R.W., 1995. A risk-benefit assessment of risperidone in schizophrenia. Drug Saf. 12, 139–145.

Deng, X.F., Chemtob, S., Varma, D.R., 1996. Characterization of alpha-1D-adrenoceptor subtype in rat myocardium, rat and other tissues. Br. J. Pharmacol. 119, 269–276.

Dunn, C.J., Fitton, A., 1996. Sertindole. CNS Drugs 5, 224–230.

Eltze, M., 1996. In functional experiments, risperidone is selective, not for the B, but for the A subtype of alpha 1-receptors. Eur. J. Pharmacol. 295, 69–73.

Engel, G., Hoyer, D., 1981. BE 2254, a new high affinity radioligand for alpha 1-adrenoceptors. Eur. J. Pharmacol. 73, 221–224.

Fenger Gron, J., Mulvany, M.J., Christensen, K.L., 1995. Mesenteric blood pressure profile of conscious, freely moving rats. J. Physiol. Lond. 488, 753–760.

Furukawa, K., Rosario, D.J., Smith, D.J., Chapple, C.R., Uchiyama, T., Chess-Williams, R., 1995. Alpha 1A-adrenoceptor-mediated contractile responses of the. Br. J. Pharmacol. 116, 1605–1610.

Graham, R.M., Perez, D.M., Hwa, J., Piascik, M.T., 1996. Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ. Res. 78, 737–749.

Hyttel, J., Nielsen, J.B., Nowak, G., 1992. The acute effect of sertindole on brain 5-HT-2, D-2 and alpha-1 receptors (ex vivo radioreceptor binding studies). J. Neural. Transm. 89, 61–69.

Ipsen, M., 1995. Characteristics of sertindole concerning alpha-1-adrenoceptors and tolerance; Alpha-1-subtype specificity of small arteries. Aarhus University, Aarhus (masters thesis).

Ipsen, M., Dragsted, N., Mulvany, M.J., 1995. Functional distribution of alpha-1 adrenoceptor subtypes in mesenteric small arteries of the rat. Pharmacol. Res. 31 (Suppl.), 192, Abstract.

Kenakin, T., 1993. Pharmacologic Analysis of Drug-Receptor Interaction. Raven, New York, NY, p. 341.

Kenny, B.A., Chalmers, D.H., Philpott, P.C., Naylor, A.M., 1995. Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. 115, 981–986.

Kong, J.Q., Taylor, D.A., Fleming, W.W., 1994. Functional distribution and role of alpha-1 adrenoceptor subtypes in the mesenteric vasculature of the rat. J. Pharmacol Exp. Ther. 268, 1153–1159.

Leech, C.J., Faber, J.E., 1996. Different alpha-adrenoceptor subtypes mediate constriction of arterioles and venules. Am. J. Physiol. 270, H710–H722.

Marshall, I., Burt, R.P., Chapple, C.R., 1995. Noradrenaline contractions of human prostate mediated by alpha 1A-(alpha 1c-) adrenoceptor subtype. Br. J. Pharmacol. 115, 781–786.

Meier, E., Hyttel, J., 1992. Identification and Characterization of Binding Sites for 3-H Sertindole. 22nd Annual Meeting. Society for Neuroscience (Abstract).

Mulvany, M.J., Halpern, W., 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ. Res. 41, 19–26.

Nyborg, N.C.B., Bevan, J.A., 1988. Increased alpha-adrenergic receptor affinity in resistance vessels from hypertensive rats. Hypertension 11, 635–638.

Olesen, O.V., Thomsen, K., Jensen, P.N., Wulff, C.H., Rasmussen, N.A., Refshammer, C., Sorensen, J., Bysted, M., Christensen, J., Rosenberg, R., 1995. Clozapine serum levels and side effects during steady state treatment of schizophrenic patients: A cross-sectional study. Psychopharmacology 117, 371–378.

Piascik, P., Alexander, S., 1995. Update on biotech information sources. Am. Pharm. NS35, 8–9.

Saussy, D.L.J., Goetz, A.S., Queen, K.L., King, H.K., Lutz, M.W., Rimele, T.J., 1996. Structure activity relationships of a series of buspirone analogs at alpha-1 adrenoceptors: Further evidence that rat aorta alpha-1 adrenoceptors are of the alpha-1D-subtype. J. Pharmacol. Exp. Ther. 278, 136–144.

Sánchez, C., Arnt, J., Dragsted, N., Hyttel, J., Lemb¢l, H.L., Meier, E., Perregaard, J., Skarsfeldt, T., 1991. Neurochemical and in vivo pharmacological profile of sertindole, a limbic-selective neuroleptic compound. Drug Dev. Res. 22, 239–250.

Skarsfeldt, T., Perregaard, J., 1990. Sertindole, a new neuroleptic with extreme selectivity on A10 versus A9 dopamine neurones in the rat. Eur. J. Pharmacol. 182, 613–614.

Sleight, A.J., Koek, W., Bigg, D.C., 1993. Binding of antipsychotic drugs for alpha-1A- and alpha-1B-adrenoceptors: Risperidone is selective for alpha-1B-adrenoceptors. Eur. J. Pharmacol. 238, 407–410.

Teng, C.M., Guh, J.H., Ko, F.N., 1994. Functional identification of alpha 1-adrenoceptor subtypes in. Eur. J. Pharmacol. 265, 61–66.

Testa, R., Guarneri, L., Poggesi, E., Simonazzi, I., Taddei, C., Leonardi, A., 1995. Mediation of noradrenaline-induced contractions of rat aorta by the alpha 1B-adrenoceptor subtype. Br. J. Pharmacol. 114, 745–750.

Van der Graaf, P.H., Shankley, N.P., Black, J.W., 1996. Analysis of the activity of alpha 1-adrenoceptor antagonists in rat aorta. Br. J. Pharmacol. 118, 299–310.

Van Kammen, D.P., McEvoy, J.P., Targum, S.D., Kardatzke, D., Sebree, T.B., 1996. A randomized, controlled, dose-ranging trial of sertindole in patients with schizophrenia. Psychopharmacology 124, 168–175.

Vargas, H.M., Zhou, L., Gorman, A.J., 1994. Role of vascular alpha-1 adrenoceptor subtypes in the pressor response to sympathetic nerve stimulation in the pithed rat. J. Pharmacol. Exp. Ther. 271, 748–754.

Zhu, W., Zhang, Y., Han, C., 1997. Characterization of subtype of alpha-1-adrenoceptor mediating vasoconstriction in perfused rat hind limb. Eur. J. Pharmacol. 329, 55–61.