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The effects of tamsulosin, a high affinity antagonist at functional α_{1A}- and α_{1D}-adrenoceptor subtypes

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1 The actions of the α_1-adrenoceptor antagonist tamsulosin have been examined at functional α_1-adrenoceptor subtypes and compared with those at the human prostate receptor.

2 At the α_{1D}-adrenoceptors of the rat aorta, tamsulosin acted as a competitive antagonist with a high affinity ($pK_B = 10.1$).

3 At the α_{1B}-adrenoceptor of the rat spleen and rabbit corpus cavernosum penis, tamsulosin again acted as a competitive antagonist but with a significantly lower affinity ($pK_B = 8.9 - 9.2$).

4 Tamsulosin acted as an unsurmountable antagonist of the α_{1A}-adrenoceptor-mediated responses of the rat and human vas deferens, reducing maximal responses to phenylephrine by 20% and 50%, respectively, at an antagonist concentration of 1 nM. Responses of depolarized (100 mM KCl) rat vas deferens preparations were unaffected by 10 nM tamsulosin but this concentration reduced maximal responses to 5-hydroxytryptamine (5-HT) in this tissue.

5 When longer antagonist incubation periods ($\geqslant 60$ min) were used, tamsulosin behaved as a competitive antagonist on the human prostate with a significantly higher affinity ($pK_B = 10.0$) than obtained at the α_{1B}-adrenoceptor.

6 The data demonstrate that tamsulosin is a high affinity antagonist at functional α_1-adrenoceptors with a selectivity $\alpha_{1D} \geqslant \alpha_{1A} > \alpha_{1B}$. In some tissues the compound exhibits an additional unsurmountable antagonist action, the clinical significance of which is unknown.

Keywords: Tamsulosin; α_1-adrenoceptors; prostate; vas deferens; spleen; corpus cavernosum; receptor subtypes; aorta

Introduction

Benign prostatic hyperplasia (BPH) produces bladder outlet obstruction via two mechanisms. A mechanical or 'static' component exerted by the increased bulk of prostate in BPH and 'dynamic' influences, resulting from contractions of the prostatic smooth muscle. Pharmacotherapy can be effective by reducing prostatic size by acting via a hormonal mechanism, or by preventing α-adrenoceptor mediated contraction of the prostatic smooth muscle.

Spinal anaesthesia, which causes total neural blockade, produces a 47% reduction in urethral closure pressure (Furuya *et al*., 1982) and antagonism of α-adrenoceptors similarly results in a decrease in urethral closure pressure (Donker *et al*., 1972). A functional predominance of α_1-adrenoceptors in human prostatic muscle was first demonstrated more than 10 years ago (Hieble *et al*., 1985) and a rationale approach for symptomatic relief of BPH is therefore the use of selective α_1-adrenoceptor antagonists (for review, see Chapple, 1995). The side-effect profile of these agents is acceptable, although, by virtue of the functional involvement of α_1-adrenoceptors in the maintenance of vascular tone, reduction of blood pressure, orthostasis, astenia and light-headedness are common side-effects for this class of compounds.

Radioligand binding studies, molecular cloning studies, and functional studies have demonstrated the heterogeneity of α_1-adrenoceptors. It is now well accepted that at least three types of α_1-adrenoceptor with a high affinity for prazosin exist, namely α_{1A} (formerly termed α_{1c}), α_{1B} and α_{1D} (also previously known as α_{1a})-adrenoceptors (Hieble *et al*., 1995). In functional studies α_1-adrenoceptors with a low affinity for prazosin have also been identified and termed α_{1L} (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1995), but how these receptors relate to the cloned receptors is still not clear. Although in many animal and human tissues there seems to be a co-expression of several types of α_1-adrenoceptors, for some tissues a functional dominance of one of the subtypes has been convincingly described. Thus, contractions of the human (Furukawa *et al*., 1995) and rat (Burt *et al*., 1995) vas deferens are mediated via the α_{1A}-adrenoceptor subtype (Burt *et al*., 1995); contractile responses of the rat spleen (Burt *et al*., 1995) and rabbit corpus cavernosum penis (CCP, Furukawa *et al*., 1996) are mediated by the α_{1B}-adrenoceptor subtype, and rat aortic responses are mediated predominantly via α_{1D}-adrenoceptors (Kenny *et al*., 1995). In human prostate it has been demonstrated that the α_{1A}-adrenoceptor is dominant at the mRNA level (Price *et al*., 1993; Schalken *et al*., 1994), and functional studies support the assumption that the α_{1A}-adrenoceptor mediates contraction in this tissue (Forray *et al*., 1994; Marshall *et al*., 1995). However, the prostatic receptor has a low affinity for prazosin and has also been described as α_{1L} (Muramatsu *et al*., 1995). This anomaly may be explained by the suggestion that the α_{1A}- and α_{1L}-adrenoceptor may represent distinct pharmacological states of the same receptor (Ford *et al*., 1996b).

Recently tamsulosin has been introduced for the treatment of bladder outlet obstruction due to BPH and appears to be the first clinically available antagonist which discriminates between α_1-adrenoceptor subtypes. In radioligand binding studies this agent exhibits a selectivity for α_{1a} and α_{1d} adrenoceptors compared with α_{1b}-adrenoceptors, although estimates for the degree of selectivity vary greatly (Michel & Insel, 1994; Schwinn *et al*., 1994; Foglar *et al*., 1995). Also, *in vivo* in the dog, tamsulosin has been shown to be 10 fold more potent for the lower urinary tract than on arterial blood pressure responses (Testa *et al*., 1994b), but Kenny *et al*. (1994) could find no such selectivity for the antagonism of urethral closure pressure over arterial blood pressure responses in this species.

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In the present study the actions of tamsulosin at functional α_{1A}-adrenoceptors (human and rat vas deferens), α_{1B}-adrenoceptors (rat spleen and rabbit CCP) and α_{1D}-adrenoceptors (rat aorta) were examined and compared with those on the human prostate.

Methods

Human prostatic smooth muscle strips were obtained from patients aged 60–75 undergoing transurethral resection of the prostate (TURP) procedures for BPH. Human epididymal vasa deferentia were obtained from patients (25–38 years old) undergoing vasectomy. Resected prostate strips and vasa deferentia were preserved in Krebs-bicarbonate solution at 4°C from the time of surgery until used in functional experiments within 24 h.

Male Wistar rats, approximately 250 g, were killed by a blow to the head and exsanguinated. Male white New Zealand rabbits were killed by an overdose of anaesthetic (0.2 mg kg^{-1} sodium pentobarbitone). Rat epididymal vasa deferentia, rat hemi-spleen cut longitudinally, rat aortic strips (with endothelium removed) and rabbit corpus cavernosum penis (CCP) were isolated and set up in Krebs-bicarbonate solution of the following composition (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 by all tissues following the addition of either phenylephrine or noradrenaline was measured by means of isometric force transducers (Lectromed UF1, 57 g sensitivity) and recorded to computer via a Cambridge Electronic Design (CED) 1401 analogue to digital converter, with CHART software. Tissues were equilibrated for 60 min with several changes of bathing medium before the drugs were administered. All experiments were performed in the presence of cocaine (10 μM) and corticosterone (10 μM) to prevent neuronal and extraneuronal uptake of drugs, and propranolol (1 μM) to antagonise β-adrenoceptors.

Human prostate

Cumulative concentration-response curves were obtained with noradrenaline or phenylephrine as the agonist. Two cumulative concentration-response curves were obtained to noradrenaline with the second curve being obtained in the presence of tamsulosin (0.1 nM–30 nM). Consistent repeated concentration-response curves to phenylephrine could not be obtained on human prostate, with significant desensitization occuring to this agonist between the first and second concentration-response curves in control experiments. For this reason only a single curve was obtained on each tissue when using this agonist.

For both sets of experiments, one tissue strip from each patient was used as a control without the addition of antagonist and where appropriate was used to correct for any time-dependent change in tissue sensitivity which may occur during the course of the experiment.

Vas deferens, spleen, aorta and CCP

Cumulative concentration-response curves to phenylephrine were obtained on the rat spleen, rat aorta and rabbit CCP, whereas responses to single doses of phenylephrine were

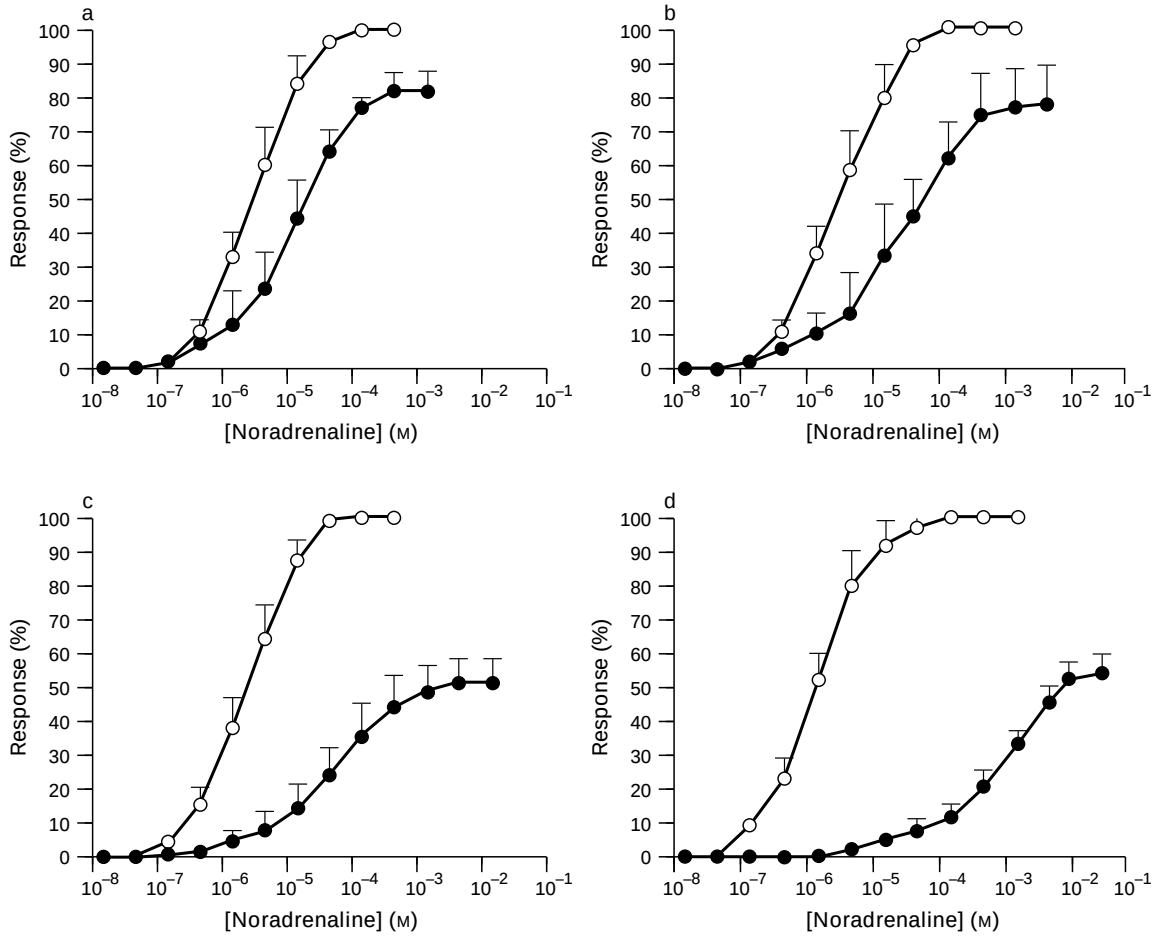

Figure 1 Concentration-response curves of human prostate to noradrenaline in the absence (○) and presence (●) of tamsulosin, (a) 1 nM, (b) 3 nM, (c) 10 nM and (d) 30 nM for 30 min. Responses in the presence of tamsulosin are plotted as a % of the maximum response obtained for the first curve. Vertical lines indicate s.e.mean for each point.

obtained on the human and rat vas deferens, the tissues being washed between each addition of drug. On most tissues concentration-response curves were obtained in the absence of antagonist and then in the presence of tamsulosin following a 60 min incubation. However, on the rat spleen multiple concentration-response curves could not be obtained and only one concentration-response curve was obtained for each tissue.

Non-cumulative concentration-response curves were also obtained to 5-hydroxytryptamine (5-HT) in the rat vas deferens, the second curves being performed in the presence of 10 nM tamsulosin. Cumulative concentration-response curves were performed to $CaCl_2$ in depolarised (100 mM KCl) rat vas deferens tissue, in the absence and presence of 10 nM tamsulosin.

Data analysis

Increases in developed tension to noradrenaline or phenylephrine were plotted as a percentage of the maximum increase for each concentration-response curve. Individual EC_{50} values were determined and are expressed as geometric means with 95% confidence limits. Differences in logarithmic EC_{50} values and maximum responses were analysed by either paired Student's *t* test or ANOVA followed by Bartlett's test where appropriate.

Schild plots were constructed and pA_2 values determined from the intercept on the abscissa scale (Arunlakshana & Schild, 1959). pK_B (−log dissociation constant) or apparent pK_B values (where the Schild slope was different to unity or the maximum response was depressed) were determined from in-

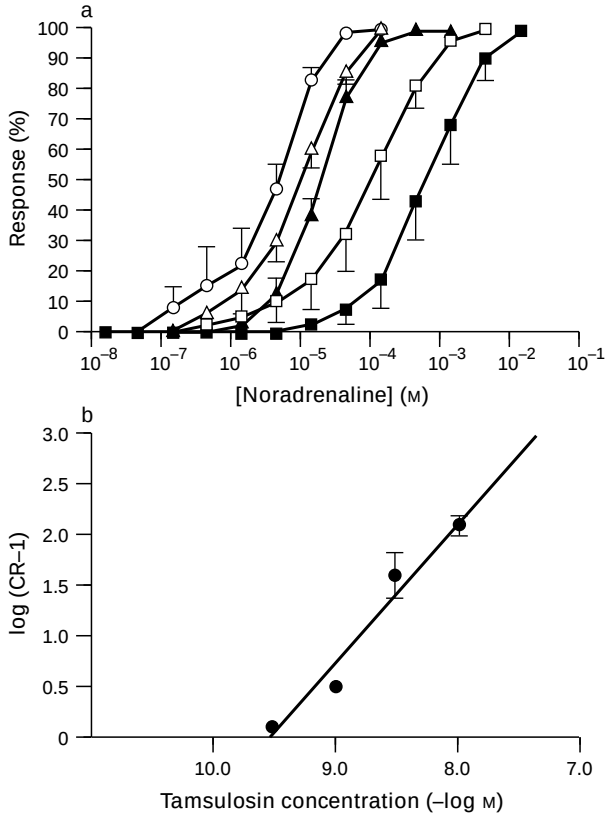

Figure 2 (a) Concentration-response curves to noradrenaline in human prostate, incubated in the absence (○), or in the presence of 0.3 nM (△), 1.0 nM (▲) 3.0 nM (□) and 10 nM (■) tamsulosin for 60 min. (b) Schild plot for the antagonism of contractile responses by tamsulosin. Vertical lines indicate s.e.mean for each point.

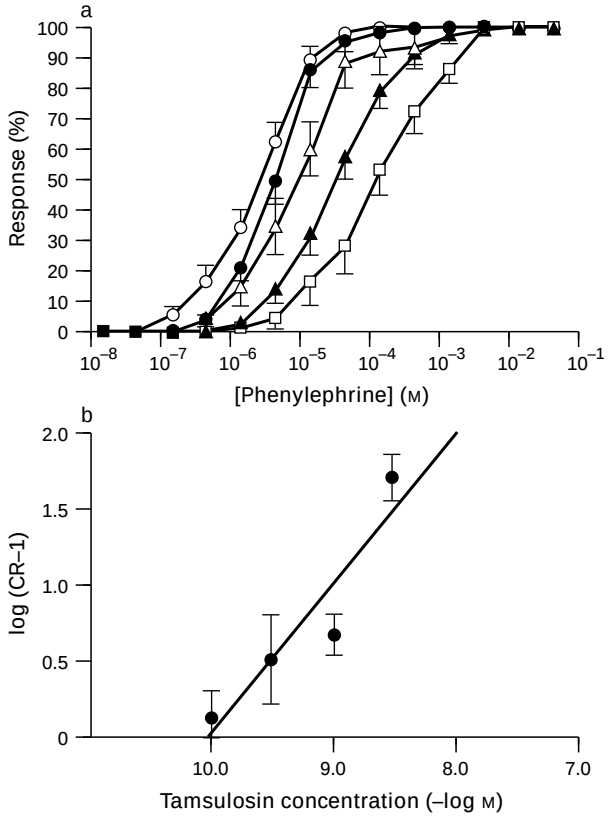

Figure 3 (a) Concentration-response curves to phenylephrine in human prostate, incubated in the absence (○), or in the presence of 0.1 nM (●), 0.3 nM (△), 1.0 nM (▲) and 3.0 nM (□ tamsulosin for 60 min. (b) Schild plot for the antagonism of contractile responses by tamsulosin. Vertical lines indicate s.e.mean for each point.

Table 1 Effect of antagonist incubation period on the apparent affinity of tamsulosin in the human prostate when noradrenaline was used as the agonist

Incubation time (min)	n	pK_B[a]	pA_2	*Schild slope*[a]	*Control max. response*[a] (g)	*Max. response in presence of tamsulosin*[a] (g)
30	29	9.7±0.1	9.2	1.5±0.2*	0.83±0.16	0.44±0.07 (30 nM)
60	21	9.9±0.4	9.5	1.2±0.2	1.14±0.60	0.67±0.24 (10 nM)
120	20	9.5±0.2	9.6	1.0±0.2	0.59±0.20	0.36±0.11 (3.0 nM)

Maximum response shown following incubation with tamsulosin is for the maximum concentration of tamsulosin used ie., 30 nM for 30 min incubation, 10 nM for 60 min incubation and 3.0 nM for 120 min incubation. [a]Results presented as mean±s.e.mean. *Schild plot is significantly ($P<0.05$) different from unity.

dividual shifts of the concentration-response curves by use of the equation:

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

where CR is the shift in concentration-response curve caused by a concentration [B] of tamsulosin.

Drugs

(−)-Tamsulosin was obtained from Yamanouchi Europe B.V. (Leiderdorp, Netherlands). (−)-Phenylephrine hydrochloride, (±)-noradrenaline hydrochloride, cocaine hydrochloride, corticosterone-21-acetate and (±)-propranolol hydrochloride were obtained commercially from Sigma (Poole, Dorset). All reagents were of Analar grade.

Table 2 Affinities of tamsulosin for α-adrenoceptors in various tissues when phenylephrine was used as the agonist

Tissue	n	pK_B[a]	pA_2	*Schild slope*[a]	*Control max. response*[a] (g)	*Max. response in presence of tamsulosin*[a] (g)
Human prostate (60 min)	10	10.0±0.4	10.0	1.00±0.26	0.68±0.44	0.45±0.09 (10 nM)
Rat spleen	22	8.9±0.5	8.9	0.99±0.11	0.28±0.05	0.33±0.11 (100 nM)
Rabbit CCP	21	9.2±0.1	9.3	0.85±0.12	3.82±1.02	4.66±0.58 (10 nM)
Rat vas deferens	22	9.7±0.1	9.4	1.36±0.01*	2.91±0.14	1.52±0.12 (10 nM)
Human vas deferens	15	9.9±0.1	12.3	0.26±0.01*	1.93±0.68	0.49±0.14 (3.0 nM)
Rat aorta	27	10.1±0.1	10.1	1.01±0.24	0.41±0.05	0.58±0.06 (100 nM)

Dissociation constants (pK_B) values were calculated from the shifts of individual concentration-response curves to phenylephrine. [a]Values are the mean±s.e.mean of n experiments obtained with a range of antagonist concentrations (prostate 0.1–3.0 nM, spleen 3.0–100 nM, CCP 1.0–10 nM, rat vas 1.0–10.0 nM, human vas 0.3–3.0 nM, aorta 1–100 nM). The pK_B values for rat and human vas deferens are apparent values since Schild plots had slopes significantly different from unity. Maximum responses in the presence of tamsulosin are for the highest concentration of tamsulosin used on that tissue. *Schild slopes significantly ($P<0.05$) different from unity.

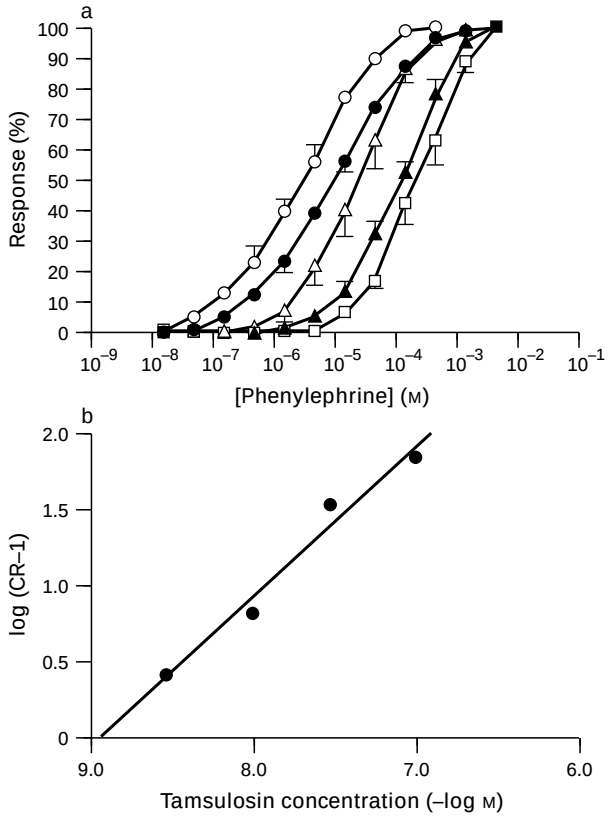

Figure 4 (a) Concentration-response curves to phenylephrine in rat spleen, incubated in the absence (○), or in the presence of 3.0 nM (●), 10 nM (△), 30 nM (▲) and 100 nM (□) tamsulosin for 60 min. (b) Schild plot for the antagonism of responses by tamsulosin. Vertical lines indicate s.e.mean for each point.

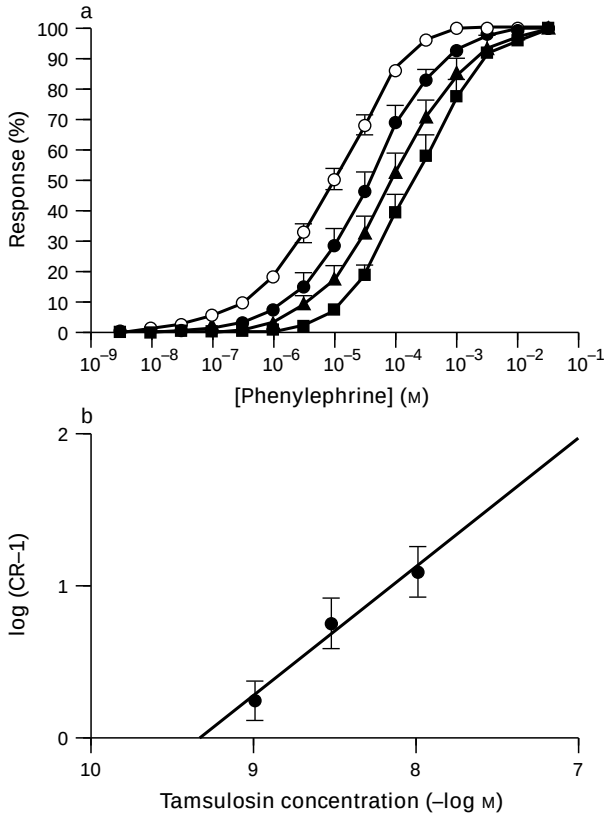

Figure 5 (a) Concentration-response curves to phenylephrine in rabbit corpus cavernosum penis (CCP), incubated in the absence (○), or in the presence of 1.0 nM (●), 3.0 nM (▲) and 10 nM (■) tamsulosin for 60 min. (b) Schild plot for the antagonism of responses by tamsulosin. Vertical lines indicate s.e.mean for each point.

Results

The effect of tamsulosin on human prostate

When incubated for 30 min with human prostate, tamsulosin (1–30 nM) caused rightward shifts of the concentration-response curves to noradrenaline giving an apparent affinity (apparent pK_B) of 9.86±0.12 (n=29). However, the Schild plot had a slope significantly (P<0.05) greater than unity (1.50±0.22) and maximum responses to noradrenaline were reduced by 49±7% (n=8) with 10 nM tamsulosin and 46±6% (n=7, P<0.05) with 30 nM (Figure 1).

When the incubation time was increased to 60 min, tamsulosin (0.3–10 nM) competitively antagonized responses to noradrenaline without significantly affecting maximum responses, giving a mean pK_B value of 9.9±0.4 (n=21). The shifts of the concentration-response curves yielded a Schild plot with a slope similar to unity (1.17±0.18) and an intercept on the abscissa scale of 9.5 (Figure 2, Table 1).

Incubating human prostate with tamsulosin (0.1–3.0 nM) for 120 min yielded similar results to those obtained with the 60 min incubation period. Tamsulosin acted as a competitive antagonist with a high affinity (pK_B=9.54±0.17, n=20) for the α_1-adrenoceptor of the human prostate.

Similar results were obtained when phenylephrine was used as the agonist. Tamsulosin (0.1–3.0 nM), again incubated with tissues for 60 min, had a high affinity with a mean pK_B of 10.00±0.40 (n=39). Maximum responses were not altered significantly and the Schild plot had a slope of 1.00±0.26 and an intercept of 10.0 (Figure 3, Table 2).

The effect of tamsulosin on α_{1B}-adrenoceptors

On the rat spleen tamsulosin produced concentration-dependent rightward shifts of the concentration-response curves to phenylephrine producing a Schild plot with an intercept of 8.9 and a slope of 0.99±0.11, which was not significantly different from unity (Figure 4, Table 2). Maximum responses were not affected by tamsulosin, the maximal response to phenylephrine being 0.28±0.05 g for control tissues and 0.33±0.11 g in the presence of 100 nM tamsulosin. Similarly in the rabbit CCP, tamsulosin (1.0–10 nM) produced rightward-shifts of the phenylephrine concentration-response curves producing a Schild plot with an intercept of 9.3 and a slope of 0.85±0.12 (Figure 5). A mean pK_B of 9.21±0.09 (n=21) was obtained with individual shifts. Again maximum responses to phenylephrine were not reduced by tamsulosin (Table 2).

The effect of tamsulosin at α_{1A}-adrenoceptors

On both the rat vas deferens and human vas deferens tamsulosin reduced maximum responses to phenylephrine, 1 nM tamsulosin reducing maximal responses by 51% from 1.58±0.31 g to 0.77±0.18 g (P<0.05, n=6) in the human vas deferens and by 20% from 3.11±0.24 g to 2.49±0.22 g (n=8) in the rat vas deferens (Figure 6).

However, tamsulosin (0.3–3.0 nM in human vas deferens and 1.0–10.0 nM in rat vas deferens) also caused rightward shifts of the concentration-response curves to phenylephrine, in humans vas deferens 1 nM tamsulosin produced a shift of 11.3 fold, yielding an apparent pK_B value of 10.02±0.03. In

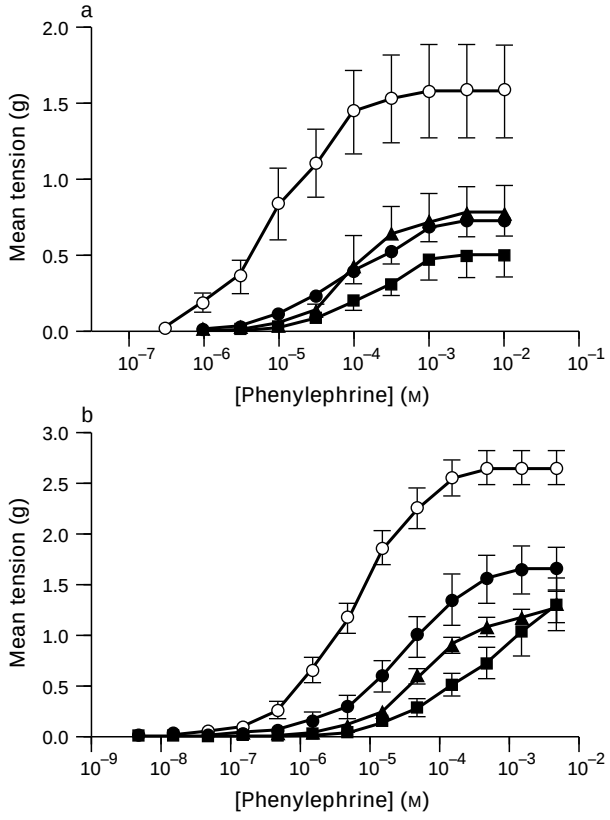

Figure 6 (a) Concentration-response curves to phenylephrine in human vas deferens, incubated in the absence (○), or in the presence of 0.3 nM (●), 1.0 nM (▲) and 3.0 nM (■) tamsulosin for 60 min. (b) Concentration-response curves to phenylephrine in rat vas deferens, incubated in the absence (○), or in the presence of 1.0 nM (●), 3.0 nM (▲) and 10 nM (■) tamsulosin for 60 min. In (a) and (b) responses are plotted as the mean developed tension in g; vertical lines show s.e.mean.

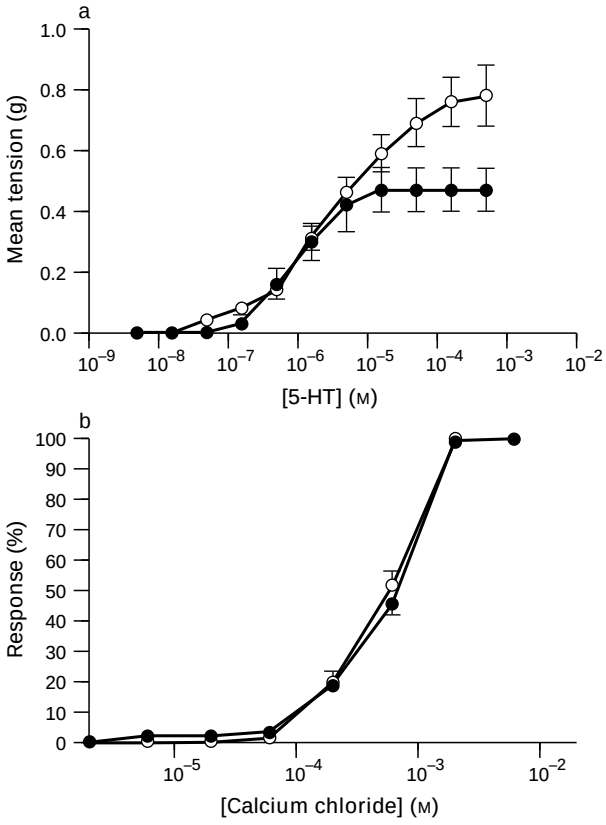

Figure 7 (a) Concentration-response curves to 5-HT in rat vas deferens, incubated in the absence (○) or presence (●) of 10.0 nM tamsulosin for 60 min. (b) Concentration-response curves to $CaCl_2$ in depolarized rat vas deferens, incubated in the absence (○), or presence (●) of 10.0 nM tamsulosin for 60 min. In (a) responses are plotted as the mean developed tension expressed in g with vertical lines showing s.e.mean.

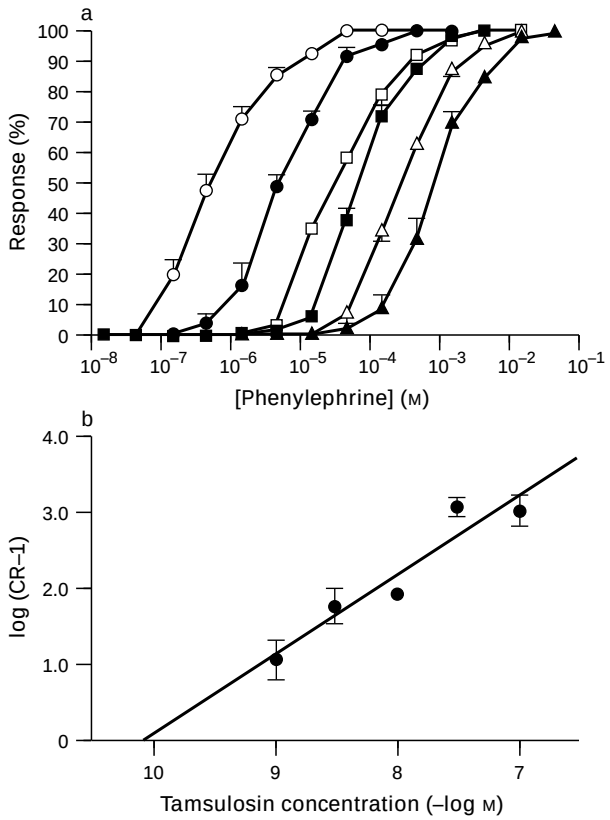

Figure 8 (a) Concentration-response curves to phenylephrine in rat aortic strips incubated in the absence (○), or in the presence of 1 nM (●), 3 nM (□), 10 nM (■), 30 nM (△), 100 nM (▲) tamsulosin for 60 min. (b) Schild plot for the antagonism by tamsulosin. Vertical lines indicate s.e.mean for each point.

the rat vas deferens 1 nM tamsulosin produced a rightward shift of the phenylephrine concentration-response curve of 4.7 fold which yielded an apparent mean pK_B value of 9.52 ± 0.08 ($n=8$).

Tamsulosin (10 nM) reduced maximal responses to 5-HT in the rat vas deferens by $44.0 \pm 4.9\%$ from 0.77 ± 0.13 g to 0.47 ± 0.09 g ($n=5$) (Figure 7a). Control curves to 5-HT showed no such reduction in maximal responses when a second time-matched control was performed, the first control maximal response being 0.67 ± 0.12 g and the second control maximal responses being 0.71 ± 0.17 g.

Tamsulosin (10 nM) had no significant effect on the $CaCl_2$ curves performed in depolarised rat vas deferens tissue, with maximal responses being 1.25 ± 0.11 g for control curves and 1.16 ± 0.21 g in the presence of tamsulosin (Figure 7b).

The effect of tamsulosin on α_{1D}-adrenoceptors

When incubated for 60 min with rat aorta, tamsulosin (1–100 nM) caused rightward shifts of the concentration-response curves to phenylephrine, giving a Schild plot with a slope not significantly different from unity (1.01 ± 0.24) and an intercept of 10.1 (Figure 8).

Maximum responses to phenylephrine were not reduced by incubation with tamsulosin, maximal responses being 0.41 ± 0.05 g for controls, 0.44 ± 0.09 g in the presence of 10 nM tamsulosin, 0.45 ± 0.08 g in the presence of 30 nM tamsulosin and 0.58 ± 0.06 g in the presence of 100 nM tamsulosin.

Discussion

At present three α_1-adrenoceptor subtypes have been identified which display a high affinity for prazosin. All three have been cloned and are termed α_{1a}, α_{1b} and α_{1d}-adrenoceptors (Hieble *et al*., 1995) and a fourth α_1-adrenoceptor (the α_{1L} subtype) has been proposed, based on functional studies, which possesses a low affinity for prazosin ($pA_2 < 9$) (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1995).

The cloned α_{1a}-adrenpceptor subtype, previously termed the α_{1c}-subtype, is thought to be equivalent to the functional α_{1A}-adrenoceptor subtype (Ford *et al*., 1994). This α_{1A}-adrenoceptor has a high affinity for prazosin, WB4101, benoxathian, phentolamine, 5-methylurapidil and (+)-niguldipine (Ford *et al*., 1994) and has been characterized in radioligand binding studies in the rat submaxillary gland and in functional studies in the rat and human vas deferens (Burt *et al*., 1995; Furukawa *et al*., 1995). Some investigators have described the receptor of the rat vas deferens as having a low affinity for prazosin and thus being the α_{1L}-receptor (Muramatsu *et al*., 1995). However, others disagree and where the affinities of a range of antagonists have been studied, the affinities correlated well with those obtained at the α_{1a}-adrenoceptor (Burt *et al*., 1995).

The functional α_{1B}-adrenoceptor present in rabbit CCP (Furukawa *et al*., 1996) and spleen (Burt *et al*., 1995) has a low affinity for WB4101, benoxathian and (+)-niguldipine and is also more sensitive to inactivation by the alkylating agent chloroethylclonidine (CEC) than either α_{1A}- or α_{1D}-adrenoceptors (Han *et al*., 1995). It is clear that the pharmacological properties of the functional α_{1B}-adrenoceptor present in tissues such as the rat spleen, correlates well with those of the cloned α_{1b}-adrenoceptor subtype (Burt *et al*., 1995).

The cloned α_{1d}-adrenoceptor is similar to the α_{1A}-adrenoceptor in having a high affinity for WB4101 and benoxathian, but has a relatively lower affinity for 5-methylurapidil and phentolamine. Highly selective competitive antagonists for the α_{1d}-adrenoceptor have recently become available and have been used to demonstrate that contractile responses of the rat aorta are mediated primarily via a functional α_{1D}-adrenoceptor (Kenny *et al*., 1995), although the possible involvement of other subtypes cannot be precluded (Van der Graaf *et al*., 1994).

In recent years attention has focused on the α_1-adrenoceptor present in the human prostate. Correlation studies with a range of α_1-antagonists have demonstrated that this tissue possesses a receptor which most clearly resembles that of the cloned α_{1a}-adrenoceptor (Forray *et al*., 1994; Marshall *et al*., 1995). However, two recent studies have suggested that some differences may exist between these receptors (Chess-Williams *et al*., 1996; Ford *et al*., 1996a), the prostatic receptor possibly representing a splice variant of the α_{1A}-adrenoceptor (Hirasawa *et al*., 1995). α_1-Adrenoceptor antagonists which do not discriminate between the α_1-adrenoceptor subtypes have been used for several years to treat the bladder outlet obstruction associated with BPH, but vascular side-effects can limit the use of maximally therapeutic doses. Tamsulosin, recently introduced for the treatment of BPH is unusual in discriminating between α_1-adrenoceptor subtypes (Han *et al*., 1995). Generally the selectivity of tamsulosin has been found to be $\alpha_{1a} \geqslant \alpha_{1d} > \alpha_{1b}$ adrenoceptors, although the degree of selectivity is a matter of debate. In radioligand binding studies estimates of the selectivity for α_{1a}-adrenoceptors compared to α_{1b}-adrenoceptors range from 3.9 to 38 fold (Michel *et al*., 1993; Kenny *et al*., 1994), and the variation in estimates of the selectivity of tamsulosin for α_{1a}-over α_{1d}-adrenoceptors range from 3 fold (Michel *et al*., 1993) to 20 fold (Testa *et al*., 1994a).

The data of the present study demonstrate that the selectivity of tamsulosin extends to functional α_1-adrenoceptors. The potency of tamsulosin was greatest on the rat aorta where responses are mediated via α_{1D}-adrenoceptors. The antagonist was only marginally less potent on the rat and human vas deferens (α_{1A}) and was least potent on the rat spleen where responses are mediated via α_{1B}-adrenoceptors. This confirms the selectivity of tamsulosin at native adrenoceptors as $\alpha_{1D} \geqslant \alpha_{1A} > \alpha_{1B}$; this has been obtained previously in radioligand binding studies in native tissues (Testa *et al*., 1994a). However, the antagonism of responses by tamsulosin differed

in the tissues examined. Although the antagonism appeared to be competitive in the rat aorta and spleen as evinced by Schild slope values similar to unity and a lack of effect on maximum responses, on rat and human vas deferens, tamsulosin acted as an unsurmountable antagonist reducing maximum responses to phenylephrine at concentrations as low as 1 nM. To investigate this action further the effects of tamsulosin on responses of the rat vas deferens to calcium and 5-HT were examined. Responses of depolarized tissues to calcium were unaffected by tamsulosin (10 nM) indicating that the drug was not acting to reduce calcium entry through voltage-operated calcium channels or interfering with contractile protein activation by calcium. However, responses of the rat vas deferens to 5-HT were reduced by tamsulosin suggesting that, at least in this tissue, tamsulosin has the ability to reduce responsiveness in addition to an effect at α_{1A}-adrenoceptors. This conclusion is supported by data from binding experiments to cloned α_{1a}-adrenoceptors where the binding kinetics of tamsulosin offer no evidence of an irreversible action (K.P. Minneman, personal communication).

On human prostate when a 30 min antagonist equilibrium period was used; tamsulosin also appeared to reduce maximal responses to noradrenaline and Schild plots were steep, indicating non-equilibrium conditions. When the antagonist equilibrium period was increased, Schild slopes were reduced to unity and although there was a trend towards a decrease in the maximal response with higher concentrations of tamsulosin, none of these changes was significant. The affinity values for tamsulosin at the human prostatic receptor were high ($pA_2 \approx 10.0$) and agree closely with affinity values obtained for this agent at cloned α_{1a} ($pK_i = 10.6$, Michel & Insel, 1994) and native α_{1A}-adrenoceptor ($pK_i = 9.9/10.0$, Michel *et al*., 1993). Since the human prostatic receptor has a low affinity for prazosin and has thus been described as the α_{1L}-receptor (Muramatsu *et al*., 1995), the present data suggest that tamsulosin also has a high affinity for the α_{1L}-adrenoceptor. This would agree with the conclusions of Ford *et al*. (1996b) who have suggested that the α_{1A}- and the α_{1L}-subtypes are different states of the same receptor which differ in their affinity for prazosin, but not tamsulosin which has a high affinity for both states of the α_{1A}-receptor.

It has been proposed that contraction of human arteries is mediated via α_{1B}-adrenoceptors (Hatano *et al*., 1994) and in our study tamsulosin exhibited lowest affinity for this receptor subtype, being 10 fold selective for the human prostate compared with the rat spleen (α_{1B}). A similar difference in affinity has been obtained for tamsulosin at α_1-adrenoceptor binding sites in human prostate and human aorta (Yamada *et al*., 1994).

The premise that this functional selectivity may have clinical relevance is supported by the observation that tamsulosin provides equivalent clinical efficacy to other selective α_1-adrenoceptor antagonists (terazosin, doxazosin and alfuzosin), whilst producing significantly fewer adverse effects (Kawabe, 1995); Abrams *et al*., 1995; Lepor, 1995).

The present study therefore demonstrates that tamsulosin is a highly potent α_1-adrenoceptor antagonist and is selective for functional α_{1D} and α_{1A} (and α_{1L}?)-adrenoceptors compared with α_{1B}-adrenoceptors. The high potency of this drug on the human prostate relative to its potency at α_{1B}-adrenoceptors may explain the clinical efficacy of tamsulosin. The significance of the unsurmountable action of tamsulosin on the human vas deferens which was observed at very low concentrations has yet to be established.

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