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α_{1L}-Adrenoceptors mediate noradrenaline-induced contractions of the guinea-pig prostate stroma

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Abstract

The α_1-adrenoceptor subtype mediating noradrenaline-induced contractions of the guinea-pig isolated prostatic smooth muscle was investigated. Noradrenaline produced concentration-dependent contractions of the tissue with a mean pD_2 value of 5.26 ± 0.03 ($n = 20$). These contractions were antagonised by prazosin, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB-4101), *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (RS-17053) and (*R*)-5-[2-[[2-(2-ethoxyphenoxy)ethyl]amino]-2-methylethyl]-2-methoxybenzensulfonamide methanesulphonate hydrate (tamsulosin). Mean pA_2 or apparent pK_B estimates for the antagonism of noradrenaline were 8.15 ± 0.05 for prazosin; 8.83 ± 0.11 for WB-4101, 7.18 ± 0.14 for RS-17053 and 10.11 ± 0.12 for tamsulosin. The relatively low estimates of the apparent dissociation constant for all antagonists except tamsulosin indicate that an α_{1L}-adrenoceptor mediates noradrenaline-induced prostatic smooth muscle contraction in the guinea-pig prostate gland.

Keywords: α_{1L}-Adrenoceptor; Prostate stroma; (Guinea-pig)

1. Introduction

Benign prostatic hyperplasia is a major disorder in ageing men. It is essentially a stromal disease in which symptoms including urinary outflow obstruction can be relieved by blockade of α-adrenoceptors within the tissue (Caine et al., 1975; see reviews by Kirby, 1989; Eri and Tveter, 1995; Hieble and Ruffolo et al., 1996). The rationale for this use depends on the findings that the tone of the smooth muscle is increased by noradrenaline (Caine et al., 1975) released from the dense sympathetic innervation supplying the prostate stroma (Chapple et al., 1991; Guh et al., 1995). Numerous clinical trials have now indicated the efficacy of treatment with α_1-adrenoceptor antagonists; despite this, controversy continues as to the nature of the adrenoceptor subtype that mediates these contractions.

There are three cloned α_1-adrenoceptor subtypes α_{1a}, α_{1b}, α_{1d}, all of which exhibit a high affinity for prazosin (see Bylund et al., 1998). These three α_1-adrenoceptor subtypes have been characterised fully; but an additional subtype, the α_{1L} which may be a variant, or a low affinity state of the α_{1A}, has been proposed on the basis of functional studies (Muramatsu, 1992; Ford et al., 1997; Bylund et al., 1998). Distinguishing features of this receptor include low affinity for prazosin and low sensitivity to *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (RS-17053) (Ford et al., 1996). However, it has a high affinity, as does the α_{1A}-adrenoceptor, for (*R*)-5-[2-[[2-(2-ethoxyphenoxy)ethyl]amino]-2- methylethyl] -2- methoxybenzensulfonamide methanesulphonate hydrate (tamsulosin) (Noble et al., 1997; Kava et al., 1998; Martin, 1999). In radioligand binding and molecular studies of human prostate, the α_{1A}-adrenoceptor predominates, but it has been proposed that the α_{1L}-adrenoceptor mediates the contractile effects of noradrenaline (Muramatsu et al., 1994; Ford et al., 1996).

The guinea-pig prostate gland may provide a suitable experimental model of human prostatic smooth muscle

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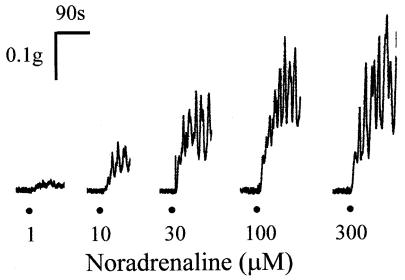

Fig. 1. A representative trace showing the contractile effects of increasing concentrations of noradrenaline in the isolated preparation of the guinea-pig prostate.

because, as in the human, it (1) has a substantial stromal component with a high proportion of smooth muscle (Ricciardelli et al., 1989), (2) undergoes similar age-related histological changes, with the possible lack of discrete nodule formation (Horsfall et al., 1994), and (3) is affected similarly by gonadal steroids (Maini et al., 1997). The guinea-pig prostate gland also receives dense sympathetic innervation (Ohkawa, 1983; Lamano Carvalho et al., 1986; Dhami and Mitchell, 1994; Lau et al., 1998).

Our recent functional studies with prazosin (Lau et al., 1998) suggest that noradrenaline, acting at α_1-adrenoceptors, as in the human, is the major neurotransmitter mediating field stimulation-induced contraction of stromal smooth muscle. Similar conclusions have been reached by Ohkawa (1983), Haynes and Hill (1997) and Najbar-Kaszkiel et al. (1997). The subtype of α_1-adrenoceptor mediating the effects of noradrenaline on the smooth muscle of the prostate stroma, has not, however, been determined in the guinea-pig. Accordingly, the aim of this investigation was to undertake a characterisation of the receptor.

2. Methods

2.1. Functional experiments

Prior approval for animal experimentation was obtained from the Monash University Standing Committee on Ethics in Animal Experimentation (SCEAE Approval No. 95/141).

Prostate glands were dissected from male guinea-pigs (Monash strain, 450–950 g) as described previously (Lau

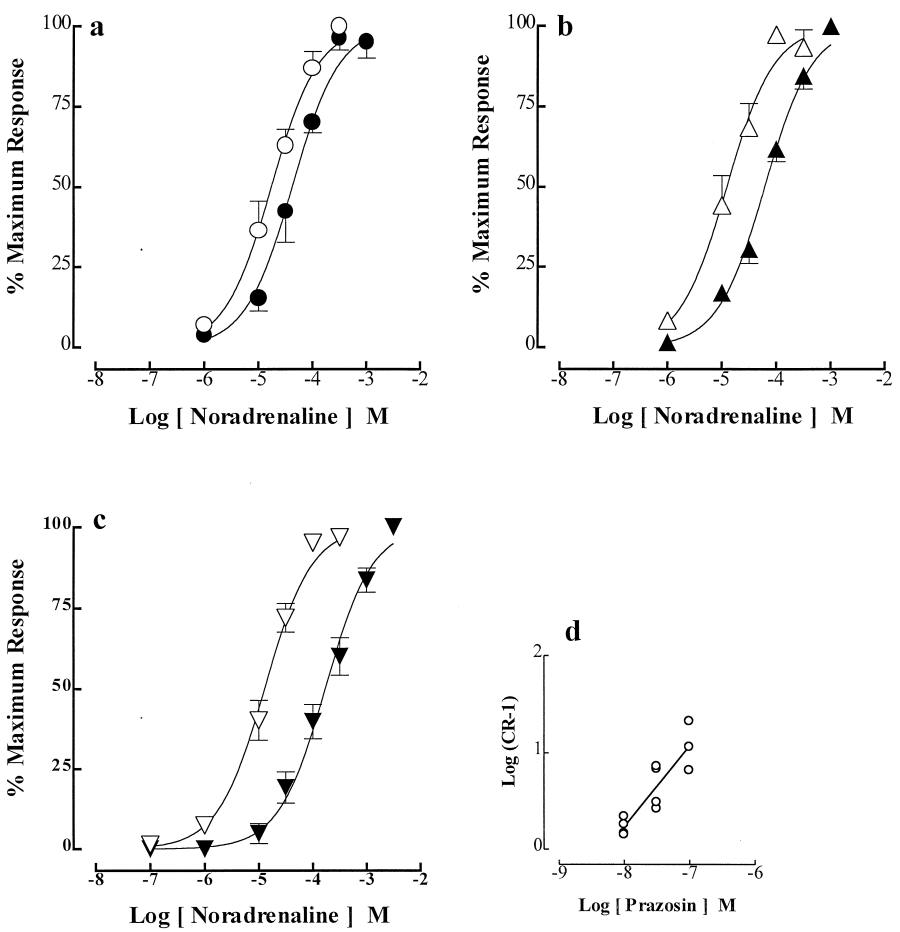

Fig. 2. Log concentration–response curves to noradrenaline on isolated preparations of guinea-pig prostate in the absence (open symbols) and presence (filled symbols) of (a) 10 nM prazosin, (b) 30 nM prazosin and (c) 100 nM prazosin. Values are mean ± S.E.M. of $n = 4$. (d) Schild regression analysis with concentration ratio (CR) estimated from individual preparations of the guinea-pig prostate.

et al., 1998). Three to four preparations per animal were set up (under 0.5 g force) in 10 ml organ baths containing Krebs–Henseleit solution comprising (mM): NaCl 118.1, KCl 4.87, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCl_2 \cdot 2H_2O$ 2.5 (pH 7.4, maintained at 37°C; bubbled with 5% CO_2 in O_2). This was supplemented with 10 μM cocaine, 10 μM β-oestradiol, to block neuronal and extraneuronal uptake of noradrenaline, respectively, and 1 μM propranolol, 0.1 μM idazoxan and 0.1 μM atropine to minimise the contribution of β- and α_2-adrenoceptors, and muscarinic receptors to responses to noradrenaline. The prostatic preparations were allowed to equilibrate for 60 min with bath medium changes every 10 min. Isometric contractions of the prostatic smooth muscle were recorded with *Grass FT03C* force-displacement transducers connected to a *MacLab* data acquisition system (*Chart 3.3*) interfaced with a *Macintosh LC575* computer.

Discrete log concentration–response curves were constructed to noradrenaline (0.1 μM to 1 mM), with a contact time of 90 s for each concentration on a 10-min dose-cycle. Two curves were constructed with an interval of 1 h between them in the absence of an antagonist and then a third curve was constructed in the presence of one concentration of an antagonist or vehicle after a 60-min preincubation period. The antagonists were prazosin (10, 30 and 100 nM), 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB-4101; (10, 30 and 100 nM), RS-17053 (30, 100 and 300 nM) and tamsulosin (0.3, 1 and 3 nM). The vehicles were distilled water for prazosin and tamsulosin, and 0.01% ethanol and 0.01% dimethylsulphoxide, for WB-4101 and RS-17053, respectively.

The peak contractile response in milligrams to each concentration of noradrenaline was recorded and expressed as a percentage of the maximum response of the second concentration–response curve (in the absence of antagonist) to noradrenaline.

Noradrenaline pD_2 estimates and antagonist-induced shifts in the position of the third curve relative to the second curve were determined using *GraphPad PRISM 2.0*. Antagonist dissociation constants, expressed as pA_2 or apparent pK_B values, were determined as follows. If the Schild regression analyses indicated that the slope was not significantly different from unity, the slope was constrained to 1 and pA_2 values were determined according to the method of Arunlakshana and Schild (1959). Otherwise, mean apparent dissociation constants (K_B) for each antagonist concentration were calculated from concentration ratios for each experiment using the equation: apparent

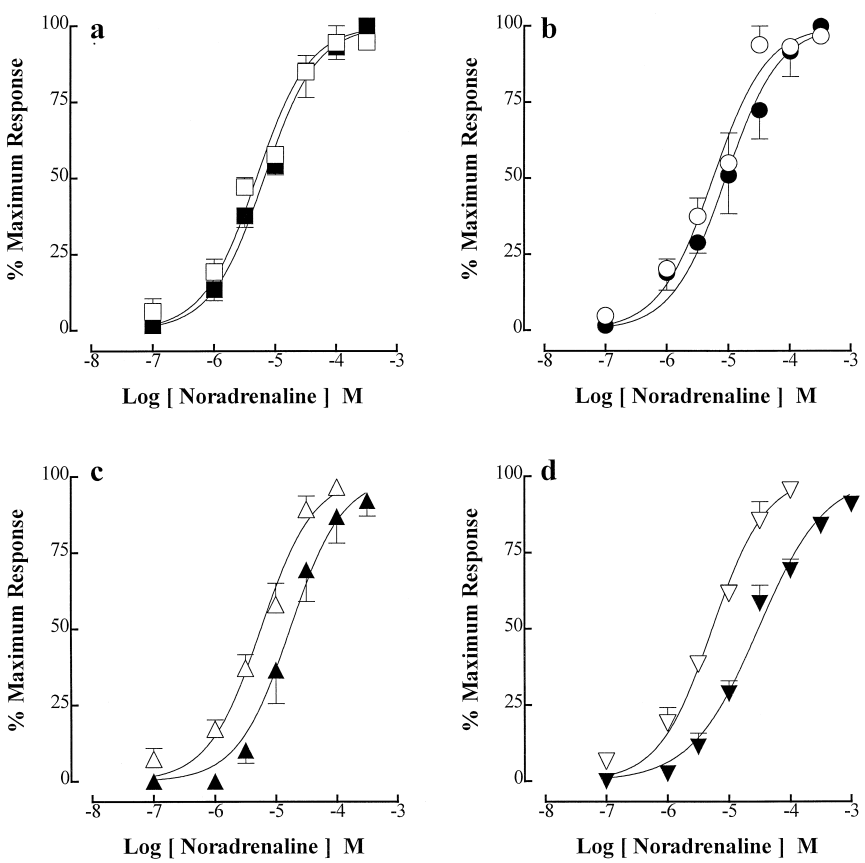

Fig. 3. Log concentration–response curves to noradrenaline on isolated preparations of guinea-pig prostate in the absence (open symbols) and presence (filled symbols) of (a) 0.01% DMSO, (b) 30 nM RS-17053, (c) 100 nM RS-17053 and (d) 300 nM RS-17053. Values are mean ± S.E.M. of $n = 4–5$.

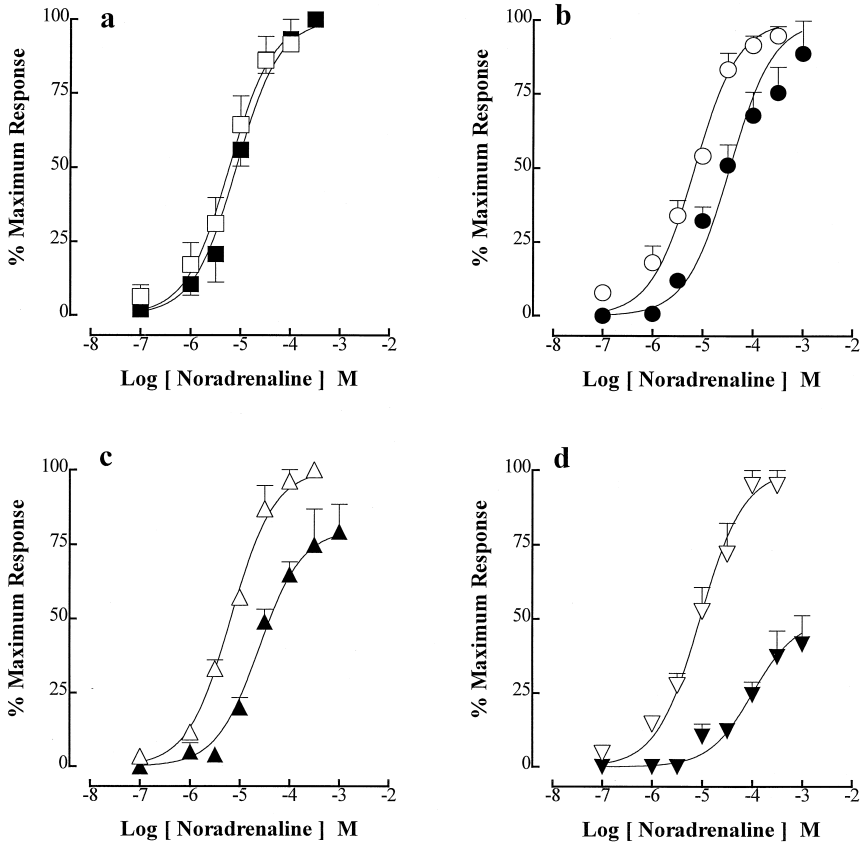

Fig. 4. Log concentration–response curves to noradrenaline on isolated preparations of guinea-pig prostate in the absence (open symbols) and presence (filled symbols) of (a) distilled water, (b) 0.3 nM tamsulosin (c) 1 nM tamsulosin and (d) 3 nM tamsulosin. Values are mean ± S.E.M. of $n = 4$.

K_B = (antagonist concentration)/(concentration ratio − 1), and converted to negative logarithms (pK_B; Furchgott, 1972).

2.2. Drugs

The following drugs were used: (−)-arterenol (noradrenaline) bitartrate, atropine sulphate, cocaine hydrochloride, β-oestradiol and DL-propranolol hydrochloride (Sigma), idazoxan (Reckitt and Colman, Hull, UK), prazosin hydrochloride (Pfizer, NSW, Australia), 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB-4101) (RBI, Natick, MA, USA), *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (RS-17053) (Tocris, Bristol, UK) and (*R*)-5-[2-[[2-(2-ethoxyphenoxy) ethyl] amino] - 2 - methylethyl] - 2 - methoxybenzensulfonamide methanesulphonate hydrate (tamsulosin) (Yamanouchi Pharm; gift from Dr. J.P. Hieble, SmithKline

Table 1
Mean affinity estimates, expressed as pA_2 or apparent pK_B values, for prazosin, WB-4101, RS-17053 and tamsulosin at α_1-adrenoceptors in the guinea-pig isolated prostate, with noradrenaline as the agonist

Antagonist	Concentration (nM)	*n*	pA_2 or pK_B (mean ± S.E.M)[a]	Slope (mean ± S.E.M.)
Prazosin	10, 30, 100	4	8.15 ± 0.05[a]	0.85 ± 0.11
WB-4101	10, 30, 100	4	8.83 ± 0.11[a]	0.95 ± 0.29
RS-17053	30	4	No shifts	
	100, 300	4, 5	7.18 ± 0.14	
Tamsulosin	0.3	4	10.11 ± 0.12[b]	
	1, 3	4		

[a] Since the Schild slope was not significantly different (Student's *t*-test, $P > 0.05$) from unity, pA_2 values for prazosin and WB-4101 were determined with slope constrained to 1.

[b] Tamsulosin, at 1 and 3 nM, caused a significant decrease to the maximal responses to noradrenaline (see Fig. 4). *n* = Number of animals.

Beecham). Stock solutions of noradrenaline (100 mM), WB-4101 (1 mM) and RS-17053 (10 mM) were prepared in catecholamine diluent, ethanol and dimethylsulphoxide, respectively. All other drugs were dissolved in distilled water.

3. Results

Noradrenaline produced concentration-dependent contractions of isolated preparations of guinea-pig prostate gland (Fig. 1). The pD_2 estimate for noradrenaline was 5.26 ± 0.03 ($n = 20$ animals); vehicles (distilled water, $n = 4$; 0.01% ethanol, $n = 4$ and 0.01% dimethylsulphoxide, $n = 5$) were without effect on these estimates (two-way Analysis of Variance, $P > 0.05$). The log concentration–response curves to noradrenaline were shifted significantly to the right ($P < 0.05$) in a parallel fashion by prazosin (10, 30 and 100 nM; Fig. 2), WB-4101 (10, 30 and 100 nM) and RS-17053 (100 and 300 nM; Fig. 3). RS-17053 (30 nM) did not produce significant shifts. As shown in Fig. 4, tamsulosin (0.3, 1 and 3 nM) produced significant rightward shifts in the log concentration–response curves to noradrenaline; however, at 1 and 3 nM, maximum responses to noradrenaline were decreased by $20.7 \pm 9.1\%$ and $53.3 \pm 6\%$, respectively ($n = 4$, Student's *t*-test, $P < 0.05$). Mean affinity estimates for prazosin, WB-4101, RS-17053 and tamsulosin at α_1-adrenoceptors in the guinea-pig isolated prostate were determined and shown in Table 1.

4. Discussion

The estimates of the affinities of the antagonists for the receptor activated by application of noradrenaline are similar to those reported to antagonise the effects of noradrenaline on human fibromuscular stroma. Ford et al. (1996) reported estimates of 6.89 for RS-17053, 8.94 for prazosin and 8.9 for WB-4101; while Muramatsu et al. (1994) reported estimates of 8.3 and 8.4 for the latter two antagonists. Comparable estimates have recently been reported for antagonists including WB 4101 and prazosin on the effects of noradrenaline on the rat prostate stroma (Hiraoka et al., 1999). In the latter study, it was concluded that, as in the human, an α_{IL}-adrenoceptor subtype mediated the effects of noradrenaline. Our finding that tamsulosin (Honda and Nagagawa, 1986) yielded a mean pK_B estimate of 10.11 versus noradrenaline is also consistent with this conclusion since it exhibits a similar affinity at α_{1A}- and α_{IL}-adrenoceptors (Kava et al., 1998). In addition, our finding that this antagonist, at higher concentrations, reduced the maximal responses to noradrenaline is reminiscent of its effects on the human prostate (Ford et al., 1996; Muramatsu et al., 1998). This finding also indicates that the low estimates we obtained for the other adrenoceptor antagonists do not simply reflect a species difference in their affinities for α-adrenoceptors as reported several years ago by Ruffolo et al. (1982). The present data raised the possibility that an α_{IL}-adrenoceptor mediates noradrenaline-induced contractions of guinea-pig prostate smooth muscle, indicating it may be suitable as a model tissue for screening drug actions at prostatic α_1-adrenoceptors.

Interestingly, the rightward shifts induced by prazosin in the positions of the log frequency–response curve to electrical field stimulation of nerve terminals present in the guinea pig stroma (Lau et al., 1998), were comparable in magnitude with those seen for the log concentration–response curve to exogenous noradrenaline. This observation is consistent with the possibility that the receptor mediating the effects of neurally released noradrenaline may also be of the α_{IL}-adrenoceptor subtype. It was for that reason, and in accord with previous investigations by Ford et al. (1996) that we chose noradrenaline, rather than phenylephrine for use as the agonist in the present study. In our earlier study of the effects of prazosin on field stimulation-induced contractions, inhibitors of catecholamine uptake and antagonists of β- and α_2-adrenoceptors, and of muscarinic receptors were not included in the bathing medium. Accordingly, further investigation of the interaction of additional α_1-adrenoceptor antagonists on responses to noradrenaline released from sympathetic terminals within the prostate tissue will, however, be required to establish whether the α_{IL}-adrenoceptor also mediates the effects of noradrenaline released from sympathetic nerve terminals within the prostate gland.

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References

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bylund, D.B., Bond, R.A., Clark, D.E., Eikenburg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffolo, R.R. Jr., Strosberg, A.D., Trendelenburg, U.G., 1998. Adrenoceptors. In: Girdlestone, D. (Ed.), The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media, London, pp. 58–74.

Caine, M., Raz, S., Zeigler, M., 1975. Adrenergic and cholinergic receptors in the human prostate, prostate capsule, and bladder neck. Br. J. Urol. 27, 193–202.

Chapple, C.R., Crowe, R., Gilpin, S.A., Gosling, J., Burnstock, G., 1991. The innervation of the human prostate gland — the changes associated with benign enlargement. J. Urol. 146, 1637–1644.

Dhami, D., Mitchell, B.S., 1994. Chemical coding of neurons projecting to pelvic viscera in the male guinea-pig: a study by retrograde transport and immunohistochemistry. Histochem. J. 26, 262–270.

Eri, L.M., Tveter, K.J., 1995. Alpha-blockade in the treatment of symptomatic benign prostatic hyperplasia. J. Urol. 154, 923–934.

Ford, A.P.D.W., Arredondo, N.F., Blue, D.R. Jr., Bonhaus, D.W., Jasper, J., Kava, M.S., Lesnick, J., Pfister, J.R., Shieh, A., Vimont, R.L., Williams, T.J., McNeal, J.E., Stamey, T.A., Clarke, D.E., 1996. RS-17053 (N-[2-(2-cyclopropylmethoxyphenoxy)-ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functional α_1-adrenoceptors in human prostate: implications for adrenoceptor classification. Mol. Pharmacol. 49, 209–215.

Ford, A.P.D.W., Daniels, D.V., Chang, D.J., Gever, J.R., Jasper, J.R., Lesnick, J.D., Clarke, D.E., 1997. Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α_1-adrenoceptor classification. Br. J. Pharmacol. 121, 1127–1135.

Furchgott, R.F., 1972. The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Handbook of Experimental Pharmacology. Blaschko, H., Muscholl, E. (Eds.), Catecholamines 33 Springer-Verlag, Berlin, pp. 283–335.

Guh, J.-H., Chueh, S.-C., Ko, F.-N., Teng, C.-M., 1995. Characterization of α_1-adrenoceptor subtypes in tension responses of human prostate to electrical field stimulation. Br. J. Pharmacol. 115, 142–146.

Haynes, J.M., Hill, S.J., 1997. β-Adrenoceptor-mediated inhibition of α_1-adrenoceptor-mediated and field stimulation-induced contractile responses in the prostate of the guinea-pig. Br. J. Pharmacol. 122, 1067–1074.

Hieble, J.P., Ruffolo, R.R. Jr., 1996. The use of alpha-adrenoceptor antagonists in the pharmacological management of benign prostatic hypertrophy: an overview. Pharmacol. Res. 33, 145–160.

Hiraoka, Y., Ohmura, T., Oshita, M., Watanabe, Y., Morikawa, K., Nagata, O., Kato, H., Taniguchi, T., Muramatsu, I., 1999. Binding and functional characterization of α_1-subtypes in the rat prostate. Eur. J. Pharmacol. 366, 119–126.

Honda, K., Nagagawa, C., 1986. α_1-Adrenoceptor antagonist effects of the optical isomers of YM-12617 in rabbit lower urinary tract and prostate. J. Pharmacol. Exp. Ther. 239, 512–516.

Horsfall, D.J., Mayne, K., Ricciardelli, C., Rao, M., Skinner, J.M., Henderson, D.W., Marshall, V.R., Tilley, W.D., 1994. Age-related changes in guinea-pig prostatic stroma. Lab. Invest. 70, 753–763.

Kava, M.S., Blue, D.R., Vimont, R., Clarke, D.E., Ford, A.P.D.W., 1998. α_{1L}-Adrenoceptor mediation of smooth muscle contraction in rabbit bladder neck: a model for lower urinary tract tissues of man. Br. J. Pharmacol. 123, 1359–1366.

Kirby, R.S., 1989. Alpha-adrenoceptor inhibitors in the treatment of benign prostatic hyperplasia. Am. J. Med. 87, 26S–30S.

Lamano Carvalho, T.L., Hodson, N.P., Blank, M.A., Watson, P.F., Mulderry, P.K., Bishop, A.E., Gu, J., Bloom, S.R., Polak, J.M., 1986. Occurrence, distribution and origin of peptide-containing nerves of guinea-pig and rat male genitalia and the effects of denervation on sperm characteristics. J. Anat. 149, 121–141.

Lau, W.A.K., Ventura, S., Pennefather, J.N., 1998. Pharmacology of neurotransmission to the smooth muscle of the rat and the guinea-pig prostate glands. J. Auton. Pharmacol. 18, 349–356.

Maini, A., Archer, C., Wang, C.Y., Haas, G.P., 1997. Comparative pathology of benign prostatic hyperplasia and prostate cancer. In Vivo 11, 293–300.

Martin, D.J., 1999. Preclinical pharmacology of α_1-adrenoceptor antagonists. Eur. Urol. 36 (1), 35–41.

Muramatsu, I., 1992. A pharmacological perspective of α_1-adrenoceptors: subclassification and functional aspects. In: Fujiwara, M., Sugimoto, T., Kogure, K. (Eds.), α_1-Adrenoceptors: Signal Transduction, Ionic Channels and Effector Organs. Excerpta Medica, Tokyo, pp. 193–202.

Muramatsu, I., Oshita, M., Ohmura, T., Kigoshi, S., Akino, H., Gobara, M., Okada, K., 1994. Pharmacological characterization of α_1-adrenoceptor subtypes in the human prostate: functional and binding studies. Br. J. Urol. 74, 572–577.

Muramatsu, I., Taniguchi, T., Okada, K., 1998. Tamsulosin: α_1-adrenoceptor subtype-selectivity and comparison with terazosin. Jpn. J. Pharmacol. 78, 331–335.

Najbar-Kaszkiel, A.T., Di Iulio, J.L., Li, C.G., Rand, M.J., 1997. Characterisation of excitatory and inhibitory transmitter systems in prostate glands of rats, guinea-pigs, rabbits and pigs. Eur. J. Pharmacol. 337, 251–258.

Noble, A.J., Chess-Williams, R., Couldwell, C., Furukawa, K., Uchyiuma, T., Korstanje, C., Chapple, C.R., 1997. The effects of tamsulosin, a high affinity antagonist at functional α_{1A}- and α_{1D}-adrenoceptor subtypes. Br. J. Pharmacol. 120, 231–238.

Ohkawa, H., 1983. Sympathetic neuromuscular transmission in the smooth muscle of guinea-pig prostate gland. Int. J. Fertil. 28, 68–77.

Ricciardelli, C., Horsfall, D.J., Skinner, J.M., Henderson, D.W., Marshall, V.R., Tilley, W.D., 1989. Development and characterization of primary cultures of smooth muscle cells from the fibromuscular stroma of the guinea-pig prostate. In Vitro Cell. Dev. Biol. 25, 1016–1024.

Ruffolo, R.R. Jr., Waddell, J.E., Yaden, E.L., 1982. Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. J. Pharmacol. Exp. Ther. 221, 309–314.