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Characterisation of the functional α-adrenoceptor subtype in the isolated female pig urethra

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Abstract

The aim of the present study is to characterise the contraction-mediating functional α-adrenoceptor of the female pig urethra. α-Adrenoceptor reference agonists were used to contract the isolated female pig urethra. The relative intrinsic activity was noradrenaline (1.0), phenylephrine (0.91), methoxamine (0.74), (±)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethane-sulfonanilide hydrochloride (NS-49) (0.68), oxymetazoline (0.60), dopamine (0.50), clonidine (0.43), midodrine (0.32), ephedrine (0.30), 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304) (0.11), and phenylpropanolamine (0.11). The 21 competitive antagonists used caused parallel rightward shifts in the α-adrenoceptor agonist concentration–response curves, giving linear Schild-plots with slopes not significantly different from unity, suggesting that contraction was mediated by a single receptor. The antagonist pK_B values calculated were *R*(−)-tamsulosin (9.68), risperidone (9.19), 2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-4,4-dimethyl-1,3(2*H*,4*H*)-isoquinolinedione (AR-C 239) (9.09), 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB-4101) (8.87), *N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide monomethanesulfonate (Rec 15/2739/3) (8.81), 5-methylurapidil (8.59), prazosin (8.57), benoxathian (8.56), *S*(+)-tamsulosin (8.27), indoramin (8.11), doxazosin (7.96), alfuzosine (7.82), phentolamine (7.70), terazosin (7.52), spiperone (7.48), oxymetazoline (7.40), 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378) (7.05), corynanthine (6.98), rauwolscine (6.40), yohimbine (6.22), and *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (RS 17053) (6.07). Correlation of subtype-selective antagonist pK_B values was best with published values for the $\alpha_{1a/1A}$-adrenoceptor subtype. Therefore, the present results suggest that contraction of the female pig urethra is caused by activation of the α_{1A}-adrenoceptor.

Keywords: Adrenoceptor subtype; BMY 7378; Corynanthine; Prazosin; Spiperone; Stress incontinence

1. Introduction

Urethral tone in the human is largely maintained by activation of postsynaptic α-adrenoceptors (Andersson, 1993). The low intraurethral pressure and the urethral closure pressure in women with stress incontinence are increased by α-adrenoceptor agonists. Consequently, stress incontinence can be treated with α-adrenoceptor agonists such as ephedrine (cf. Boston, 1928), midodrine and phenylpropanolamine (cf. Gillberg et al., 1998), some with vascular side-effects.

Three human α_1- and three human α_2-adrenoceptor subtypes have been cloned (cf. Bylund et al., 1994, 1998; Hieble et al., 1995). The existence of a fourth α_1 subtype, designated α_{1L}, has been postulated (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990); however, it has not been cloned and may represent a particular conformational state of the α_{1A}-adrenoceptor (Ford et al., 1997; Bylund et al., 1998). In the human female urethra different levels of α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor expression have been suggested using RNase protection assay and in situ hybridisation (Takahashi et al., 1996; Takeda et al., 1996; Nasu et al., 1998).

The pig urethra is also contracted by the α-adrenoceptor agonists noradrenaline and phenylephrine (Persson and Andersson, 1992; Bridgewater et al., 1993, 1995). The aim of the present study is to characterise the contraction-mediating functional α-adrenoceptor of the female pig urethra.

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2. Materials and methods

2.1. Tissue preparation

The urethra en bloc with the urinary bladder, vagina, uterus and ovaries from female pigs (75–100 kg carcass weight) was obtained fresh at the local abattoir (Farmek Scan, Uppsala, Sweden), placed on ice and transported less than 3 km to the laboratory. The urethra was cleansed of adherent fat, connective tissue and mucosa. Longitudinal muscle pieces (50–200 mg, about $3 \times 5 \times 15$ mm) of the urethra about 3 cm distal from the ureters where the maximal intraurethral pressure can be expected (cf. Bridgewater et al., 1993) were prepared and placed in Tyrode solution. Six to twelve muscle strips were obtained from each urethra.

2.2. Physiological solution

The Tyrode solution contained (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, D-glucose 5.6, ascorbate 0.114, desipramine 0.0006, normetanephrine 0.01, propranolol 0.001, and was aerated with 5% CO_2 in O_2 to give pH 7.4 at 37°C.

2.3. Set-up of the preparation

The preparation was mounted in a 10-ml glass organ bath (Radnoti, Monrovia, CA, USA) under a passive isometric tension of 10 mN, equivalent to 1 g weight, in Tyrode solution at 37°C. The tissue was allowed to rest for 30–60 min until the baseline stabilised. During this period, the resting tension was repeatedly readjusted to 10 mN.

Isometric contraction of the preparation was registered by a force displacement transducer (Grass FT03) and recorded continuously on a polygraph (Grass 7D).

Two types of organ bath set-up were used. In the 'classical' one, the Tyrode solution was changed at 10–20 min intervals by emptying and refilling. In the other set-up type, the organ bath was superfused with Tyrode solution at a rate of 1 ml min^{-1} (cf. Stjärne et al., 1979; Alberts, 1995a,b; Bridgewater et al., 1995).

2.4. Experimental protocol

The urethral strip was primed with a submaximal agonist concentration to ascertain the contractility of the preparation, except in experiments with the agonists oxymetazoline, clonidine and UK 14,304. To regain the initial resting tension the preparation was allowed to rest for about 60 min.

For construction of concentration–response curves agonist was added in a cumulative manner to the organ bath until the contraction did not increase any further (Fig. 1). After completion of the first curve the agonist was removed and the preparation was allowed to rest for 80–300 min in order to regain the initial resting tension. Then the preparation was incubated with antagonist for 60 min and a second curve was made in its presence.

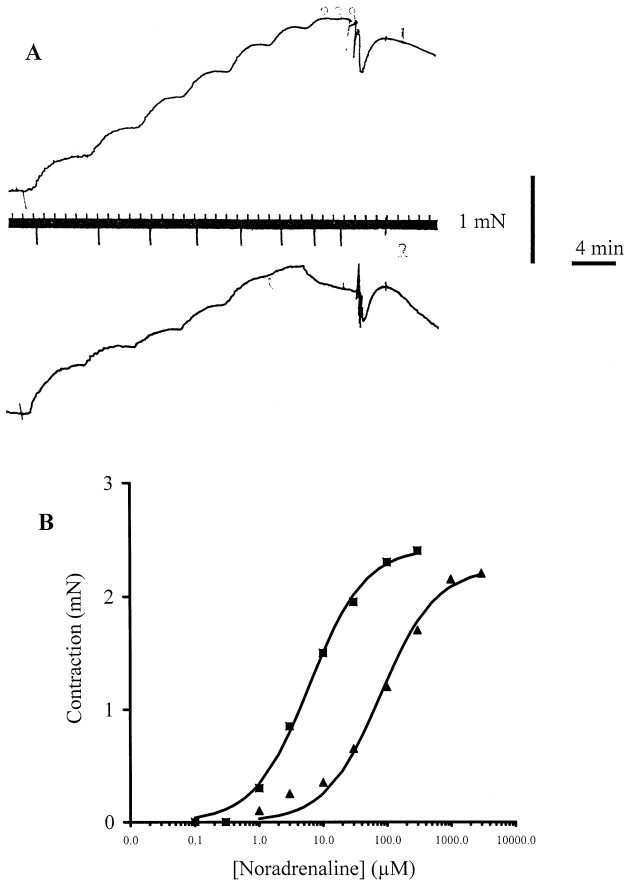

Fig. 1. (A) Cumulative concentration–response curve recordings for noradrenaline in the isolated female pig urethra. Two simultaneous, parallel recordings from two organ baths are shown. Agonist was added at downward marks on the time scale at increasing concentrations (0.1, 0.3, 1, 3, 10, 30, 100 μM), followed by wash. (B) Plot of the two cumulative concentration–response curves obtained in a preparation in the absence and presence of prazosin (0.1 μM).

2.5. Calculation of agonist EC_{50} values

The agonist concentration–response curve was characterised assuming one binding site by an equation describing a hyperbolic function containing two constants, $y = (P_1 x)(P_2 + x)^{-1}$, where y is the evoked contraction, x is the agonist concentration, P_1 is the maximal contraction asymptotically approached at 'infinitely' high agonist concentration, i.e., the 'maximal contraction', and P_2 is the agonist concentration yielding half of the 'maximal contraction', EC_{50}. The constants were calculated from each curve using an iterative non-linear regression computer program (Fig. P for Windows, Version 3.1, Biosoft, Cambridge, UK; cf. Stjärne et al., 1979; Alberts, 1992).

The agonist EC_{50} values reported are arithmetic means of EC_{50} values obtained in (n) number of concentration–response curves.

Competitive reference antagonists that cause a parallel rightward shift of the concentration–response curves without depression of the 'maximal contraction' were used (cf. Fig. 1). Therefore, a criterion for inclusion of data in the results was that the 'maximal contraction' of the second concentration–response curve was $100 \pm 20\%$ of the first, control, curve. Each antagonist concentration was tested in at least three preparations.

2.6. Calculation of antagonist pA_2 and pK_B values

The antagonist pA_2 values were determined from the Schild plots (Schild, 1947, 1949; Arunlakshana and Schild, 1959), $-\log$[antagonist] (i.e., pB) vs. $\log(dr-1)$ (cf. Alberts, 1993) where 'dr' is the dose-ratio ((EC$_{50}$ in the presence of antagonist) $\times$ (EC$_{50}$ in the absence of antagonist)$^{-1}$) obtained in each preparation. Individual $\log(dr-1)$ values so obtained were used for calculation of the pA_2 and pK_B values. The means of (n) number of $\log(dr-1)$ values are plotted in Fig. 2.

The Schild-plot of a competitive antagonist is by definition linear with a slope of unity (Kenakin, 1997). Under these conditions, when $\log(dr-1)$ is zero, pA_2 equals pK_B, as shown by the logarithmic Schild equation ($p(dr-1) = pB - pK_B$) (Kenakin, 1997). Therefore, if the slope was not significantly different from unity, as indicated by the 95% confidence interval (Fig. P for Windows), it was constrained to unity to calculate pK_B.

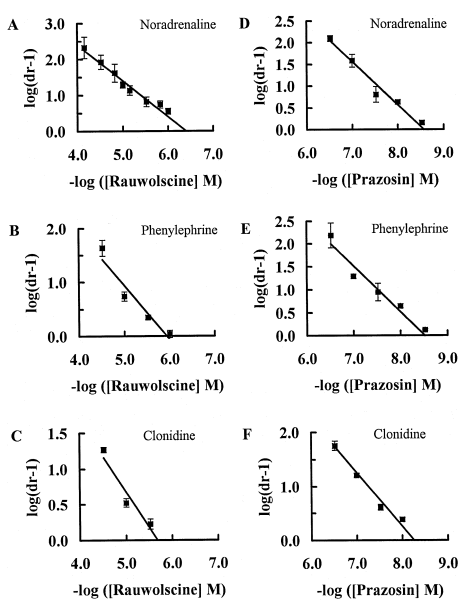

Fig. 2. Schild-plots for the antagonists, (A) rauwolscine vs. noradrenaline, (B) rauwolscine vs. phenylephrine, (C) rauwolscine vs. clonidine, (D) prazosin vs. noradrenaline, (E) prazosin vs. phenylephrine, and (F) prazosin vs. clonidine. For (n) values, see Section 3.

When oxymetazoline was used as agonist, the antagonist dissociation constant (K_B) values were calculated on the basis of the dose-ratio of a single antagonist concentration using the Schild equation: $K_B = [\text{antagonist}] \times (dr-1)^{-1}$ (cf. Furchgott, 1972; Alberts, 1993).

Data are expressed as mean $\pm$ S.E.M. throughout. Statistical evaluation was done with Student's *t*-test or with approximate Student's *t*-test (cf. Daniel, 1991).

2.7. Correlation analysis

Correlation coefficients (r) and slopes of the linear-regression lines were calculated between the negative logarithm of the K_B values (pK_B) and the negative logarithm of published constants. Only subtype-selective antagonists were used in the analysis. Analysis was done with data sets where all three α_1-adrenoceptor subtypes are reported in the same study. Analysis was done with at least three of the same subtype-selective compounds having been tested both in the previous and in the present study. The statistic 't' for a relationship was calculated (Kenakin, 1997).

2.8. Drugs and chemicals

The following chemical compounds were used: 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB-4101), carbamylcholine chloride (carbachol), corynanthine hydrochloride, dopamine hydrochloride, (−)-ephedrine hydrochloride, histamine dihydrochloride, methoxamine hydrochloride, midodrine hydrochloride, (−)-noradrenaline bitartrate, DL-normetanephrine hydrochloride, oxymetazoline hydrochloride, L-phenylephrine hydrochloride, DL-propranolol hydrochloride, serotonin creatine sulfate, yohimbine hydrochloride (Sigma/Aldrich, St. Louis, MO, USA), (±)-alfuzosine hydrochloride (Synthélabo, Tours, France), 5-methylurapidil (Byk Gulden, Konstanz, Germany), 2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-4, 4-dimethyl-1, 3(2*H*,4*H*)-isoquinolinedione (AR-C 239 Cl), clonidine hydrochloride (Boehringer Ingelheim, Ingelheim, Germany), desipramine hydrochloride, phentolamine methane sulphonate (Novartis, Basel, Switzerland), doxazosin mesylate, prazosin hydrochloride (Pfizer, New York, USA), indoramin hydrochloride (Wyeth Lederle, Taplow, Maidenhead, Berkshire, UK), benoxathian hydrochloride, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378), rauwolscine hydrochloride, risperidone, spiperone hydrochloride, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304) (Research Biochemicals, Natick, MA, USA), *N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide monomethanesulfonate (Rec 15/2739/3) (Recordati, Milan, Italy), *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dime-

Table 1
Parameters describing the contractile effect of agonists in the isolated female pig urethra

Agonist	EC_{50} (μM) mean ± S.E.M.	pEC_{50}	*n*	Maximal contraction (%) mean ± S.E.M.	*n*	*P*
Noradrenaline	2.0 ± 0.11	5.69	1069	100		
Phenylephrine	6.7 ± 0.44	5.18	135	91 ± 6.0	5	> 0.05
Methoxamine	15.5 ± 1.6	4.81	3	74 ± 1.4	3	< 0.001
NS-49	30.6 ± 3.9	4.51	15	68 ± 4.0	9	< 0.001
Oxymetazoline	0.65 ± 0.03	6.18	78	60 ± 5.7	6	< 0.001
Dopamine	169 ± 34	3.77	9	50 ± 4.9	9	< 0.001
Clonidine	1.5 ± 0.06	5.84	142	43 ± 3.1	17	< 0.001
Midodrine	172 ± 72	3.76	3	32 ± 14	3	< 0.01
Ephedrine	894 ± 202	3.05	6	30 ± 1.3	6	< 0.001
UK 14,304	6.9 ± 1.9	5.16	11	11 ± 1.8	6	< 0.001
Phenylpropanolamine	1425 ± 60	2.85	3	11 ± 2.1	13	< 0.001
Carbachol	2.3 ± 0.8	5.64	3	12 ± 1.3	3	< 0.001
Histamine	128 ± 53	3.89	8	26 ± 8.7	8	< 0.001

The 'maximal contraction' of the second concentration–response curve is expressed relative to the 'maximal contraction' of the first curve made in the same preparation with noradrenaline (0.1–300 μM). The second curve was made with phenylephrine (1–300 μM), methoxamine (0.1–1000 μM), NS-49 (1–300 μM), oxymetazoline (0.1–30 μM), dopamine (1–300 μM), clonidine (1–100 μM), midodrine (3–1000 μM), ephedrine (1–10 mM), UK 14,304 (0.1–300 μM), phenylpropanolamine (0.1–10 mM), carbachol (0.1–100 μM), and histamine (1–3000 μM). Statistical difference (*P*) from 'maximal contraction' for noradrenaline is shown.

thyl-1*H*-indole-3-ethanamine hydrochloride (RS 17053) (Tocris, Bristol, UK), terazosin hydrochloride dihydrate (Abbott, North Chicago, IL, USA), (±)-phenylpropanolamine hydrochloride (norephedrine) (Pharmacia code 45 32 58, batch 9071UA) (Alps Pharmaceuticals, Furukawa-Cho, Gifu Prefecture, Japan), *R*(−)- and *S*(+)-tamsulosin (*R*(−)-YM617, batch CA003028, *S*(+)-YM617, batch CA003033), (±)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethane-sulfonanilide hydrochloride ((±)-NS-49, formerly PNO-49B) (cf. Obika et al., 1995) (prepared in the Department of Medicinal Chemistry, Pharmacia and Upjohn, Uppsala), L(+)-ascorbic acid (Merck, Darmstadt, Germany). All other chemicals were of analytical grade.

Noradrenaline, phenylephrine, NS-49, oxymetazoline, dopamine, clonidine, midodrine, ephedrine, phenylpropanolamine, histamine, and serotonin were dissolved and diluted in ascorbate (0.114 mM). UK 14,304 was dissolved in dimethylsulfoxide. A stock solution of

Table 2
Antagonist pK_B values in the isolated female pig urethra determined against the agonist noradrenaline

Antagonist	pK_B (mean ± S.E.M.)	*n*	Antagonist concentrations	Slope
Rauwolscine	6.40 ± 0.05	49	1–70 μM	0.92
Prazosin	8.57 ± 0.05	23	3–300 nM	0.99
R(−)-Tamsulosin	9.68 ± 0.06	14	0.3–3 nM	0.78
Risperidone	9.19 ± 0.06	16	1–10 nM	0.91
AR-C 239	9.09 ± 0.06	12	1.76–10 nM	0.68
WB-4101	8.87 ± 0.11	24	3–100 nM	0.98
Rec 15/2739	8.81 ± 0.06	16	3–30 nM	1.03
5-Methylurapidil	8.59 ± 0.63	22	10–300 nM	0.92
Benoxathian	8.56 ± 0.06	15	3–30 nM	0.78
S(+)-Tamsulosin	8.27 ± 0.05	15	10–100 nM	0.93
Indoramin	8.11 ± 0.05	22	30–300 nM	0.93
Doxazosin	7.96 ± 0.03	13	30–300 nM	0.90
Alfuzosine	7.82 ± 0.05	14	30–300 nM	0.81
Phentolamine	7.70 ± 0.05	14	30–300 nM	1.03
Terazosin	7.52 ± 0.04	17	100–1000 nM	0.96
Spiperone	7.48 ± 0.05	17	100–1000 nM	0.94
Oxymetazoline	7.40 ± 0.05	26	30–1000 nM	0.89
BMY 7378	7.05 ± 0.07	15	0.1–3 μM	1.01
Corynanthine	6.98 ± 0.04	13	0.3–3 μM	0.87
Yohimbine	6.22 ± 0.10	15	0.6–10 μM	0.92
RS 17053	6.07 ± 0.05	15	1–3 μM	0.88

prazosin was made in methanol and diluted at least a thousand-fold in Tyrode. 5-Methylurapidil was dissolved in hydrochloric acid (0.1 M). Risperidone was dissolved in ethanol and diluted at least a hundred thousand-fold in Tyrode. RS 17053 was dissolved in dimethylsulfoxide or ethanol and diluted at least a thousand-fold in Tyrode. All other compounds were dissolved in water or Tyrode solution.

3. Results

3.1. Agonist relative intrinsic activity and EC_{50} values

The 'maximal contraction' compared to noradrenaline in the same preparation for phenylephrine, methoxamine, NS-49, oxymetazoline, dopamine, clonidine, midodrine, ephedrine, UK 14,304, phenylpropanolamine, carbachol, and histamine were determined in the female pig urethra in vitro. The EC_{50} and pEC_{50} values were also calculated (Table 1). Serotonin (1–300 μM) relaxed the female pig urethra (data not shown).

3.2. α-Adrenoceptor antagonist pK_B values

The pK_B value of the α_2-adrenoceptor antagonist rauwolscine was determined against the non-selective α-adrenoceptor agonist noradrenaline (pK_B: 6.40 ± 0.05, $n = 49$), the putative α_1-adrenoceptor agonist phenylephrine (pK_B: 5.94 ± 0.05, $n = 20$), and the putative α_2-adrenoceptor agonists clonidine (pK_B: 5.68 ± 0.04, $n = 16$) and oxymetazoline (apparent pK_B 5.94 ± 0.12 calculated from the 3 μM antagonist dose-ratio 4.0 ± 0.56; $n = 4$).

The pK_B value of the α_1-adrenoceptor antagonist prazosin was determined against noradrenaline (pK_B: 8.57 ± 0.05, $n = 23$), phenylephrine (pK_B: 8.52 ± 0.07, $n = 43$), clonidine (pK_B: 8.26 ± 0.03, $n = 19$), and oxymetazoline (apparent pK_B 8.16 ± 0.18, calculated from the 0.03 μM antagonist dose-ratio 7.1 ± 1.9; $n = 4$).

The 21 competitive antagonists used caused parallel rightward shifts in the α-adrenoceptor agonist concentration–response curves (cf. Fig. 1). Antagonist-induced rightward shifts in the agonist EC_{50} values were used to construct Schild-plots (cf. Fig. 2) and to calculate antagonist pK_B values (Table 2).

4. Discussion

4.1. Agonist relative intrinsic activity and EC_{50} values

All agonists shown in Table 1, except phenylephrine, had significantly lower intrinsic activity than noradrena-

Table 3
Correlation of pK_B values (from Table 2) obtained in the pig urethra with constants for cloned and native α_1-adrenoceptors

Correlation coefficient (r)			Slope			Constant compared	Compound	Reference
α_{1a}	α_{1b}	α_{1d}	α_{1a}	α_{1b}	α_{1d}			
0.94[c]	0.37	0.69[a]	1.21e	0.37f	0.65g	pK_i	RT W Rec 5MU B ST In PA S BMY	Kenny et al., 1995, 1996
0.94[c]	0.91[b]	0.63	1.26e	1.04f	0.79g	pK_i	RT W Rec 5MU ST In BMY	Kenny et al., 1997
0.95[b]	0.18	0.85[a]	0.98e	0.21f	1.27g	pK_i	RT W Rec 5MU In PA S	Testa et al., 1995
0.93[b]	0.84[a]	0.63	1.13e	0.70f	0.70g	pK_i	W 5MU In PA Ox C	Tseng-Crank et al., 1995
0.98[b]	0.93[a]	0.90[a]	1.06e	1.17f	1.46g	pK_i	RT W 5MU PA Ox	Obika et al., 1995
0.97[a]	0.85	0.76	1.00e	0.93f	1.36g	pK_i	W 5MU PA Ox	Weinberg et al., 1994
0.93	0.47	0.99	1.32e	0.97f	2.96g	pK_i	W 5MU In	Forray et al., 1994
0.96[c]	0.11	−0.08	1.35ei	0.09fn	−0.12gq	pK_i	W 5MU B In PA S BMY	Marshall et al., 1995
0.96[b]	0.76	0.82	1.03eik	0.68fmn	1.45gq	pK_i	W 5MU PA Ox	Michel et al., 1995
0.97[c]	0.68[a]	0.43	1.35i	0.64n	0.62q	pK_i	W 5MU B In PA S Ox BMY C	Goetz et al., 1994, 1995
0.85[a]	0.15	0.70	0.70k	0.12m	1.06q	pK_i	W 5MU B PA S Ox	Laz et al., 1994
0.89[a]	0.84	0.89[a]	1.15e	1.02n	1.52q	pK_i	RT W 5MU In PA Ox	Foglar et al., 1995
0.91[a]	0.90[a]	0.72	1.51i	1.04n	0.86q	pK_i	RT W 5MU In PA BMY	Ford et al., 1996
0.87	0.79	0.74	1.05i	0.90m	1.39q	pK_i	RT 5MU ST PA Ox	Michel and Insel, 1994
0.80	0.09	0.65	0.97i	0.13n	1.16q	pK_i	W 5MU PA S Ox	Faure et al., 1994
0.97[a]	0.94	0.87	1.30i	0.75m	1.35q	pK_D	W PA Ox C	Lomasney et al., 1991
1.00[a]	0.87	0.93	1.46i	1.05n	1.90q	pK_i	W 5MU Oxy	Han et al., 1995
0.94[c]	0.69[a]	0.54	1.17	0.52	0.47	pA_2	RT R AR-C W 5MU B In PA S BMY	Eltze, 1994, 1996
0.78	−0.52	0.97[a]	0.73	−0.72	0.93	pK_i	W 5MU PA S	Testa et al., 1993
0.88[a]	0.25	0.67	1.00	0.30	1.26	pK_i	W 5MU B PA S Ox	Ford et al., 1994
0.93[a]	0.28	0.66	1.16	0.41	1.09	pK_i	W 5MU PA S Ox	Blue et al., 1995

Subtype selective antagonists: WB-4101 (W), phentolamine (PA), oxymetazoline (Ox), corynanthine (C), 5-methylurapidil (5MU), spiperone (S), *R*(−)-tamsulosin (RT), *S*(+)-tamsulosin (ST), benoxathian (B), indoramin (In), BMY 7378 (BMY), Rec 15/2739 (Rec), risperidone (R), AR-C 239 (AR-C).

e: human α_{1a}, f: human α_{1b}, g: human α_{1d}, i: bovine α_{1a}, k: rat α_{1a}, m: rat α_{1b}, n: hamster α_{1b}, q: rat α_{1d}.

Rabbit liver α_{1A}, rat liver α_{1B}, rat hippocampus α_{1D} (Testa et al., 1993). Rat vas deferens α_{1A}, guinea-pig spleen α_{1B}, rat aorta α_{1D} (Eltze, 1994, 1996).

The statistical significance (P) for a correlation is: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

line, and were therefore partial agonists in the female pig urethra. Serotonin, in contrast, relaxed this preparation.

Agonist EC_{50} values in the isolated female pig urethra were calculated (Table 1). In preliminary experiments noradrenaline also contracted the isolated guinea-pig urethra (EC_{50}: 20 ± 2.5 μM, $n = 23$) (P.A.C. Bergström, P. Alberts, unpublished), and the isolated human female urethra (EC_{50}: 8.3 ± 2.0 μM, $n = 24$) (P. Alberts, P.A.C. Bergström, M.G. Fredrickson, unpublished).

4.2. Antagonist pK_B values: characterisation of the functional α-adrenoceptor

The purpose of the present study is to characterise the functional α-adrenoceptor of the female pig urethra. Pharmacological receptor classification takes advantage of drug selectivity, i.e., selective compounds have higher pK_B values for some receptor(s). Most adrenoceptor agonists and antagonists bind to both α_1- and α_2-adrenoceptors. For example, the partial α_2-adrenoceptor agonists oxymetazoline and clonidine have been shown to bind also to all three α_1-adrenoceptor subtypes (Minneman et al., 1994).

The antagonist Schild-plots obtained were linear in the concentration-range tested with slopes not significantly different from negative unity, suggesting that contraction was mediated by a single receptor. The pK_B values for the α_1-adrenoceptor antagonist prazosin (8.16–8.57) and the α_2-adrenoceptor antagonist rauwolscine (5.68–6.40) were similar, independent of whether the non-selective α-adrenoceptor agonist noradrenaline, the α_1-adrenoceptor agonist phenylephrine, or the α_2-adrenoceptor agonists oxymetazoline or clonidine was used (Table 2). This finding also suggests that all four agonists activated the same receptor.

The pK_B values in the pig urethra for prazosin and the other α_1-adrenoceptor antagonists matched the affinity for α_1-adrenoceptors, while the pK_B values for rauwolscine did not match the affinity for α_2-adrenoceptors (cf. Bylund et al., 1994). Therefore, the functional receptor in the pig urethra is of the α_1-adrenoceptor type.

With regard to the three cloned α_1-adrenoceptor subtypes, prazosin and rauwolscine are non-selective. The majority of subtype selective compounds, *R*(−)- and *S*(+)-tamsulosin, risperidone, AR-C 239, WB-4101, Rec 15/2739, 5-methylurapidil, benoxathian, indoramin, phentolamine, oxymetazoline (and RS 17053; just a narrow concentration range yielded a parallel shift in the agonist concentration–response curve; not used in the correlation analysis, cf. Marshall et al., 1996) are α_{1A}-selective, while spiperone is α_{1B}-selective, and BMY 7378 and corynanthine are α_{1D}-selective (cf. references in Table 3).

Correlation analysis of subtype-selective antagonist pK_B values (Table 2) was done using published values for cloned and native α_1-adrenoceptors (Table 3, Fig. 3). The analysis suggests poor correlation with the $\alpha_{1b/1B}$-adrenoceptor subtype. In most instances, 18 of 21, correlation

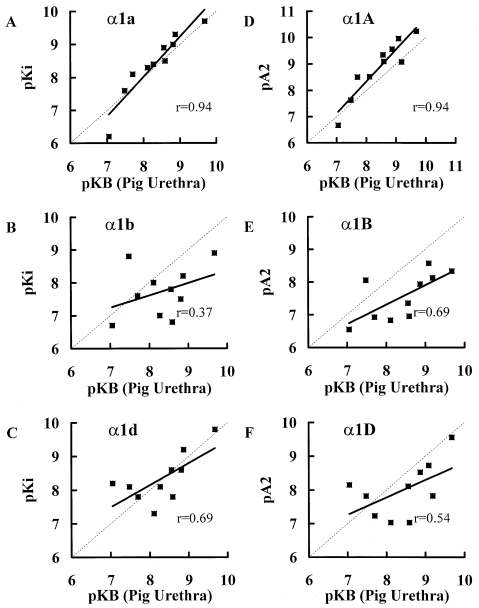

Fig. 3. Correlation (log–log) plots between pK_B values (from Table 2) obtained in the pig urethra with constants for the cloned human (A) α_{1a}-adrenoceptor, (B) α_{1b}-adrenoceptor, and (C) α_{1d}-adrenoceptor genes stably expressed in rat-1 fibroblasts, for *R*(−)- and *S*(+)-tamsulosin, WB-4101, Rec 15/2739, 5-methylurapidil, benoxathian, indoramin, phentolamine, spiperone and BMY 7378 (data from Kenny et al., 1995, 1996), and native (D) α_{1A}-adrenoceptors in rat vas deferens, (E) α_{1B}-adrenoceptors in guinea-pig spleen, and (F) α_{1D}-adrenoceptors in rat aorta for *R*(−)-tamsulosin, risperidone, AR-C 239, WB-4101, 5-methylurapidil, benoxathian, indoramin, phentolamine, spiperone and BMY 7378 (data from Eltze, 1994, 1996). Data were taken from Table 3. The correlation coefficient (r) is shown.

was best with the $\alpha_{1a/1A}$-adrenoceptor subtype, in one $\alpha_{1a/1A}$ was equal to $\alpha_{1d/1D}$, and in two correlation was best with the $\alpha_{1d/1D}$-adrenoceptor. In eight studies, BMY 7378 and corynanthine were used, which are selective for the α_{1D}- over the α_{1A}-adrenoceptor subtype (Lomasney et al., 1991; Eltze, 1994, 1996; Goetz et al., 1994, 1995; Marshall et al., 1995; Tseng-Crank et al., 1995; Ford et al., 1996; Kenny et al., 1995, 1996, 1997). In all these cases (Table 3), correlation was best with the $\alpha_{1a/1A}$-adrenoceptor subtype.

This finding is in agreement with the suggestions that the functional subtype in the rat and rabbit urethra is the α_{1A}-adrenoceptor (Chess-Williams et al., 1994; Auguet et al., 1995). The α_{2A}-adrenoceptor has been suggested to be the functional presynaptic autoreceptor in the guinea-pig urethra (Alberts, 1992, 1993, 1995a; Trendelenburg et al., 1997).

In conclusion, the present results suggest that the functional receptor in the female pig urethra is of the α_{1A}-adrenoceptor subtype.

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