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Comparison of adrenoceptor subtype expression in porcine and human bladder and prostate

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Abstract We have quantified and characterized α_1-, α_2- and β-adrenoceptor subtypes in porcine bladder detrusor and bladder neck, human bladder detrusor, and porcine and human prostate. α_1-, α_2- and β-adrenoceptor were identified in radioligand binding studies using [^{3}H]prazosin, [^{3}H]RX 821002 and [^{125}I]iodocyanopindolol, respectively, as the radioligands. In porcine male and female detrusor and bladder neck and male prostate, adrenoceptors were detected in the order of abundance $\beta > \alpha_2 \gg \alpha_1$ (not detectable), with no major differences between the sexes or between detrusor and bladder neck. In human detrusor and prostate the order of abundance was $\beta > \alpha_2 \gg \alpha_1$ (not detectable) and $\beta \gg \alpha_1 > \alpha_2$, respectively. The α_2-adrenoceptors in all tissues were homogeneously of the α_{2A}-subtype as evidenced by competition binding studies with yohimbine, prazosin, ARC 239 and oxymetazoline. The β-adrenoceptors represented a mixed population with a dominance of the β_2-subtype in all tissues as demonstrated by competition binding with ICI 118,551 and CGP 20,712A. We conclude that pigs may be a suitable model for studies of detrusor function with respect to adrenoceptor expression. They may be less suitable for studies of bladder neck or prostate function.

Key words Pig · Human · α_2-Adrenoceptor · β-Adrenoceptor · Bladder · Prostate

Introduction

Urinary incontinence and other forms of disturbed micturition are a major cause of morbidity which may gain even greater importance with the growing portion of the elderly in the population [20]. Normal bladder function depends on a coordinated balance between pressure developed by the detrusor muscle and resistance developed by the bladder neck, the urethra and, in males, the prostate. In the filling phase relaxation of the detrusor and contraction of the sphincters are required, while in the micturition phase contraction of the detrusor and relaxation of the sphincters are necessary. This is coordinated by the integration of excitatory, inhibitory and sensory nerves in control centers in the spinal cord and brain [7]. While contraction of the detrusor muscle is mainly controlled by cholinergic mechanisms [1], sympathetic pathways may also contribute to the regulation of detrusor tone and possibly even more importantly may be involved in the control of resistance of the lower urinary tract [1].

The sympathetic nervous system acts on three classes of receptors (α_1, α_2 and β) with at least three subtypes each which are designated α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2C}, β_1, β_2 and β_3 [3, 10]. At the neuroeffector junction between sympathetic nerves and smooth muscle cells, α_1- and α_2-adrenoceptors generally mediate enhancements of contraction, while β-adrenoceptors mostly mediate smooth muscle relaxation; prejunctional α_2-adrenoceptors may additionally attenuate smooth muscle contraction by inhibiting the release of contraction-causing neurotransmitters. Such observations have also been made in smooth muscle of urogenital tissues [1]. While recent research has accumulated large amounts of information regarding expression of α_1-adrenoceptor subtypes in the human prostate [12, 17, 22], only limited information is available on subtypes of other adrenoceptors in the prostate and on subtypes of all types of adrenoceptors in the bladder.

Advances in physiological and pathophysiological knowledge on micturition will require the use of adequate animal models. An optimal animal model should not only be large enough to allow detailed urodynamic measurements and have sufficient anatomical similarity with humans but should also exhibit a similar profile of receptor subtype expression. Therefore, we have compared expression of subtypes of α_1-, α_2- and β-adrenoceptors in

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porcine and human bladder and prostate in radioligand saturation and competition binding studies.

Material and methods

Tissue sources and preparation

Human bladder detrusor and prostate samples were obtained from patients undergoing cystectomy due to bladder cancer; all specimens were from macroscopically tumor-free areas. Porcine bladder detrusor, bladder neck and prostate were obtained from Goettingen minipigs (20–50 kg) which had been obtained from Ellegard (Aarhus, Denmark). All human and porcine tissue samples were macroscopically freed from surrounding adipose and connective tissues, blotted dry, rapidly frozen in liquid nitrogen and stored at −80°C.

The tissue samples were thawed in ice-cold binding buffer A [50 mM TRIS, 10 mM $MgCl_2$, 0.5 mM ethylenediaminetetraacetate (EDTA), pH 7.5] and minced with scissors. They were homogenized with an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) for 10 s at full speed and thereafter twice for 20 s each at two-thirds speed. The homogenates were centrifuged for 20 min at 50 000 *g* at 4°C. The pellets were resuspended in buffer, rehomogenized shortly (10 s at full speed) and washed by an additional centrifugation step. The final pellets were resuspended and rehomogenized in the appropriate binding buffer (see below).

Radioligand binding

α_1-Adrenoceptors, α_2-adrenoceptors and β-adrenoceptors were identified by [^{3}H]prazosin, [^{3}H]RX 821002 and [^{125}I]iodocyanopindolol (ICYP) binding, respectively, as previously described [6, 15, 16]. Briefly, experiments for α_1- and α_2-adrenoceptors were performed in binding buffer A (see above) and those for β-adrenoceptors in binding buffer B (10 mM TRIS, 150 mM NaCl at pH 7.4) in a total assay volume of 1000 µl (α_1-adrenoceptors) or 250 µl (α_2- and β-adrenoceptors). The protein content typically was 50–100 µg/assay for α-adrenoceptors and 20–40 µg/assay for β-adrenoceptors. The mixtures were incubated for 45 min at 25°C (α_1-adrenoceptors), 60 min at 25°C (α_2-adrenoceptors) or 90 min at 37°C (β-adrenoceptors). Incubations were terminated by rapid vacuum filtration over Whatman GF/C filters followed by two washes of the filters each with 10 ml ice-cold incubation buffer. Non-specific binding was defined as binding in the presence of 10 µM phentolamine (α_1- and α_2-adrenoceptors) or 100 µM isoprenaline (β-adrenoceptors). In saturation experiments six concentrations of radioligand were tested, and in competition experiments a single concentration of radioligand was competed for by 21 narrowly spaced concentrations of subtype-selective drugs.

Data analysis

Saturation binding experiments were analyzed by fitting of rectangular hyperbolic functions to the experimental data. Competition binding experiments were analyzed by fitting of mono- and biphasic sigmoidal functions to the experimental data; a biphasic fit was accepted only if it resulted in a significant improvement of the fit as judged by an *F*-test. All curve fitting was performed by the InPlot computer program (GraphPAD Software, San Diego, CA). Statistical significance of differences between groups was assessed by two-tailed unpaired *t*-tests using the InStat computer program (GraphPAD Software) with a $P < 0.05$ considered as significant.

Chemicals

[^{3}H]Prazosin (specific activity 70–80 Ci/mmol) and [^{125}I]iodocyanopindolol (specific activity 2200 Ci/mmol) were obtained from New England Nuclear (Dreieich, Germany), and [^{3}H]RX 821002 (2-methoxy-idazoxan; specific activity 40–70 Ci/mmol) was from Amersham (Braunschweig, Germany). (-)-Isoprenaline HCl, phentolamine HCl, prazosin HCl and oxymetazoline HCl were obtained from Sigma (Munich, Germany). CGP 20,712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanololmethane-sulfonate) was a kind gift from Ciba-Geigy (Basel, Switzerland), ICI 118,551 (erythro-D,L-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-*ol* HCl) from Zeneca (Planckstadt, Germany), and ARC 239 (2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3(2H,4H)-isochinolindion dihydrochlride) from Dr. Karl Thomae GmbH (Biberach, Germany). All drugs were dissolved at 10 mM in dilute HCl (10 mM), except for prazosin, which was dissolved at 10 mM in dimethylsulfoxide, and diluted further in the respective binding buffers (see above).

Results

α_1-Adrenoceptors

Despite intensive attempts we were unable to identify quantifiable specific [^{3}H]prazosin binding in porcine bladder detrusor, bladder neck or prostate (data not shown). In human bladder detrusor we found a small difference between total and nonspecific binding, but this was too small to allow reliable quantification and/or characterization of bladder α_1-adrenoceptors (data not shown). These negative data are not related to technical problems since we have recently described the characterization of human prostate α_1-adrenoceptors using identical methods [17].

α_2-Adrenoceptors

Specific, saturable, high-affinity binding of [^{3}H]RX 821002 to α_2-adrenoceptors was detected in all porcine

Table 1 α_2-Adrenoceptors in porcine and human urogenital tissues. Data are means ± SEM of the indicated number of experiments (*n*)

	n	K_d (pM)	B_{max} (fmol/mg protein)
Porcine tissues			
Male bladder detrusor	5	506 ± 121	26.1 ± 11.2
Male bladder neck	5	563 ± 135	26.2 ± 9.5
Female bladder detrusor	6	572 ± 115	14.8 ± 1.5
Female bladder neck	6	754 ± 216	24.0 ± 5.5
Prostate	6	823 ± 163	12.3 ± 5.4
Human tissues			
Bladder detrusor	6	509 ± 71	41.6 ± 6.4
Prostate	4	561 ± 49	17.9 ± 1.5

and human tissues investigated. The K_d values in all tissues ranged between 500 and 850 pM (Table 1). Receptor densities were 15–25 fmol/mg protein in porcine bladder and ≈ 40 fmol/mg protein in human bladder. In porcine bladder α_2-adrenoceptor densities were not significantly different between male and female animals or in either sex between bladder neck and detrusor region (Table 1). The α_2-adrenoceptor density in porcine and human prostate was 12 and 18 fmol/mg protein, respectively (Table 1).

To identify the α_2-adrenoceptor subtypes in porcine bladder detrusor, bladder neck and prostate and in human bladder detrusor and prostate, competition binding experiments with appropriate subtype-selective drugs were performed. In these experiments a rank order of potency of yohimbine > oxymetazoline > prazosin ≈ ARC 239 was detected in all tissues (Figs. 1, 2, Table 2). While competition curves were somewhat shallow in some cases, biphasic competition curves did not consistently yield significantly better fits for any of the subtype-selective compounds. Thus, shallow competition curves were most likely related to a small signal/

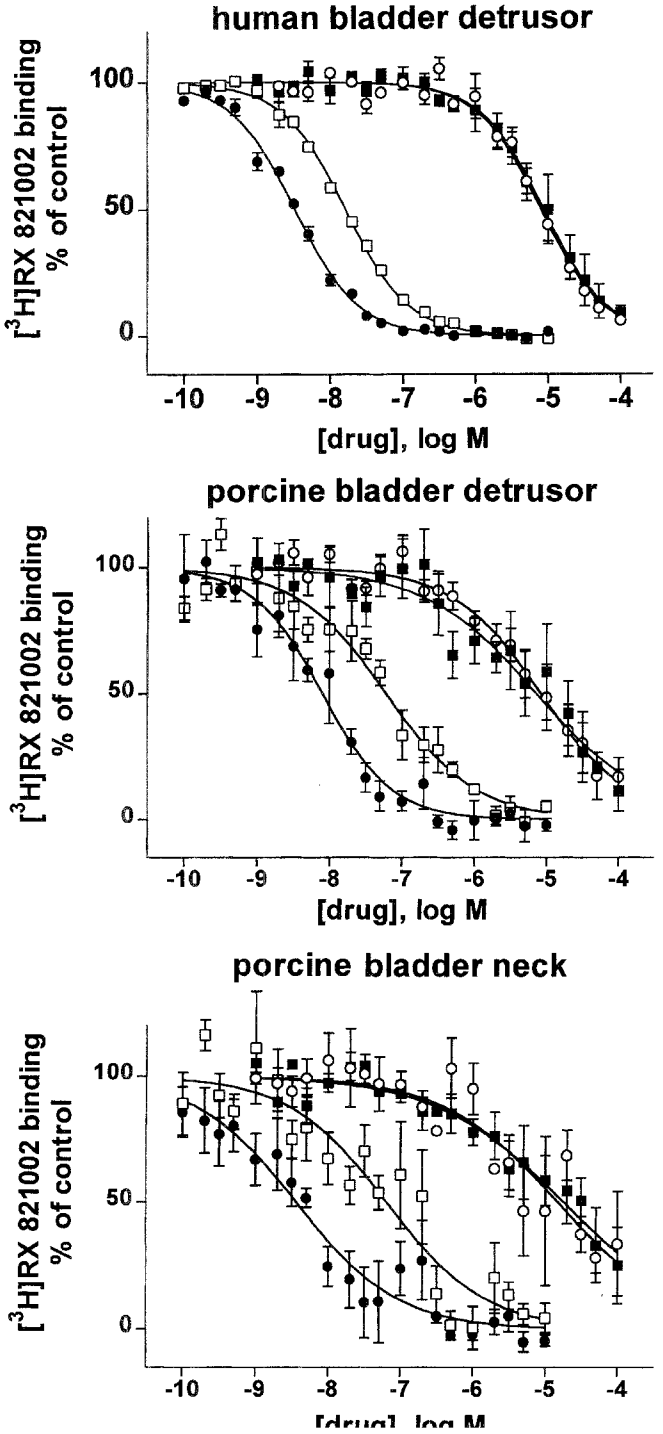

Fig. 1 Competition of yohimbine (*filled circles*), prazosin (*open circles*), ARC 239 (*filled squares*) and oxymetazoline (*open squares*) for [^{3}H]RX 821002 binding to human bladder detrusor (*upper panel*), porcine bladder detrusor (*middle panel*) and bladder neck (*lower panel*). Data are expressed as percentage of binding in the absence of competitor (control) and are means ± SEM of three to five experiments. A numerical analysis of these data is given in Table 2

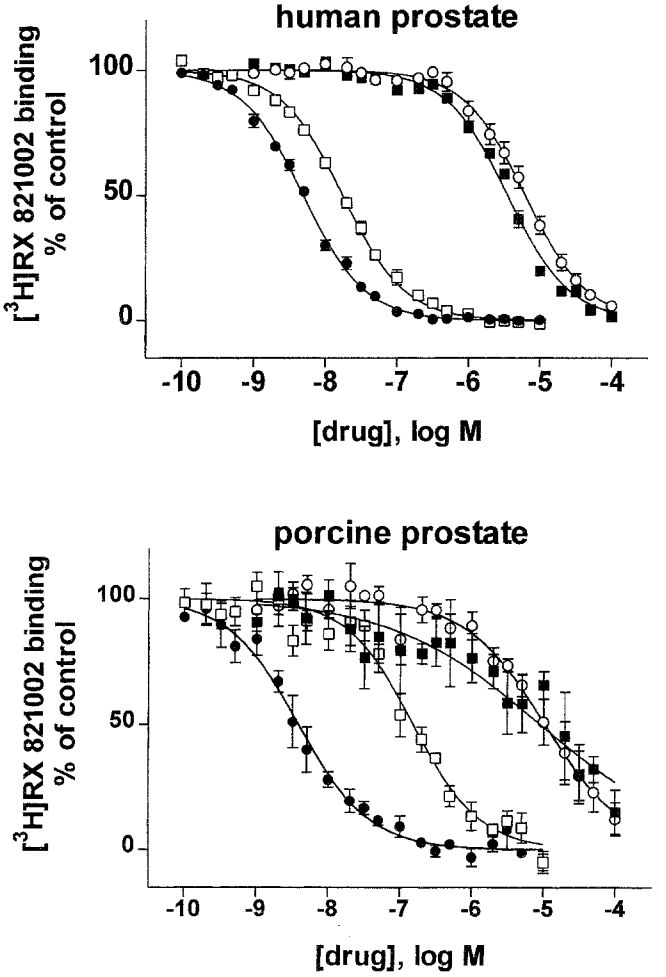

Fig. 2 Competition of yohimbine (*filled circles*), prazosin (*open circles*), ARC 239 (*filled squares*) and oxymetazoline (*open squares*) for [^{3}H]RX 821002 binding to human prostate (*upper panel*) and porcine prostate (*lower panel*). Data are expressed as percentage of binding in the absence of competitor (control) and are means ± SEM of three to five experiments. A numerical analysis of these data is given in Table 2

Table 2 α_2-Adrenoceptor subtypes in porcine and human urogenital tissues. Data are means ± SEM of the indicated number of experiments (*n*). A graphical representation of the data is given in Figs. 1–2

	n	Hill slope	−log K_i
Yohimbine			
Porcine bladder detrusor	5	0.82 ± 0.05	8.66 ± 0.14
Porcine bladder neck	5	0.88 ± 0.10	8.55 ± 0.11
Porcine prostate	5	0.79 ± 0.10	8.52 ± 0.34
Human bladder detrusor	3	1.03 ± 0.02	8.89 ± 0.04
Human prostate	3	0.98 ± 0.02	8.76 ± 0.02
Prazosin			
Porcine bladder detrusor	5	0.88 ± 0.12	5.76 ± 0.16
Porcine bladder neck	5	0.83 ± 0.11	5.70 ± 0.15
Porcine prostate	4	1.02 ± 0.14	5.47 ± 0.14
Human bladder detrusor	4	1.25 ± 0.16	5.77 ± 0.05
Human prostate	3	1.08 ± 0.06	5.57 ± 0.07
ARC 239			
Porcine bladder detrusor	4	0.80 ± 0.16	5.83 ± 0.17
Porcine bladder neck	4	0.79 ± 0.21	5.53 ± 0.28
Porcine prostate	4	0.66 ± 0.19	5.51 ± 0.41
Human bladder detrusor	3	0.99 ± 0.05	5.69 ± 0.10
Human prostate	3	1.21 ± 0.08	5.88 ± 0.02
Oxymetazoline			
Porcine bladder detrusor	4	0.91 ± 0.10	7.88 ± 0.22
Porcine bladder neck	4	0.93 ± 0.24	7.85 ± 0.26
Porcine prostate	4	1.00 ± 0.15	7.30 ± 0.12
Human bladder detrusor	3	0.86 ± 0.04	8.28 ± 0.02
Human prostate	3	0.79 ± 0.09	8.25 ± 0.03

noise ratio. These data indicate the presence of a homogeneous population of α_2-adrenoceptors. The rank order of potency and the absolute affinities of the test drugs are in good agreement with other data on porcine and human α_{2A}-adrenoceptors [24] and indicate the presence of a homogeneous population of this subtype.

β-Adrenoceptors

Specific, saturable, high-affinity ICYP binding was detected in all tissues investigated. The K_d values were between 10 and 60 pM in porcine tissues and between 100 and 200 pM in human bladder and prostate (Table 3). The β-adrenoceptor density was 30–45 fmol/mg protein in porcine bladder; within porcine bladder similar values were found in male and female pigs and within each sex in bladder detrusor and bladder neck. In human bladder 60 fmol/mg protein of β-adrenoceptors was found (Table 3). In porcine and human prostate ≈ 60 and ≈ 280 fmol/mg protein of β-adrenoceptors were detected, respectively (Table 3).

The highly β_1-adrenoceptor-selective antagonist CGP 20,712A competed for ICYP binding to porcine bladder and prostate with consistently shallow curves which were significantly better explained by a two-site model (Table 4, Fig. 3). Nonlinear regression analysis of these curves revealed the presence of 15–20% high-affinity sites (β_1-adrenoceptors) in bladder detrusor and bladder neck, with the remaining 80–85% of sites having low affinity, i.e. being β_2-adrenoceptors. Based on similar experiments, the β_1/β_2-adrenoceptor ratio in porcine prostate was 42:58%. In contrast, the highly β_2-adrenoceptor-selective antagonist ICI 118,551 competed for ICYP binding to porcine tissues with steeper curves which could not be resolved into two phases, and calculated ICI 118,551 affinities were rather low (Table 4, Fig. 3); calculation of β_1/β_2-adrenoceptor ratios was not possible for these experiments.

In human bladder detrusor the β_2-selective ICI 118,551 competed for ICYP binding with consistently shallow and biphasic competition curves, indicating the coexistence of β_1- and β_2-adrenoceptors in a 21:79%

Table 3 β-Adrenoceptors in porcine and human urogenital tissues. Data are means ± SEM of the indicated number of experiments (*n*)

	n	K_d (pM)	B_{max} (fmol/mg protein)
Porcine tissues			
Male bladder detrusor	6	27 ± 10	46.2 ± 6.5
Male bladder neck	6	32 ± 8	35.5 ± 5.1
Female bladder detrusor	6	9 ± 2	30.7 ± 4.7
Female bladder neck	6	18 ± 4	36.1 ± 3.4
Prostate	6	55 ± 20	57.8 ± 16.8
Human tissues			
Bladder detrusor	9	166 ± 68	59.8 ± 8.0
Prostate	8	139 ± 41	280.2 ± 47.4

Table 4 β-Adrenoceptor subtypes in porcine and human urogenital tissues. Data are means ± SEM of the indicated number of experiments (*n*). A graphical representation of the data is given in Figs. 3–4 (* Mean value derived from the only two experiments in which data were significantly better fitted by a two-site model)

	n	$-\log K_{i\ high}$	$-\log K_{i\ low}$	% high-affinity sites
CGP 20,712A competition				
Porcine bladder detrusor	3	9.36 ± 0.17	5.52 ± 0.13	16 ± 9
Porcine bladder neck	3	8.69 ± 0.26	5.22 ± 0.28	18 ± 4
Porcine prostate	3	9.01 ± 0.20	5.03 ± 0.11	42 ± 14
Human bladder detrusor	5	8.68*	5.16 ± 0.14	3 ± 2
Human prostate	9	8.49 ± 0.37	5.21 ± 0.06	22 ± 6
ICI 118,551 competition				
Porcine bladder detrusor	3	–	6.83 ± 0.08	0
Porcine bladder neck	3	–	6.98 ± 0.03	0
Porcine prostate	3	–	6.85 ± 0.14	0
Human bladder detrusor	5	8.46 ± 0.15	5.97 ± 0.34	79 ± 9
Human prostate	9	8.17 ± 0.28	6.23 ± 0.32	56 ± 6

ratio (Fig. 3, Table 4). In contrast only two out of five competition curves for the β_1-selective CGP 20,712A were significantly better fitted by a two-site than a one-site model, with the remaining curves yielding relatively steep competition curves of low affinity (Fig. 3, Table 4). In an analysis of the overall data the β_1/β_2-adrenoceptor ratio was 3:97%, and 7:93% when only the biphasic CGP 20,712A competition experiments were included in the analysis (Table 4).

In human prostate, ICI 118,551 competed for ICYP binding with biphasic curves from which a β_1/β_2-adrenoceptor ratio of 44:56% was calculated (Fig. 4, Table 4). The competition curves for CGP 20,712A were also shallow and biphasic in human prostate, and a β_1/β_2-adrenoceptor ratio of 22:78% was calculated from these experiments (Fig. 4, Table 4).

Discussion

While the sympathetic nervous system plays an important role in control of the lower urinary tract in a variety of species including man, its role in human bladder detrusor function has been much discussed, partly because of the paucity of adrenergic innervation of human bladder detrusor muscle. An increased importance of adrenergic control in some pathophysiological settings has been suggested, e.g. by findings of markedly increased α-adrenoceptor expression in patients with bladder hyperactivity without neurological disorders. A concise review of adrenoceptor expression and function in bladder and lower urinary tract has been presented recently [1]. The present study has characterized the adrenoceptor subtype expression at the protein level in porcine bladder detrusor, bladder neck and prostate and human bladder detrusor and prostate using saturation and competition radioligand binding experiments. These investigations were performed to determine whether pigs are similar to humans with regard to urogenital adrenoceptor expression and may be useful large animal models for this parameter. In the following, results on bladder and prostate will be discussed separately.

Bladder detrusor

In both porcine and human bladder detrusor the rank order of adrenoceptor abundance was $\beta > \alpha_2 \gg \alpha_1$, with the latter not being detectable in quantifiable amounts in either species. A preponderance of β- over α-adrenoceptors in bladder detrusor has also been indicated by several previous studies [1]. Overall the α_2- and β-adrenoceptor densities in the present study, determined as fmol/mg protein, were 50–100% greater in the human than in the porcine bladder, which may relate to the paucity of adrenergic innervation of the human bladder (see above). While human bladder samples had been obtained from patients of either sex, our pig data show that no major sex differences exist with regard to α_2- or β-adrenoceptor expression in the bladder.

Our competition binding experiments with appropriate subtype-selective drugs [3] demonstrated that the α_2-adrenoceptors in porcine and human detrusor represented a homogeneous population of α_{2A}-adrenoceptors. Competition binding experiments on β-adrenoceptors using subtype-selective drugs [3] demonstrated that β_1- and β_2-adrenoceptors coexist in detrusor muscle of both species, but the β_2-subtype represents at least 80% of the total β-adrenoceptors. On a quantitative level this analysis was hampered by two problems. Firstly, our data indicate that ICI 118,551 does not discriminate β_1- and β_2-adrenoceptors in pigs although it does so in all other species investigated [3]. Similarly a lack of good differentiation between β_1- and β_2-adrenoceptors by ICI 118,551 in pigs has also been seen in previous studies with vascular endothelium and smooth muscle [8, 18]. Secondly, in both human tissues the percentage of β_1-adrenoceptors as detected by the β_1-selective CGP 20,712A was greater than that detected by the β_2-selective ICI 118,551. Thus, the abundance of β_2-adrenoceptors in the porcine tissues based on the CGP 20,712A competition curves (≈85%) may even be underestimated and the true abundance of β_2-adrenoceptors in the human tissues may lie somewhere between the values seen with ICI 118,551 and those with CGP 20,712A. Despite these technical problems, which also apply to porcine bladder neck and porcine and

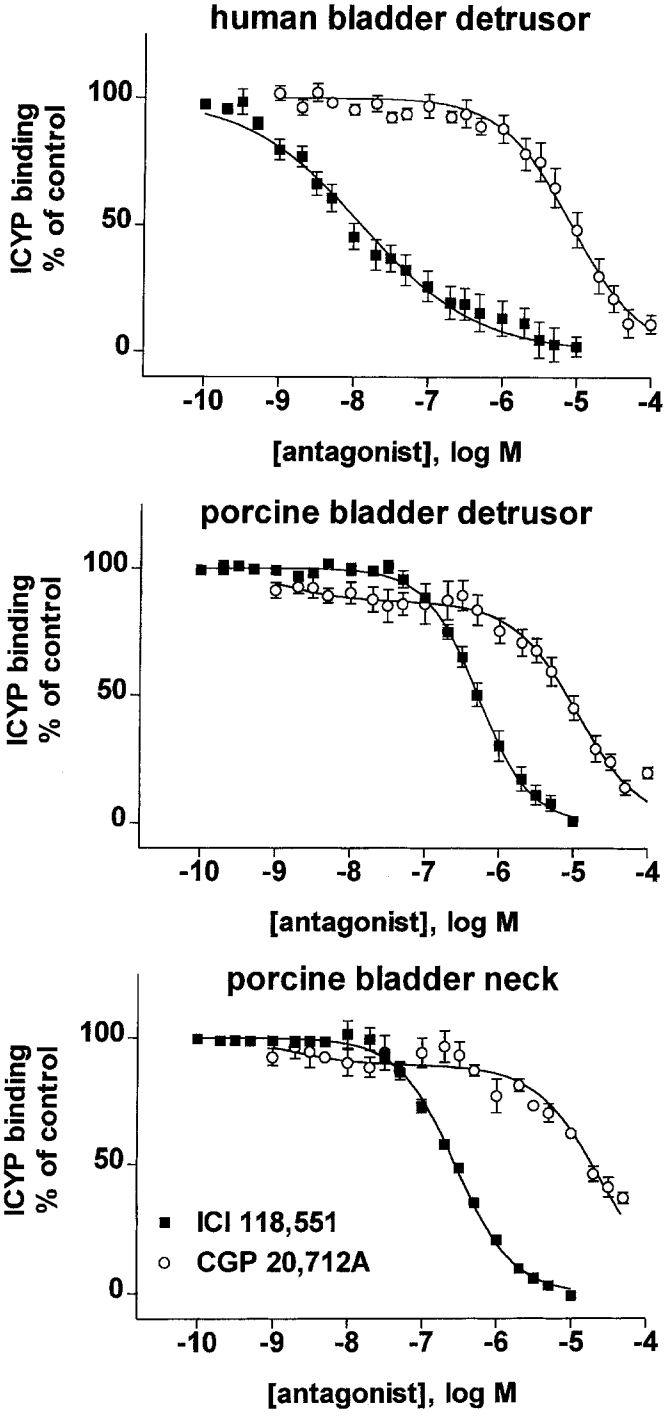

Fig. 3 Competition of ICI 118,551 (*filled squares*) and CGP 20,712A (*open circles*) for [^{125}I]ICYP binding to human bladder detrusor (*upper panel*), porcine bladder detrusor (*middle panel*), and bladder neck (*lower panel*). Data are expressed as percentage of binding in the absence of competitor (control) and are means ± SEM of three and five experiments in pigs and humans, respectively. A numerical analysis of these data is given in Table 4

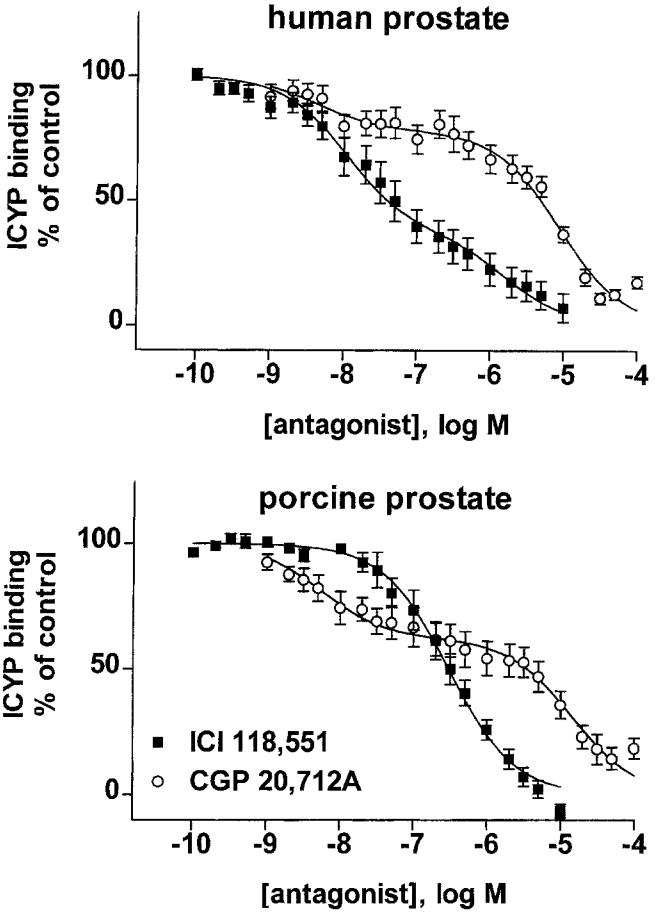

Fig. 4 Competition of ICI 118,551 (*filled squares*) and CGP 20,712A (*open circles*) for [^{125}I]ICYP binding to human prostate (*upper panel*) and porcine prostate (*lower panel*). Data are expressed as percentage of binding in the absence of competitor (*control*) and are means ± SEM of three and nine experiments in pigs and humans, respectively. A numerical analysis of these data is given in Table 4

human prostate, our data clearly indicate that the β_2-adrenoceptor is the dominating β-adrenoceptor subtype in porcine and human bladder detrusor. This conclusion is in line with data from many species showing a dominance of β_2-adrenoceptors in bladder detrusor, with the only exception being the guinea pig where β_1-adrenoceptors dominate [1]. Specifically a dominance of the β_2-subtype has also been demonstrated by others in human bladder detrusor [13].

On a functional level isolated bladder detrusor strips have shown little contraction in response to α-adrenoceptor stimulation; while this may be related to minimal receptor densities for the α_1-adrenoceptors, postjunctional α_2-adrenoceptors do not appear to couple well to detrusor smooth muscle contraction [1]. However, prejunctional α_2-adrenoceptors may be important in inhibiting neurotransmitter release, and activation of these receptors has been shown to cause concentration-dependent inhibition of rat detrusor contraction [14]. β-Adrenoceptors, which are more abundant in bladder detrusor than α-adrenoceptors, may contribute to urine storage function by inhibiting the reflex activation of the detrusor during bladder filling [1]. In summary our data confirm that human bladder detrusor contains mostly β-adrenoceptors of the β_2-subtype. They extend previous findings by demonstrating that the α_2-adrenoceptors belong to the α_{2A}-subtype, and that porcine detrusor has

a very similar profile of adrenoceptor expression to human detrusor although at a somewhat lower level. The functional role of the postjunctional α_2-adrenoceptors in human bladder remains to be identified.

Bladder neck

Our experiments in porcine bladder neck have yielded results which were quite similar to those from porcine bladder detrusor. Thus, the overall rank order of adrenoceptor abundance was $\beta > \alpha_2 \gg \alpha_1$, with the latter not being detectable in quantifiable amounts, and no major sex differences. The α_2-adrenoceptors represented a homogeneous population of the α_{2A}-subtype while the β-adrenoceptors presented a mixed population of the β_1- and β_2-subtype. Human bladder neck tissue unfortunately was not available for our study. In contrast to the situation in pigs described here, previous studies have detected α_1-adrenoceptors in bladder neck of rabbits [1] and humans [13]. In the latter study it was even reported that α_1-adrenoceptors dominate over α_2-adrenoceptors in human bladder base by a 4:1 margin. It should also be noted that in rabbit bladder neck the α_1/α_2-adrenoceptor ratio may be sex dependent [1], while no major sex differences were seen in α_2-adrenoceptor density in porcine bladder neck in the present study. Data from several species including man indicate that α-adrenoceptors in general and α_1-adrenoceptors in particular are important for control of contraction of the bladder neck sphincter [1]. Thus, even in the absence of human bladder neck data in the present study, it can be concluded that pigs are not a suitable model species for studies of bladder neck function with regard to adrenoceptor expression and function.

Prostate

In prostatic membranes we have detected considerable differences between pigs and humans with regard to adrenoceptor expression. Thus, in porcine prostate 58 and 12 fmol/mg protein of β- and α_2-adrenoceptors, respectively, but no α_1-adrenoceptors were detected. In contrast our present and previous data generated with identical methods [17] have found 280, 18 and 28 fmol/mg protein of β-, α_2- and α_1-adrenoceptors, respectively, in human prostate. α_1-Adrenoceptor subtypes in the human prostate have been the focus of much recent debate, but will not be discussed in this context since no α_1-adrenoceptors were found in porcine prostate and since our data on human prostate α_1-adrenoceptors have been presented recently [17].

Our competition binding data demonstrate that the α_2-adrenoceptors of porcine and human prostate belong predominantly if not exclusively to the α_{2A}-subtype. Messenger RNA for α_{2A}-adrenoceptors has been detected in human prostate in RNAse protection assays [19] and by reverse transcriptase polymerase chain reaction [5]; the latter study has also detected mRNA for the α_{2B}- and the α_{2C}-adrenoceptor in human prostate. The discrepancy between mRNA detection for α_{2B}- and α_{2C}-adrenoceptors and lack of detection of corresponding protein may be related to the extremely high sensitivity of the polymerase chain reaction and/or to the fact that mRNA abundance does not always correctly predict presence of the corresponding protein. Our competition binding data on β-adrenoceptors have found a dominance of the β_2-subtype in pigs and humans, but the contribution of β_1-adrenoceptors was substantial and certainly greater than in bladder tissue. Messenger RNA for the β_3-adrenoceptor has been found in human prostate in RNAse protection assays [2], while we have not found evidence for β_3-adrenoceptors at the protein level in porcine or human prostate in our study. This could be related to the above differences between mRNA and protein detection in general but also to the fact that our assay conditions were not in favor of β_3-adrenoceptor detection. Since ICYP has only moderate affinity for this subtype [21], high radioligand concentrations are required for detection. Due to marked increases in nonspecific binding at high ligand concentrations, these could not be used in the present study.

From a functional point of view little is known about the role of α_2-adrenoceptors in the prostate. Contraction of human prostate is mediated exclusively by α_1-adrenoceptors [9], and autoradiographic studies have shown that human prostatic α_2-adrenoceptors do not reside in stroma; rather they are found on vascular smooth muscle and glandular cells [4, 11]. In contrast human prostatic β-adrenoceptors at least partially exist on stroma cells since they can mediate prostatic smooth muscle relaxation [23]. Taken together, human and porcine prostate differ considerably in adrenoceptor subtype expression on a quantitative and qualitative level. Therefore, from the adrenoceptor point of view, pigs do not appear to be a useful species to serve as a model system for studies of human prostatic function.

In conclusion we have demonstrated that human and porcine bladder and prostate express α_{2A}-, β_1- and β_2-adrenoceptors; human prostate additionally expresses α_1-adrenoceptors. While pigs have a similar adrenoceptor subtype expression profile to humans in bladder detrusor, marked differences can be found for bladder neck and prostate. This may be related to the enormously different anatomy of the closure mechanism of the two species, particularly in males. Thus, with regard to adrenoceptors pigs do not appear very suitable for investigations of bladder neck or prostate function. However, they may serve as a model system for studies of bladder detrusor dysfunction, e.g. in neurogenic bladder or myelomeningocele patients. Such studies are currently in progress in our laboratories.

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