British Journal of Pharmacology (1997) 122, 1009–1014

Quantification and distribution of α_1-adrenoceptor subtype mRNAs in human vas deferens: comparison with those of epididymal and pelvic portions

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1 This study was intended to quantify the amounts of the α_1-adrenoceptor subtype mRNAs in human vas deferens, and demonstrate the receptor subtype responsible for the vas contraction.

2 The RNase protection assay showed that the mean total amount of α_{1a} mRNA was 7.4 ± 2.2 pg/5 μg of poly $(A)^+$ RNA (97.0% of the total α_1 mRNA) in the epididymal portion (E-vas) and 4.9 ± 0.8 pg/5 μg of poly $(A)^+$ RNA (96.3% of the total) in the pelvic portion (P-vas). The E-vas showed a tendency to have a greater α_{1a} mRNA abundance than the P-vas ($P = 0.11$). The α_{1b} and α_{1d} mRNAs were absent or of extremely low abundance.

3 By an *in situ* hybridization, the α_{1a} and α_{1d} mRNAs were recognized in the smooth muscle cells of the E-vas and the P-vas, and the distribution pattern the same in both tissues. The α_{1b} mRNA positive site was scarcely detectable in both vas portions.

4 In a functional study, *l*-phenylephrine produced concentration-dependent contraction in the E-vas ($E_{max} = 2.24 \pm 0.70$ g; $pD_2 = 5.32 \pm 0.09$) and the P-vas ($E_{max} = 2.46 \pm 0.46$ g; $pD_2 = 5.07 \pm 0.12$). KMD-3213, a novel α_{1A}-adrenoceptor-selective antagonist, caused parallel rightward shifts of the concentration–response curves for *l*-phenylephrine. Apparent pK_B values were 9.90 ± 0.16 for the E-vas and 9.71 ± 0.17 for the P-vas. There was no significant difference in E_{max}, pD_2 or pK_B estimates between the two portions.

5 We have found that α_{1a} mRNA is predominant in the human vas deferens, and confirmed that contraction of this organ is mediated by the α_{1A}-adrenoceptor.

Keywords: α_1-Adrenoceptor subtype mRNA; human vas deferens; RNase protection assay; *in situ* hybridization

Introduction

Three α_1-adrenoceptor subtypes (α_{1a}, α_{1b} and α_{1d}) have been cloned and their sequence determined (Bruno *et al*., 1991; Hirasawa *et al*., 1993; Schwinn *et al*., 1995). In 1994, as separate nomenclatures have been used for the pharmacologically identified and cloned receptors, a new nomenclature was proposed (Ford *et al*., 1994) and recommended by the International Union of Pharmacology (Hieble *et al*., 1995). This nomenclature is followed in our paper.

Using antisense RNA probes prepared from the cloned genes, various investigators have detected mRNAs of these subtypes in many organs and tissues (Price *et al*., 1993; 1994; Weinberg *et al*., 1994; Moriyama *et al*., 1996; Nasu *et al*., 1996). The vas deferens has a muscular coat (tunica muscularis) that is made up of a middle circular layer surrounded by inner and outer longitudinal muscle layers (Neaves, 1975). The thickness of the muscle layers gradually decreases along the length of the ductus deferens (Paniagua *et al*., 1982). Adrenergic nerves are observed in all three layers of the tunica muscularis, but their density is the greatest in the outer longitudinal layer (McConnell *et al*., 1982), and noradrenaline appears to be the main mediator of contractile responses via adrenoceptors (Holmquist *et al*., 1990).

Many mechanisms contribute to the transport of spermatozoa in the ductus deferens (Guha *et al*., 1975; Neaves, 1975). Among these, muscle peristaltic contraction induced by adrenergic neurotransmitters is important in propelling the ductal contents (Bruschini *et al*., 1997; Lipschultz *et al*., 1981). A defect in the contractility of the vas deferens is thought to account for some cases of infertility. Recently, Furukawa *et al*. (1995) reported that the contractile response to *l*-phenylephrine is mediated by the α_{1A}-adrenoceptor subtype in human vas deferens, and pointed out that this tissue is readily available for functional studies of this receptor.

The vas deferens may be divided into the following five portions: (1) the epididymal portion within the tunica vaginalis, (2) the scrotal portion, (3) the inguinal division, (4) the retroperioneal or pelvic portion, and (5) the ampulla (Lich *et al*., 1978). The regional variation in purinergic and adrenergic responses has been examined in the vas deferens of various experimental animals, and noradrenaline was found to contribute more to contraction in the epididymal portion (E-vas) than in the pelvic portion (P-vas) (Sneddon & Machaly, 1992). However, there is no such report for human vas deferens.

The purpose of this study was to quantify the amounts of the α_1-adrenoceptor subtype mRNAs and to estimate the ratio of these mRNAs in the two main parts of the human vas deferens (E-vas and P-vas), and demonstrate the subtype responsible for the vas contraction. This information is relevant to the design of pharmacological stimulators of the contraction of vas deferens.

Methods

Specimens

Thirty-nine cases of men with prostatic cancer or bladder cancer showing no invasion to vas deferens (mean age ±s.d.;

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67.5±9.6 years) were selected for this study with full informed consent. They underwent radical prostatectomy and/or castration for prostatic cancer, and total cystectomy for invasive bladder cancer. They had received no treatment with any α_1-adrenoceptor antagonist for at least 1 week before the operation. Tissue samples (about 3 cm segments) of the E-vas or the P-vas were obtained from the specimens removed during operation, and processed for the following procedures after the corresponding specimens has been shown to be tumour-free. Samples for the RNase protection assay were quickly frozen in liquid nitrogen and stored at −130°C. Samples for *in situ* hybridization were embedded in the O.C.T. compound, quickly frozen in liquid nitrogen and stored at −80°C until experiment. For functional studies, about 1 cm length of strips of vas deferens were kept in Krebs solution (described below) at 4°C and used within 24 h for this study.

RNase protection assay

Preparation of RNA Total RNA was extracted from the E-vas and the P-vas by the method of Chomczymski & Sacchi (1987) with slight modifications. Frozen tissue samples were homogenised in liquid nitrogen with a mortar and pestle and then extracted with guanidine isothiocyanate buffer (25 mM sodium acetate, pH 4.0, containing 4 M guanidine isothiocyanate, 0.5% sodium *N*-lauroyl sarcosinate, and 0.1 M 2-mercaptoethanol). Four to nine RNA samples of the E-vas (mean age ±s.d.: group 1, 76.1±5.7; group 2, 69.6±6.4; group 3, 56.4±5.0) or the P-vas (mean age ±s.d.: group 1, 73.4±6.4; group 2, 68.9±6.6; group 3, 53.4±4.4) were mixed together into three groups with age matching, because each tissue sample has only a low RNA content (Table 1). Each RNA sample was treated batchwise with an oligo (dT) resin (oligotex dT30 super™, Takara Co. Ltd, Otsu, Japan) and the bound poly $(A)^+$ RNA was eluted and stored at −80°C until required for RNase protection assays. RNA purity was estimated from the ratio of its absorbance at 260 nm to its absorbance at 280 nm.

Preparation of RNA probes and sense RNA Each of the three α_1-adrenoceptor subtype cDNA clones was isolated from a human prostate cDNA library as previously described (Hirasawa *et al*., 1993). An α_{1a} fragment (275 bp; nucleotides 1443–1717), an α_{1b} fragment (230 bp; nucleotides 2086–2315), and an α_{1d} fragment (260 bp; nucleotides 1321–1580) were inserted into pBluescript vector (Stratagene Cloning Systems, La Jolla, CA, USA). Antisense RNA probes were transcribed with T7 RNA polymerase from DNA templates in the presence of [α-^{32}P]UTP, and sense RNA to be used as standard for the quantification of mRNA was transcribed with T3 RNA polymerase from DNA templates in the absence of [α-^{32}P]UTP. The specificity of these probes was confirmed by Northern blot hybridization of total RNA extracted from the transfected Chinese hamster ovary (CHO) cell lines, as described elsewhere (Nasu *et al*., 1996).

Procedure of RNase protection assay Poly $(A)^+$ (5 μg) was dissolved in hybridization buffer (40 mM PIPES, pH 6.4, containing 80% (v/v) deionised formamide, 0.4 M NaCl, 2 mM EDTA) and added to each of the three radiolabelled antisense RNA probes (1×10^6 d.p.m.) described above. The hybridization mixture was incubated at 42°C for 14 h, then treated with RNase A (4 μg ml^{-1}) and RNase T1 (70 U ml^{-1}) at 37°C for 30 min. The samples were subsequently treated with proteinase K (100 μg ml^{-1}) at 37°C for 15 min and electrophoresed on a 5% polyacrylamide denaturing gel containing 8 M urea. The imaging plate was exposed to the dried gel and the bands quantified with an imaging analyser (BAS2000, Fuji Film Co., Tokyo, Japan). Standard solutions of *in vitro* transcribed sense RNA, prepared by serially diluting a stock solution whose concentration had been spectrophotometrically determined, were simultaneously subjected to the RNase protection assay, and a separate standard curve was generated for each probe (Nasu *et al*., 1996). The amount of mRNA corresponding to each of the three α_1 subtypes expressed in the vas deferens tissues was determined from these standard curves.

In situ *hybridization*

Preparation of RNA probes and sense RNA The C-terminal of each of the three α_1-adrenoceptor subtype cDNAs, an α_{1a} fragment (623 bp; nucleotides 1443–2065), an α_{1b} fragment (595 bp; nucleotides 2086–2680) and an α_{1d} fragment (611 bp; nucleotides 1321–1931), were selected as a probe, and inserted into a pST19 vector (Boehringer Mannheim GmbH, Mannheim, Germany). Antisense RNA probes were transcribed with T7 RNA polymerase, and sense RNA probes were transcribed with SP6 RNA polymerase from DNA templates in the presence of UTP labelled with digoxigenin.

Procedure of in situ *hybridization* Frozen tissues were cut at a thickness of 10 μm, fixed in 4% paraformaldehyde and rinsed in 2×SSC (15 mM sodium citrate, pH 7.0, containing 0.15 M NaCl). The sections were then treated with 50 μg ml^{-1} of proteinase K, acetylated with 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine, containing 0.15 M NaCl and prehybridized for 2 h at 45°C in hybridization buffer (10 mM Tris-HCl, pH 7.4, containing 0.6 M NaCl, 1 mM EDTA, 16% (v/v) formamide, Denhardt's solution, 0.125 mg ml^{-1} salmon sperm DNA, 0.25 mg ml^{-1} yeast tRNA and 10% (w/v) dextran thionate). Sections were then hybridized with digoxigenin-labelled probes for 16 h at 45°C. They were rinsed in 2×SSC to remove the excess probes, and treated with 20 μg ml^{-1} of RNase A. They were then incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (×5000) at room temperature for 30 min, and the probes bounded were visualized in the substrate solution (mixture of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M Tris-HCl, pH 9.5, containing 0.1 M NaCl and 50 mM $MgCl_2$) at room temperature for 4 h. Finally, the sections were counterstained with nuclear fast red, air dried and mounted in xylene-based mounting media.

Table 1 Distribution of α_1-adrenoceptor subtype mRNAs in human epididymal and pelvic vas deferens

Type of vas *Group (no. of cases)*	*Total α_1 mRNA*[a] (pg)	*Subtype mRNA*[a] (pg, %) α_{1a}	α_{1b}	α_{1d}
Epididymal				
1 (8)	9.3	9.1 (98%)	ND[b]	0.2 (2%)
2 (9)	5.2	4.9 (94%)	ND[b]	0.3 (6%)
3 (5)	8.2	8.1 (99%)	ND[b]	0.1 (1%)
Pelvic				
1 (4)	6.0	5.8 (97%)	ND[b]	0.1 (3%)
2 (8)	4.6	4.4 (96%)	ND[b]	0.3 (4%)
3 (5)	4.8	4.6 (96%)	ND[b]	0.2 (4%)

Poly $(A)^+$ RNA (5 μg) from human vas deferens was used for the RNase protection assay. Mean age of each group was described in Methods. [a] The values given for the mRNA represent the weight (pg) of the single-stranded protected fragment only. [b] ND: not detected.

Functional study

Drugs (−)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl]amino]propyl]indoline-7-carboxamide (KMD-3213), a newly synthesized α_1-adrenoceptor antagonist was provided by Kissei Pharmaceutical Co. Ltd (Matsumoto, Japan). Corticosterone (Sigma, St. Louis, MO, USA) was dissolved in dimethylsulphoxide and diluted with physiological saline to appropriate concentrations. Other drugs were dissolved in physiological saline and adjusted to appropriate concentrations. KMD-3213 was dissolved in dimethylsulphoxide, and was diluted to appropriate concentrations with Hartmann's solution of the following composition (w/v%): NaCl, 0.60; KCl, 0.03; $CaCl_2$, 0.02; sodium lactate, 0.31. The other antagonists were prepared with physiological saline (0.9% NaCl solution) to appropriate concentrations.

Procedure of functional study The connective tissue was carefully removed from the vas segments. Each segment (about 1 cm lengths) was suspended longitudinally under 1 g of resting tension in an organ bath maintained at 37°C containing 10 ml of Krebs solution of the following composition: NaCl, 111 mM; KCl, 5.9 mM; $CaCl_2$, 2.5 mM; $MgCl_2$, 1.2 mM; NaH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; glucose, 11.5 mM. Each bath was continuously bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2. After 1 h of equilibration, the tension developed following the addition of *l*-phenylephrine was measured by means of isometric force transducers. Non-cumulative concentration–response curves for *l*-phenylephrine were constructed by adding threefold incremental concentrations of *l*-phenylephrine, with washing for 10–20 min between each addition. After the initial concentration–response curve had been obtained, the tissues were washed several times before being incubated with 1 nM of KMD-3213 for 30 min. Then, a further non-cumulative concentration–response curve for *l*-phenylephrine in the presence of the same concentration of KMD-3213. Only two concentration–response curves were constructed for on each tissue. In all experiments, the Krebs solution also contained 1 μM propranolol to block β-adrenoceptors, and 0.1 μM yohimbine to block α_2-adrenoceptors, as well as 0.1 μM of desipramine and 10 μM corticosterone to inhibit intra- and extraneuronal uptake, respectively.

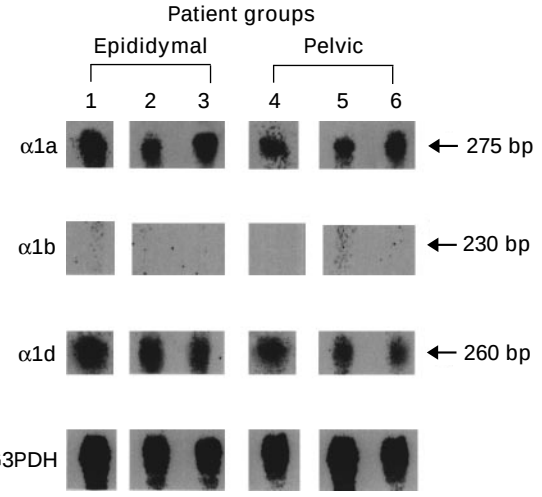

Figure 1 RNase protection assay of α_1-adrenoceptor subtype mRNAs in human epididymal and pelvic vas deferens (E-vas and P-vas). Poly $(A)^+$ RNAs (5 μg each) from the E-vas (lanes 1–3) and the P-vas (lanes 4–6) was hybridized with radiolabelled antisense RNA probes specific for α_{1a}, α_{1b}, α_{1d} or glyceraldehyde-3-phosphate dehydrogenase (G3PHD). After RNase digestion, protected fragments were separated on a 5% polyacrylamide gel containing 8 M urea. All gels were exposed to an imaging plate overnight. Lane 1 and 4, group 1; lane 2 and 5, group 2; lane 3 and 6, group 3. Mean age of each group was described in Methods.

The concentration ratios between EC_{50} values (50% effective concentration) of control curves and those of KMD-3213-treated curves were calculated. The negative logarithm of the dissociation constant (pK_B) of each antagonist was determined from the following equation:

$$pK_B = \log(\text{concentration ratio} - 1) - \log(\text{antagonist [M]}).$$

Results

RNase protection assay

RNA samples from both the E-vas and the P-vas were analysed to determine the amounts of the α_1-adrenoceptor subtype mRNAs (Figure 1). Radiolabelled sense RNA probes for each subtype detected virtually none of the other mRNA species expressed in human vas deferens (data not shown). The mean total α_1 mRNA abundance was 7.6 ± 2.1 pg/5 μg of poly $(A)^+$ RNA in the E-vas and 5.1 ± 0.8 pg/5 μg of poly $(A)^+$ RNA in the P-vas, revealing no significant difference in the total α_1 mRNA abundance between the two portions ($P = 0.07$). The mean total amount of α_{1a} mRNA was 7.4 ± 2.2 pg/5 μg of poly $(A)^+$ RNA in the E-vas and 4.9 ± 0.8 pg/5 μg of poly $(A)^+$ RNA in the P-vas, indicating no significant difference between the α_{1a} mRNA abundance in these portions ($P = 0.11$). Nonetheless, there was a tendency for the E-vas to have a greater α_{1a} mRNA abundance than the P-vas. The mean α_{1d} mRNA level was 0.2 ± 0.1 pg/5 μg of poly $(A)^+$ RNA in the E-vas and 0.2 ± 0.0 pg/5 μg of poly $(A)^+$ RNA in the P-vas, whereas α_{1b} mRNA was not detected in any of the tissue samples. There was no significant difference in the expression amount of the three α_1 subtype mRNAs among three groups of RNA mixture with age matching. Thus, the α_{1a} mRNA represented $97.0 \pm 2.6\%$ of the total α_1 mRNA in the E-vas and $96.3 \pm 0.3\%$ in the P-vas, and the α_{1d} mRNA accounted for $3.0 \pm 2.6\%$ of the total α_1 mRNA in the E-vas and $3.6 \pm 0.6\%$ in the P-vas (Table 1).

In situ *hybridization*

Compared with the definite staining with antisense probes, hybridization with sense probes yielded no detectable staining. The α_{1a} mRNA was definitely recognized in the smooth muscle layer of both the E-vas and the P-vas, in which the positive sites were primarily the smooth muscle cells. The α_{1d} mRNA was also recognized in the muscle layer but the intensity of staining was low. In contrast, α_{1b} mRNA positive site was scarcely detectable in the muscle layer of the E-vas and the P-vas. The distribution of α_{1a} and α_{1d} mRNA was diffuse, and no difference in the expression pattern was observed between them (Figure 2).

Functional study

Figure 3 shows the concentration–response curves for *l*-phenylephrine in the absence and in the presence of 1 nM of KMD-3213 in the E-vas and the P-vas. *l*-Phenylephrine produced concentration-dependent contraction on both the E-vas and the P-vas, with pD_2 values of 5.32 ± 0.09 and 5.07 ± 0.12, respectively. The maximum contractile response (E_{max}) values were 2.24 ± 0.70 g in the E-vas and 2.46 ± 0.46 g in the P-vas (Table 2). Those values were not significantly different between both portions. KMD-3213 at a concentration of 1 nM caused parallel rightward shifts of the concentration–response curves to *l*-phenylephrine in both portions. Apparent pK_B values for KMD-3213 were 9.90 ± 0.16 in the E-vas and 9.71 ± 0.17 in the P-vas. There was no significant difference between the respective pD_2 values, the respective E_{max} values or the respective pK_B values for the two portions of the vas deferens (Table 2). However, KMD-3213 (1 nM) reduced the E_{max} values to *l*-

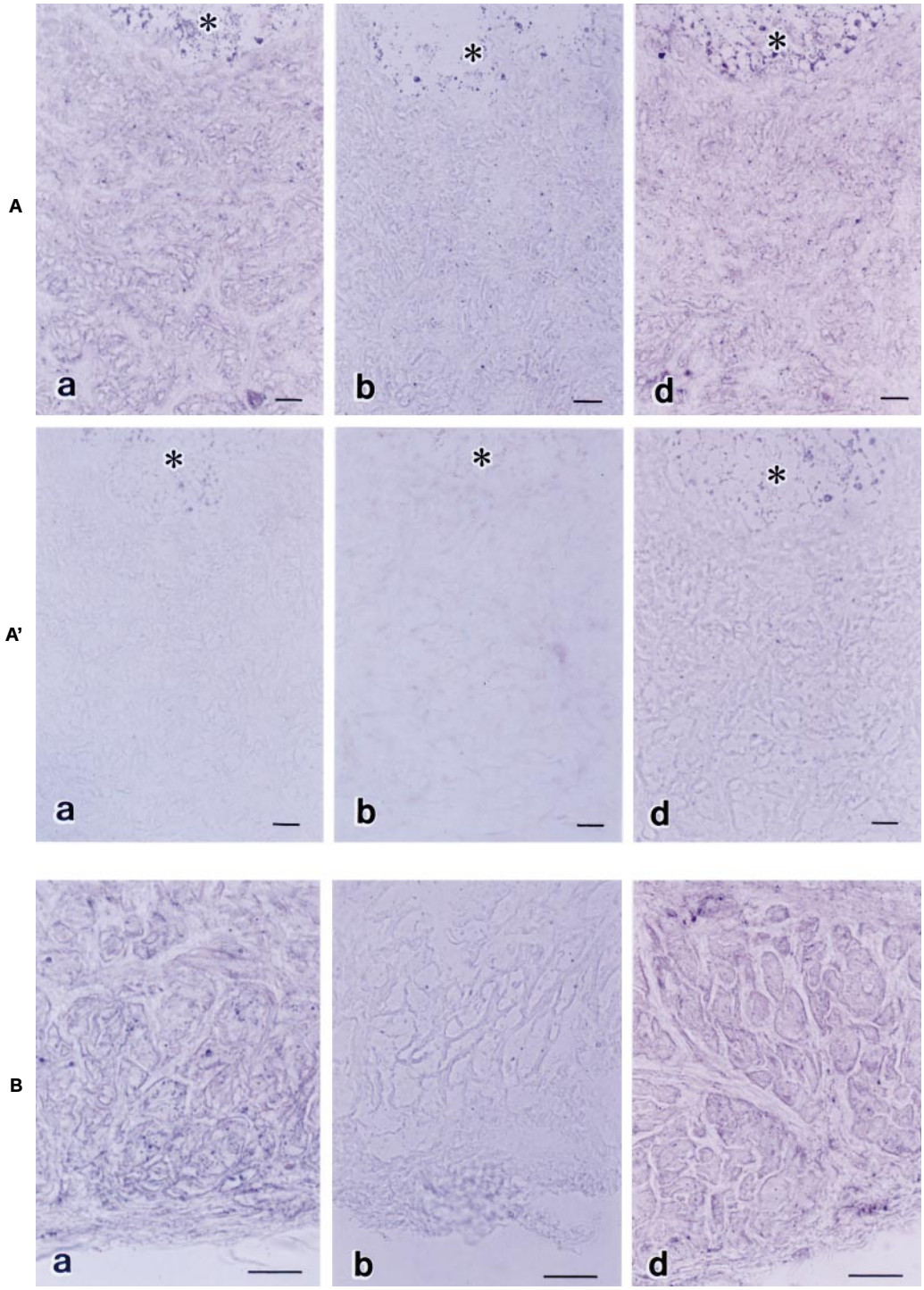

Figure 2 Microscopic views of *in situ* hybridization signals of α_1-adrenoceptor subtype mRNAs in human epididymal and pelvic vas deferens (E-vas and P-vas). The signals of α_{1a} and α_{1d} mRNA were recognized in the whole smooth muscle layers. However, those of the α_{1b} mRNA were scarcely detectable in either portion of the vas deferens. The hybridization with sense probes showed no positive staining. A and A', Surface area of the E-vas; B, adventitial area of the P-vas; A and B, antisense probe; A', sense probe; a, α_{1a} probe; b, α_{1b} probe; d, α_{1d} probe. Asterisks (*) indicate the epithelial area of the vas deferens. Bar = 50 μm.

phenylephrine by 16.4% only in the E-vas ($P<0.05$, paired t test) (Figure 3).

Discussion

The existence of three α_1-adrenoceptor subtypes has recently been demonstrated by use of molecular biological techniques, and the expression of these subtype mRNAs has previously been investigated in various human tissues (Price *et al*., 1994; Weinbert *et al*., 1994).

Functional studies of human prostate samples obtained from prostatectomy have concluded that the predominant functional receptor in this tissue has the pharmacological characteristics of the native α_{1A}-adrenoceptor, which appears to be identical to the cloned α_{1a}-adrenoceptor (Lepor *et al*., 1993; Chapple *et al*., 1994). RNase protection assay also showed that the cloned α_{1a}-adrenoceptor is the predominant receptor in the human prostate (Price *et al*., 1993; Nasu *et al*., 1996).

Investigations of the regional distribution of functional α_1-adrenoceptors in the rat vas deferens, using pharmacological

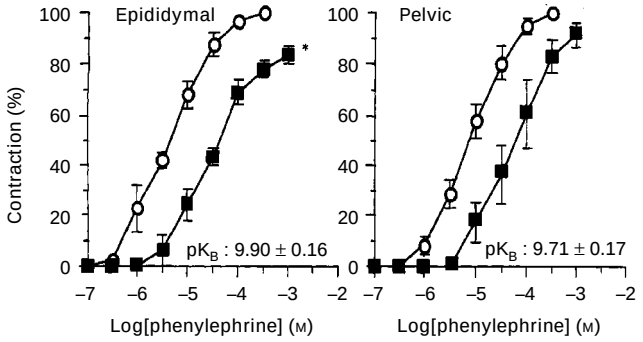

Figure 3 Concentration–response curves of human epididymal and pelvic vas deferens (E-vas and P-vas) for *l*-phenylephrine in the absence (○) and in the presence (■) of 1 nM KMD-3213. Responses were plotted as a percentage of maximum contractile response of the control response. Asterisk (*) indicates the maximum contractile responses to *l*-phenylephrine were significantly smaller than the control responses at $P<0.05$ (paired *t* test). Apparent pK_B values for KMD-3213 was 9.90±0.16 in the E-vas and 9.71±0.17 in the P-vas.

Table 2 E_{max} and pD_2 values for *l*-phenylephrine in human isolated epididymal and pelvic vas deferens

Type	*n*	E_{max} (g)		$pD_2 \pm s.e.$	
Epididymal	5	2.24±0.70	NS[a]	5.32±0.09	NS[a]
Pelvic	6	2.46±0.49		5.07±0.12	

[a]NS: statistically not significant, epididymal vas vs pelvic vas (unpaired Student's *t* test).

and molecular pharmacological techniques, revealed that the α_{1A}-adrenoceptor subtype is predominant in the E-vas (Faure *et al*., 1994; Rokosh *et al*., 1994). Regional variation in purinergic and adrenergic responses of the vas deferens was examined in various experimental animals, and noradrenaline was reported to be more responsible for contraction in the E-vas than in the P-vas (Sneddon & Machaly, 1992). Furthermore, Furukawa *et al*. (1995) recently reported that the contractile responses of human vas deferens (E-vas) to *l*-phenylephrine were mediated through the α_{1A}-adrenoceptor subtype.

However, the expression and distribution of α_1-adrenoceptor subtype mRNAs have not been confirmed yet in human vas deferens. Additionally, regional distribution of these receptor has not been studied. The aim of the present study was to characterise the α_1 subtype responsible for mediating contractile responses of the human vas deferens as well as the subtype variation in its two main portions (E-vas and P-vas). The three α_1-adrenoceptor subtypes mRNAs (α_{1a}, α_{1b} and α_{1d}) were quantified with RNase protection assay, and the distribution of each subtype was confirmed by *in situ* hybridization. Functional studies were also performed using KMD-3213, a novel α_{1A}-adrenoceptor-selective antagonist (Shibata *et al*., 1995).

We have quantified each α_1-adrenoceptor subtype mRNA expressed in the E-vas and the P-vas by using an RNase protection assay. The predominant subtype mRNA was the α_{1a} subtype in both portions of the vas deferens, where it accounted for almost 100% of the total α_1 mRNA, and the α_{1b} and the α_{1d} subtypes were absent or extremely low. These findings are supported by our *in situ* hybridization study (Figure 2). Furthermore, our functional study (Figure 3), as well as the previous work (Furukawa *et al*., 1995), also indicate the view that the α_{1A}-adrenoceptor mediates the contraction of the human vas deferens. This low abundance of α_{1d} mRNA is in contrast to its relatively high levels in human prostate (Moriyama *et al*., 1996; Nasu *et al*., 1996), which showed about 15% being α_{1d} in benign prostatic hypertrophy (BPH) and 30% being α_{1d} in non-BPH. We also found no regional variation in ratios of α_1-adrenoceptor subtype mRNAs in human vas deferens. This lack of regional variation, and the extremely low abundance of α_{1b} and α_{1d} may be characteristic of human vas deferens. In the contractile experiments, however, KMD-3213 (1 nM), an α_{1A}-adrenoceptor-selective antagonist, reduced the E_{max} values to *l*-phenylephrine by 16.4% only in the E-vas. This finding suggests that the insurmountability of KMD-3213 in the E-vas may be slightly higher than that in the P-vas.

Through *in situ* hybridization, we revealed that the α_{1a} and the α_{1d} mRNAs were primarily localised in all of the muscle layers in both E-vas and P-vas, in which there was no clear difference in localisation of either subtype. The scarce α_{1b}-adrenoceptor mRNA could be demonstrated. The staining intensities of α_{1a} and α_{1d} mRNA were not proportional to the mRNA levels determined by RNase protection assay. However, *in situ* hybridization is primarily a qualitative technique that demonstrates the location of the mRNA but not its exact amount. Thus, the findings on *in situ* hybridization are consistent with the results of the RNase protein assay and functional study.

In the experiments with human recombinant adrenoceptors, KMD-3213 showed a Ki value of 0.036 nM for the α_{1A}-adrenoceptor, but has 580- and 56-fold lower affinity for α_{1B} and α_{1D}-adrenoceptors. KMD-3213 also has a potent affinity for the native α_{1A}-adrenoceptor with a pK_B value of 10.0 in rabbit prostate and a pK_i value of 9.82 in the rat submaxillary gland (Shibata *et al*., 1995; Yamagishi *et al*., 1996), and has a lower affinity for the α_{1D}-adrenoceptor with a pA_2 value of 8.13 (Yamagishi *et al*., 1996). In the present study, the pK_B values for KMD-3213 were 9.90 in the E-vas and 9.71 in the P-vas, values that show good agreement with the pK_B value and pK_i value in the recombinant or native α_{1A}-adrenoceptor. They are also comparable to the value of tamsulosin ($pK_B = 10.0$) for the human vas deferens (Furukawa *et al*., 1995). Furakawa *et al*. (1995) concluded that the α_{1B}-adrenoceptor is not the predominant subtype mediating responses of human vas deferens, because *l*-phenylephrine produced dose-dependent contractions, the treatment with CEC failed to alter responses significantly, and WB4101, 5-methylurapidil and oxymetazoline had relatively high affinities (Furukawa *et al*., 1995). These data suggest that the α_{1A}-adrenoceptor mediates the contractile response in the human vas deferens. In the vas deferens of various experimental animals, α_1-adrenoceptor agonists induced rapid and transient contraction of the P-vas, while they induced slower and sustained contraction of the E-vas (Sneddon & Machaly, 1992). In human vas deferens, pK_B estimates for KMD-3213 were not significantly different between the E-vas and the P-vas, but there was a tendency for the E-vas to have a greater total α_1, namely, α_{1a} mRNA abundance than the P-vas. This result may be related to the observation that the thickness of the entire muscular layer gradually decreases along the length of the vas deferens (Paniagua *et al*., 1982).

In conclusion, we have found molecular biologically that α_{1a} mRNA is predominant in at least two major portions (E-vas and P-vas) of the human vas deferens. We have also confirmed that contraction of the human vas deferens is pharmacologically mediated by the α_{1A}-adrenoceptor.

This investigation was supported in part by the Human Science Foundation and a Grant-in-Aid for Scientific Research (B), No 08457421, from the Ministry of Education, Science, and Culture, Japan.

References

BRUNO, J.F., WHITTAKER, J., SONG, J. & BERELOWITZ, M. (1991). Molecular cloning and sequencing of a cDNA encoding a human α_{1A} adrenergic receptor. *Biochem. Biophys. Res. Commun.*, **179,** 1485 – 1490.

BRUSCHINI, H., SCHMIDT, R.A. & TANAGHO, E.A. (1977). Studies on the neurophysiology of the vas deferens. *Invest Urol.*, **15,** 112 – 116.

CHAPPLE, C.R., BURT, R.P., ANDERSSON, P.O., GREENGRASS, P., WYLLIE, M. & MARSHALL, I. (1994). Alpha$_1$-adrenoceptor subtypes in the human prostate. *Br. J. Urol.*, **74,** 585 – 589.

CHOMCZYMSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162,** 156 – 159.

FAURE, C., PIMOULE, C., ARBILLA, S., LANGER, S.Z. & GRAHAM, D. (1994). Expression of α_1-adrenoceptor subtypes in rat tissues: implications for α_1-adrenoceptor classification. *Eur. J. Pharmacol.*, **268,** 141 – 149.

FORD, A.P.D.W., WILLIAMS, T.J., BLUE, D.R. & CLARKE, D.E. (1994). α_1-Adrenoceptor classification: sharpening Occam's razor. *TIPS*, **15,** 167 – 170.

FURUKAWA, K., ROSARIO, D.J., SMITH, D.J., CHAPPLE, C.R., UCHIYAMA, T. & CHESS WILLIAMS, R. (1995). $\alpha 1_A$-Adrenoceptor-mediated contractile responses of the human vas deferens. *Br. J. Pharmacol.*, **116,** 1605 – 1610.

GUHA, S.K., KAUR, H. & AHMED, A.M. (1975). Mechanics of spermatic fluid transport in the vas deferens. *Med. Biol. Eng.*, **13,** 518 – 522.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P. & RUFFOLO, R.R.J. (1995). International Union of Pharmacology. *X*. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. *Pharmacol. Rev.*, **47,** 267 – 270.

HIRASAWA, A., HORIE, K., TANAKA, T., TAKAGAKI, K., MURAI, M., YANO, J. & TSUJIMOTO, G. (1993). Cloning, functional expression and tissue distribution of human cDNA for the α_{1C}-adrenergic receptor. *Biochem. Biophys. Res. Commun.*, **195,** 902 – 909.

HOLMQUIST, F., HEDLUND, H. & ANDERSSON, K.-E. (1990). Effects of the α_1-adrenoceptor antagonist R-(−)YM12617 on isolated human penile erectile tissue and vas deferens. *Eur. J. Pharmacol.*, **186,** 87 – 93.

LEPOR, H., TANG, R. & SHAPIRO, E. (1993). The alpha-adrenoceptor subtype mediating the tension of human prostatic smooth muscle. *Prostate*, **22,** 301 – 307.

LICH, R.J., HOWERTON, L.W. & AMIN, M. (1978). *Anatomy and Surgical Approach to the Urogenital Tract in the Male*. Philadelphia W.B. Saunders.

LIPSHULTZ, L.I. MCCONNELL, J. & BENSON, G.S. (1981). Current concept of the mechanisms of ejaculation. Normal and abnormal states. *J. Reprod. Med.*, **26,** 499 – 507.

MCCONNELL, J., BENSON, G.S. & WOOD, J.G. (1982). Autonomic innervation of the urogenital system: adrenergic and cholinergic elements. *Brain Res. Bull.*, **9,** 679 – 694.

MORIYAMA, N., KURIOMOTO, S., HORIE, S., NASU, K., TANAKA, T., YANO, J., HIRANO, H., TSUJIMOTO, G. & KAWABE, K. (1996). Detection of α_1-adrenoceptor subtypes in human hypertrophied prostate by *in situ* hybridization. *Histochem. J.*, **28,** 283 – 288.

NASU, K., MORIYAMA, N., KAWABE, K., TSUJIMOTO, G., MURAI, M., TANAKA, T. & YANO, J. (1996). Quantification and distribution of α1-adrenoceptor subtype mRNAs in human prostate: comparison of benign hypertrophied tissue and non-hypertrophied tissue. *Br. J. Pharmacol.*, **119,** 797 – 803.

NEAVES, W.B. (1975). Biological aspects of vasectomy. In *Handbook of Physiology, Section 7, Endocrinology*, ed. Greep, R.O. & Astwood, E.B. p. 383. Baltimore: Williams & Wilkins.

PANIAGUA, R., REGADERA, J., NISTAL, M. & ABAURREA, M.A. (1982). Histological, histochemical and ultrastructural variations along the length of the human vas deferens before and after puberty. *Acta Anat. Basel.*, **111,** 190 – 203.

PRICE, D.T., LEFKOWITZ, R.J., CARON, M.G., BERKOWITZ, D. & SCHWINN, D.A. (1994). Localization of mRNA for three distinct alpha 1-adrenergic receptor subtypes in human tissues: implications for human alpha-adrenergic physiology. *Mol. Pharmacol.*, **45,** 171 – 175.

PRICE, D.T., SCHWINN, D.A., LOMASNEY, J.W., ALLEN, L.F., CARON, M.G. & LEFKOWITZ, P.J. (1993). Identification, quantification, and localization of mRNA for three distinct alpha$_1$ adrenergic receptor subtypes in human prostate. *J. Urol.*, **150,** 546 – 551.

ROKOSH, D.G., BAILEY, B.A., STEWART, F.R., KARNS, L.R., LONG, C.S. & SIMPSON, P.C. (1994). Distribution of α_{1C}-adrenergic receptor mRNA in adult rat tissues by RNase protection assay and comparison with α_{1B} and α_{1D}. *Biochem. Biophys. Res. Commun.*, **200,** 1177 – 1184.

SCHWINN, D.A., JOHNSTON, G.I., PAGE, S.O., MOSLEY, M.J., WILSON, K.H., WORMAN, N.P., CAMPBELL, S., FIDOCK, M.D., FURNESS, L.M., PARRY-SMITH, D.J., PETER, B. & BAILEY, D.S. (1995). Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. *J. Pharmacol. Exp. Ther.*, **272,** 134 – 142.

SHIBATA, K., FOGLAR, R., HORIE, K., OBIKA, K., SAKAMOTO, A., OGAWA, S. & TSUJIMOTO, G. (1995). KMD-3213, a novel, potent, α_{1a}-adrenoceptor-selective antagonist: characterization using recombinant human α_1-adrenoceptors and native tissues. *Mol. Pharmacol.*, **48,** 250 – 258.

SNEDDON, P. & MACHALY, M. (1992). Regional variation in purinergic and adrenergic responses in isolated vas deferens of rat, rabbit and guinea-pig. *J. Auton. Pharmacol.*, **12,** 421 – 428.

WEINBERG, D.H., TRIVEDI, P., TAN, C.P., MITRA, S., PERKINS-BARROW, A., BORKOWSKI, D., STRADER, C.D. & BAYNE, M. (1994). Cloning, expression and characterization of human alpha adrenergic receptors, α_{1A}, α_{1B} and α_{1C}. *Biochem. Biophys. Res. Commun.*, **201,** 1296 – 1304.

YAMAGISHI, R., AKIYAMA, K., NAKAMURA, S., HORA, M., MASUDA, N., MATSUZAWA, A., MURATA, S., UJIIE, A., KURASHINA, Y., IIZUKA, K. & KITAZAWA, M. (1996). Effect of KMD-3213, an α_{1a}-adrenoceptor-selective antagonist, on the contraction of rabbit prostate and rabbit and rat aorta. *Eur. J. Pharmacol.*, **315,** 73 – 79.

(Received May 2, 1997
Accepted August 13, 1997)