British Journal of Pharmacology (1996) 119, 797-803

Quantification and distribution of α1-adrenoceptor subtype mRNAs in human prostate: comparison of benign hypertrophied tissue and non-hypertrophied tissue

[1]Kimio Nasu, *Nobuo Moriyama, *Kazuki Kawabe, †Gozoh Tsujimoto, Masatoshi Murai, Teruo Tanaka & Junichi Yano

Molecular Biology Department, Research Laboratories, Nippon Shinyaku Co., Ltd., Kyoto, Japan, *Department of Urology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan and †Division of Pediatric Pharmacology, National Children's Medical Research Center, Tokyo, Japan

1 There are at least three α1-adrenoceptor subtypes, α1a, α1b and α1d, in human tissues. Using an RNase protection assay, we have now determined the amount of each subtype mRNA in human prostatic tissue, for both benign prostatic hypertrophy (BPH) and non-BPH. In all tissue samples examined, the predominant subtype mRNA was α1a. The total abundance of α1-adrenoceptor mRNA in BPH samples was over six times that in non-BPH samples. This increase was mostly accounted for by α1a, which was almost nine times as abundant in BPH samples as in non-BPH samples. The abundance of α1b was almost the same between BPH and non-BPH samples, and the abundance of α1d in BPH samples was about three times that in non-BPH samples. The ratio of the numbers of the subtype mRNAs, α1a:α1b:α1d, was 85:1:14 in BPH samples and 63:6:31 in non-BPH samples.

2 *In situ* hybridization studies showed no significant differences in the tissue localization of α1-adrenoceptor subtype mRNAs between BPH and non-BPH samples. α1a and α1d were clearly detected in the interstitium of the prostate, where α1a was stained more intensely than α1d, and the positive sites were primarily smooth muscle cells. In contrast, α1b staining was very faint.

3 This increase in mRNA abundance may be directly related to the contraction of prostatic tissue that leads to obstruction of the urinary tract in BPH patients. Specifically, our data suggest that increased expression of the α1a subtype may be primarily responsible for the contraction of the prostate.

Keywords: α1-Adrenoceptor subtype mRNA; prostate; benign prostatic hypertrophy (BPH); RNase protection assay; *in situ* hybridization

Introduction

α1-Adrenoceptor subtypes are encoded by a multigene family, and three subtypes, α1a, α1b, and α1d, which are structurally and pharmacologically distinct, have been identified by molecular cloning techniques (Bylund *et al.*, 1994). α1b has been cloned and sequenced from hamster (Cotecchia *et al.*, 1988), rat (Voigt *et al.*, 1990), and human sources (Ramarao *et al.*, 1992). α1a was cloned and sequenced from human prostate, and it was tentatively designated α1C (Hirasawa *et al.*, 1993). In addition, α1d was cloned and sequenced from rat (Minneman *et al.*, 1988) and human sources (Bruno *et al.*, 1991), and it was tentatively designated α1A/D. However there was a discrepancy between the pharmacological properties of the cloned receptors and those of the pharmacologically defined receptors. To resolve the discrepancy between the pharmacological and the molecular terminology, Ford *et al.* (1994) proposed a unified nomenclature, and their proposal has been recommended by the International Union of Pharmacology (Hieble *et al.*, 1995). Thus, α1A/D, α1B and α1C are now respectively renamed α1d, α1b and α1a, and this nomenclature is adopted in the present paper.

Benign prostatic hypertrophy (BPH) is a common disease in elderly men and it leads to obstruction of the urinary tract, often by a contraction of hypertrophied prostatic tissue. This contraction is caused in part by an adrenergic response mediated by α1-adrenoceptors. Traditionally, BPH patients have been treated by transurethral resection of the prostate or by open prostatectomy. However, several α1-adrenoceptor antagonists have been found to be useful for treating difficulty in urination, and are now in clinical use (Abrams *et al.*, 1982; Kawabe *et al.*, 1990; Lepor *et al.*, 1992). Yet different antagonists have different effects on BPH, suggesting the existence of subtypes of the α1-adrenoceptor. In addition, some of these antagonists also have side effects, such as a lowering of blood pressure. Several groups have used both functional and radioligand binding assays (Michel *et al.*, 1994; Foglar *et al.*, 1995) and *in situ* hybridization (Price *et al.*, 1993b; Moriyama *et al.*, 1996) to investigate α1-adrenoceptor subtypes in the human prostate, and the results indicate that α1a is the major subtype. These findings led to the development of prostate-selective drugs that cause few side effects (Kawabe *et al.*, 1994). However, because of the difficulty of obtaining tissue samples, few studies have been performed on human prostate, and even fewer on non-BPH prostate.

In the present study, we used BPH specimens and urinary cancerous prostatic specimens (non-BPH) obtained during open surgery, and we report the quantification and comparison of α1-adrenoceptor subtype mRNAs in both BPH and non-BPH tissues by RNase protection assay and *in situ* hybridization.

Methods

Human prostatic tissues

Twelve men with BPH (mean age ± s.d., 68.7 ± 7.6 years) and four men with bladder cancer but no BPH (mean age ± s.d.,

[1]Author for correspondence at: Molecular Biology Department, Research Laboratories, Nippon Shinyaku Co., Ltd., Nishiohji Hachijo, Minami-ku, Kyoto 601, Japan

61.3 ± 9.5 years) were selected for this study, with full informed consent. Specimens of BPH tissue were obtained by suprapubic or retropubic prostatectomy and specimens of non-BPH tissue were obtained from total cystoprostatectomy for invasive bladder tumour. The non-BPH tissue we used conformed to the following criteria: (1) no evidence of prostatic enlargement, (2) no prostatic carcinoma, BPH nodules or invasion of the bladder cancer, and (3) no symptomatic prostatism. After tissue had been taken for pathological examination, samples for the RNase protection assay were quickly frozen in liquid nitrogen and stored at −130°C. For *in situ* hybridization, tissue samples were embedded in the O.C.T. compound, quickly frozen in liquid nitrogen, and stored at −80°C.

Preparation of RNA

Total RNA was extracted from three strains of transfected Chinese hamster ovary (CHO) cell line, each of which expresses one of the human α1-adrenoceptor subtypes (α1a, α1b or α1d), and from human prostatic tissues (BPH or non BPH) by the method of Chomczymski & Sacchi (1987). Poly(A)+ RNA was purified on an oligo (dT) resin (Oligotex-dT30 Super; Takara, Kyoto, Japan). Each RNA sample was quantified by its absorbance at 260 nm and stored at −80°C until it was used for RNase protection assay.

Preparation of RNA probes

Each of the three α1-adrenoceptor subtype cDNA clones was isolated from a human prostate cDNA library as previously described (Hirasawa *et al.*, 1993). Fragments corresponding to the C-terminal region of each α1-adrenoceptor subtype were selected as probes for the RNase protection assay and inserted into a pBluescript transcription vector (Stratagene, La Jolla, CA, U.S.A.). We adjusted the α1a fragment to 275 bp (nucleotides 1443–1717), the α1b fragment to 230 bp (nucleotides 2086–2315), and the α1d fragment to 260 bp (nucleotides 1321–1580) in length. Antisense RNA probes were transcribed with T7 RNA polymerase, and sense RNA probes with T3 RNA polymerase, from linearized DNA templates in the presence of [α-^{32}P]-UTP. Each probe was purified by electrophoresis on a 5% polyacrylamide gel containing 8 M urea. For *in situ* hybridization, an α1a fragment (623 bp, nucleotides 1443–2065), an α1b fragment (595 bp, nucleotides 2086–2680), and an α1d fragment (611 bp, nucleotides 1321–1931) were inserted into a pST19 transcription vector (Boehringer Mannheim GmbH, Mannheim, Germany). Antisense RNA probes were transcribed with T7 RNA polymerase, and sense RNA probes with SP6 RNA polymerase, from linearized DNA templates in the presence of digoxigenin-UTP.

RNase protection assay and standard curves

RNase protection assays were performed as described by Lee & Costlow (1987) with slight modifications. RNA from CHO cells or human prostatic tissues was hybridized with each of the radiolabelled RNA probes (1×10^6 d.p.m.) described above. After RNase treatment, protected RNA fragments were electrophoresed on a 5% polyacrylamide gel containing 8 M urea, which was then dried down. An imaging plate was exposed to the dried gel and scanned with an imaging analyzer (BAS2000; Fuji film, Tokyo, Japan). To quantify the amount of mRNA expressed, sense RNA corresponding to each antisense RNA probe was transcribed from DNA templates in the absence of [α-^{32}P]-UTP and purified by electrophoresis on a 5% polyacrylamide gel containing 8 M urea. The concentration of the purified RNA fragments was determined spectrophotometrically and standard solutions of each sense RNA species were prepared by serial dilution of a stock solution. The sample RNA and the sense RNA (1, 10, 100, or 1000 pg) were simultaneously subjected to the RNase protection assay and a separate standard curve was generated for each probe.

In situ *hybridization*

Frozen prostatic tissues were cut into slices 10 μm thick, fixed on slides with 4% paraformaldehyde, and rinsed in 2 × SSC (15 mM sodium citrate, pH 7.0, containing 150 mM NaCl). The specimens were treated with 50 μg ml^{-1} of proteinase K, acetylated with 0.25% (v/v) acetic anhydride solution in 0.1 M triethanolamine containing 150 mM NaCl, then preincubated at 50°C for 2 h in hybridization buffer (10 mM Tris-HCl, pH 7.4, containing 0.6 M NaCl, 1 mM EDTA, 16% (v/v) formamide, 1 × Denhardt's solution (Denhardt, 1966), 0.125 mg ml^{-1} salmon sperm DNA, 0.25 mg ml^{-1} yeast tRNA and 10% (w/v) dextran thionate). The specimens were then treated with digoxigenin-labelled probes for 16 h at 50°C in hybridization buffer. The slides were rinsed with 2 × SSC, and after removal of excess probe, they were treated with 20 μg ml^{-1} of RNase A. They were incubated with alkaline phosphatase conjugated anti-digoxigenin antibody, then treated with the substrate solution (100 mM Tris-HCl, pH 9.5, containing 50 mM $MgCl_2$, 100 mM NaCl, 20 mg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate, and 37.5 mg ml^{-1} nitroblue tetrazolium) for 4 h to visualize the bound probes. The stained slides were examined under a light microscope and photographed.

Results

Identification of α1-adrenoceptor subtype mRNA by RNase protection assay

The subtype selectivity of the α1a, α1b and α1d RNA probes was verified by Northern blot hybridization of total RNA extracted from the transfected CHO cell lines described above (data not shown). Each of the antisense RNA probes speci-

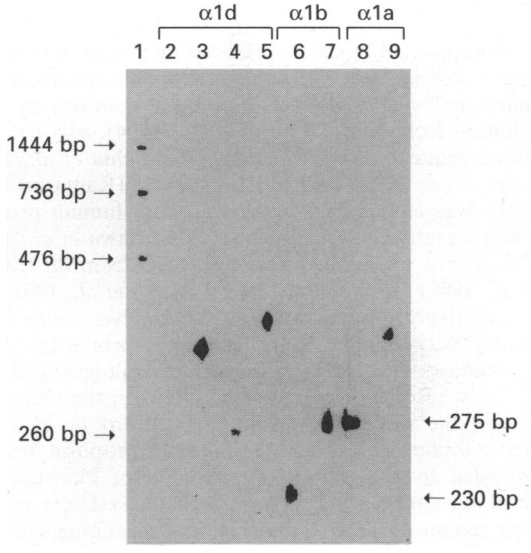

Figure 1 RNase protection assay of α1-adrenoceptor subtype mRNAs from transfected Chinese hamster ovary (CHO) cell strains. Total RNA (10 μg) from each CHO cell strain, each of which expresses one of the human α1-adrenoceptor subtypes (α1a, α1b or α1d), was hybridized with each of the three radiolabelled antisense RNA probes specific for α1a, α1b, or α1d, or the sense RNA probe specific for α1d. After RNase digestion, protected fragments were separated on a 5% polyacrylamide gel containing 8 M urea. Lane 1, size marker; lane 2, CHO cell strain α1d + α1d sense probe; lane 3, α1d sense probe; lane 4, CHO cell strain α1d + α1d antisense probe; lane 5, α1d antisense probe; lane 6, CHO cell strain α1b + α1b antisense probe; lane 7, α1b antisense probe; lane 8, CHO cell strain α1a + α1a antisense probe; lane 9, α1a antisense probe.

fically hybridized to mRNA from the CHO cell lines expressing the corresponding receptor, and no cross-hybridization was detected. In addition, none of the sense RNA probes recognized any mRNA when the membrane was exposed to an imaging plate for more than one day. Using each of the antisense RNA probes, RNase protection assays were performed on total RNA from these CHO cells. A single band was detected at the predicted position for each protected mRNA species (Figure 1, lanes 4, 6 and 8). As expected, the undigested probes, which contain part of the transcription vector, were larger than the corresponding digested fragments (Figure 1, lanes 5, 7 and 9). There was almost no background in any of the lanes, and the detection of mRNA by the antisense RNA probes was subtype specific. The α1d sense RNA probe did not hybridize to any mRNA in the total RNA of CHO cells expressing the α1d receptor (Figure 1, lane 2). Similarly, neither the α1a nor the α1b sense probes recognized any mRNA in the corresponding CHO cell lines (data not shown).

Standard curves

Sense RNAs corresponding to the α1-adrenoceptor subtype mRNAs were synthesized by *in vitro* transcription with T3 RNA polymerase in the absence of [α-^{32}P]-UTP and purified as described in Methods. The electrophoresis patterns of the RNase protection assays with various concentrations of the sense RNAs indicated that the intensity of the bands increased in a dose-dependent manner, and the band position in the gel corresponded to the predicted size of the antisense RNA probe (Figure 2a). Standard curves were constructed from RNase protection assays in which a constant amount of the antisense RNA probe was hybridized with various amounts of the corresponding sense RNA (a typical set of standard curves is shown in Figure 2b). The amount of mRNA expressed in human prostatic tissues was estimated from these standard curves.

Quantification of α1-adrenoceptor subtype mRNAs in prostatic tissue (BPH and non-BPH)

RNA from BPH and non-BPH tissue samples was analysed by the RNase protection assay to determine the amount of each α1-adrenoceptor subtype mRNA. Radiolabelled sense RNA probes for each subtype did not detect any mRNA expressed in human prostatic tissues (data not shown). Each antisense RNA probe revealed a specific protected fragment of the predicted size (Figures 3 and 4). From standard curves constructed as described above, the amounts of α1-adrenoceptor mRNA detected in the human prostate samples per 10 μg of poly(A)+ RNA were calculated (Table 1, BPH samples; Table 2, non-BPH samples).

α1a mRNA was the predominant receptor subtype mRNA in all tissue samples, both BPH and non-BPH (the expression of mRNA for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) did not vary among the samples; see Figures 3 and 4). However, total α1-adrenoceptor mRNA was more than six times as abundant in BPH tissue samples as in non-BPH samples; and most of this increase was accounted for by α1a mRNA, which was almost nine times as abundant in BPH samples as in non-BPH samples. The abundance of α1d in

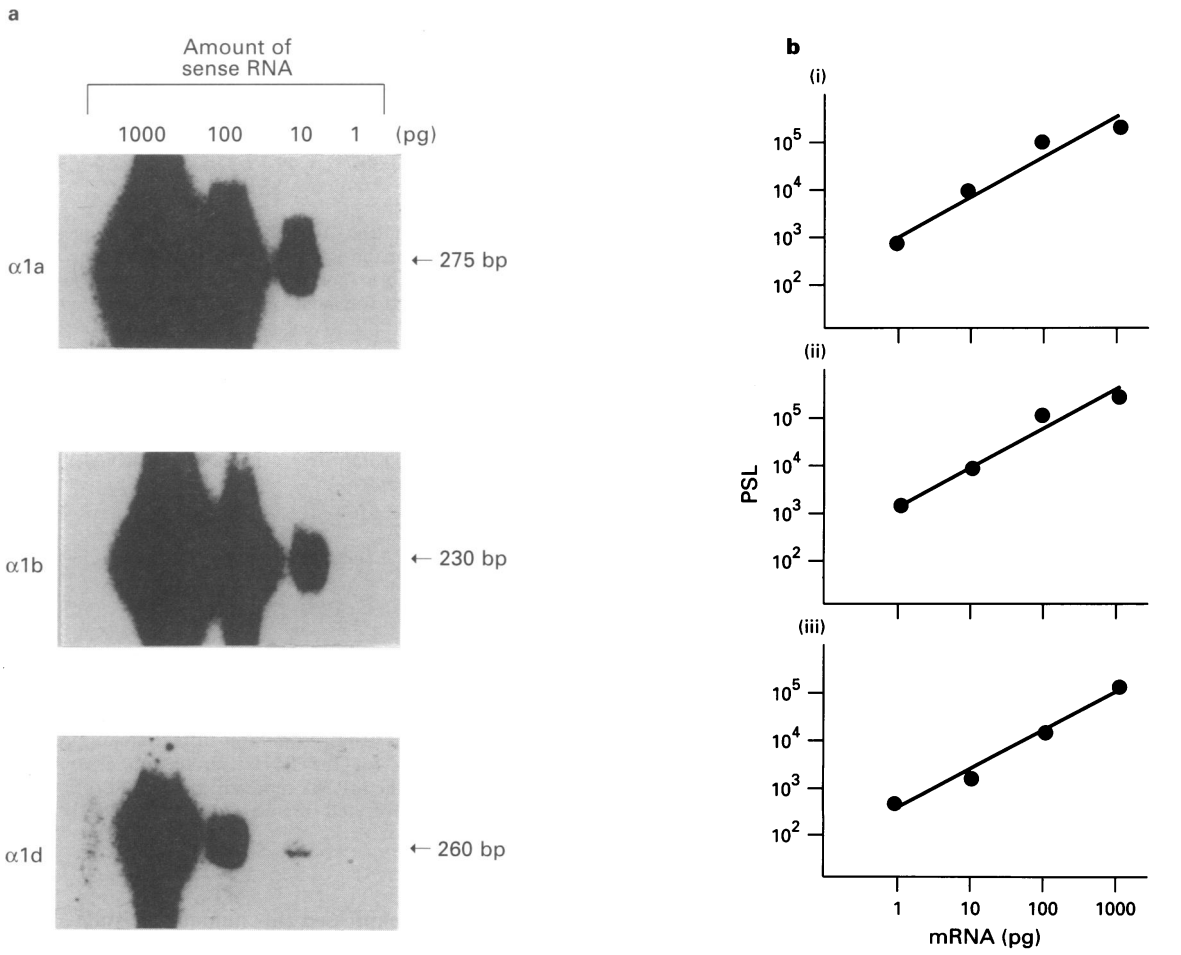

Figure 2 Representative standard curves from the RNase protection assay using each of the *in vitro* transcribed sense RNAs. Sense RNA (1, 10, 100, or 1000 pg) was subjected to the RNase protection assay and a separate standard curve was generated for each subtype probe (α1a, α1b or α1d). (a) Electrophoresis pattern; (b) standard curves ((i) α1a; (ii) α1b; (iii) α1d). (The relative intensity of the photostimulated luminescence (PSL) is linearly related to the amount of radioactivity).

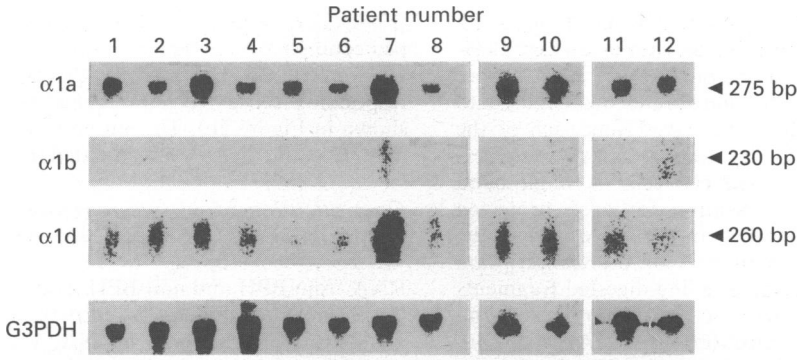

Figure 3 RNase protection assay of α1-adrenoceptor subtype mRNAs in human prostatic tissue (BPH). Poly(A)+ RNA (10 μg) from human BPH tissue was hybridized with radiolabelled antisense RNA probes specific for α1a, α1b, α1d or glyceraldehyde-3-phosphate dehydrogenase (G3PDH). After RNase digestion, protected fragments were separated on a 5% polyacrylamide gel containing 8 M urea. α1a and G3PDH gels were exposed to the imaging plate for about 4 h; α1b and α1d gels were exposed overnight.

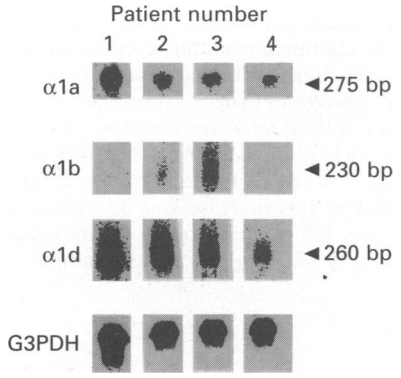

Figure 4 RNase protection assay of α1-adrenoceptor subtype mRNAs in human prostatic tissue (non-BPH). For experimental details, see the legend to Figure 3.

BPH samples was about three times that in non-BPH samples, while the abundance of α1b was almost the same in BPH and non-BPH samples.

In situ *hybridization*

We used *in situ* hybridization to investigate differences in tissue localization of α1-adrenoceptor subtype mRNAs between BPH and non-BPH tissue samples. The subtype selectivity of the α1a, α1b and α1d RNA probes for *in situ* hybridization was verified by Northern blot hybridization using total RNA extracted from the transfected CHO cell lines as described above for the RNase protection assay. There was no detectable staining upon treatment with sense RNA probes (data not shown). In both BPH and non-BPH samples, the localization of α1-adrenoceptor subtype mRNAs was very similar. α1a and α1d were clearly detected in the interstitium of the prostate, where the positive sites were primarily smooth muscle cells (Figure 5). The distribution of both α1a and α1d was diffuse, and no difference in the expression pattern was observed between the two subtypes, but α1a was stained more intensely than α1d. In contrast, α1b staining in BPH samples was very faint in the interstitium and that in non-BPH samples was essentially negative.

Table 1 Distribution of α1-adrenoceptor subtype mRNAs in human prostatic tissues (BPH)

Patient No.	*Total α1 mRNA[1] (pg)*	*Subtype mRNA[1], pg (%)* α1a	α1b	α1d
1	55.5	49.3 (89)	ND[2]	6.2 (11)
2	47.8	41.1 (86)	ND[2]	6.7 (14)
3	81.1	73.5 (91)	ND[2]	7.6 (9)
4	48.7	42.1 (86)	ND[2]	6.6 (14)
5	49.9	45.1 (90)	ND[2]	4.8 (10)
6	41.1	35.6 (87)	ND[2]	5.5 (13)
7	167	120 (76)	6.8 (4)	30.9 (20)
8	43.6	37.3 (86)	ND[2]	6.3 (14)
9	70.5	56.6 (80)	ND[2]	13.9 (20)
10	78.3	61.7 (79)	ND[2]	16.6 (21)
11	21.0	17.8 (85)	ND[2]	3.2 (15)
12	18.9	16.0 (85)	0.6 (3)	2.3 (12)
Mean (pg)	60.3 ± 38.7	49.7 ± 27.5	0.6 ± 2.0	9.2 ± 7.7
(%)		85 ± 5	1 ± 1	14 ± 4

Poly (A)$^+$RNA (10 μg) from human prostatic tissues with BPH was used for the RNase protection assay. [1]The values given for the mRNA represent the weight (pg) of the single stranded protected fragment only. [2]ND, not detected.

Table 2 Distribution of α1-adrenoceptor subtype mRNAs in human prostatic tissues (non-BPH)

Patient No.	*Total α1 mRNA[1]* (pg)	Subtype mRNA[1], pg (%) α1a	α1b	α1d
1	15.9	9.4 (59)	ND[2]	6.5 (41)
2	8.5	5.1 (60)	0.8 (9)	2.6 (31)
3	7.9	4.8 (61)	1.2 (15)	1.9 (24)
4	4.1	2.9 (71)	ND[2]	1.2 (29)
Mean (pg)	9.1 ± 4.9	5.6 ± 2.7	0.5 ± 0.6	3.1 ± 2.4
(%)		63 ± 6	6 ± 7	31 ± 7

Poly (A)$^+$RNA (10 μg) from non-BPH human prostatic tissues was used for the RNase protection assay. [1]The values given for the mRNA represent the weight (pg) of the single stranded protected fragment only. [2]ND, not detected.

Discussion

The expression of α1-adrenoceptor subtype mRNAs has previously been investigated in various human tissues, and the major subtype mRNA was found to depend on the type of tissue: α1a in heart, liver, lung and prostate, α1b in spleen, kidney and foetal brain, and α1d in aorta and cerebral cortex (Price *et al.*, 1993a; Weinberg *et al.*, 1994). The α1-adrenoceptor subtypes expressed in human prostate have been pharmacologically characterized by radioligand binding assay (Muramatsu *et al.*, 1994; Chapple *et al.*, 1994) and the expression of the three subtype mRNAs has been examined by an RNase protection assay and *in situ* hybridization (Price *et al.*, 1993b; Moriyama *et al.*, 1996). Previously published studies of

the α1-adrenoceptor subtype mRNAs have been restricted to non-BPH tissues; there are no previous studies on the comparative expression of α1-adrenoceptor subtype mRNAs in BPH and non-BPH tissues. In the present study we have used BPH tissue samples, in addition to non-BPH samples from bladder cancer patients, to quantify the α1-adrenoceptor subtype mRNAs expressed, and to investigate their localization, in prostatic tissues (the non-BPH samples were regarded as controls for the BPH samples). Thus, our study may shed light on the variation in efficacy among the α1-adrenoceptor antagonists used in the treatment of BPH.

We have quantified each α1-adrenoceptor subtype mRNA by using an RNase protection assay. Subtype-specific antisense RNA probes were selected from the same region of each α1-adrenoceptor subtype, corresponding to the C-terminal region of the receptor, and were adjusted to similar lengths. Price *et al.* (1993b) used a solution hybridization method with antisense RNA probes selected from a different region of each α1-adrenoceptor subtype to quantify the amount of each subtype mRNA. Their method may be subject to an artifactual error if the probe is not completely digested: in this method, the radioactivity of the hybridized probe after RNase treatment is measured by liquid scintillation counting without prior electrophoresis. However, in our modified method, we could eliminate this artifactual error by measuring the radioactivity of the specific band after electrophoresis on a denaturing gel.

In all tissue samples tested, the predominant subtype mRNA was α1a, as previously reported (Price *et al.*, 1993b; Moriyama *et al.*, 1996). However, we found a major difference between BPH and non-BPH samples: the total abundance of α1-adrenoceptor mRNA in BPH samples was over six times that in non-BPH samples, and the abundance of α1a mRNA in BPH samples was almost nine times that in non-BPH samples. The ratio of the numbers of the subtype mRNAs, α1a:α1b:α1d, was 85:1:14 in BPH samples and 63:6:31 in non-BPH samples. Price *et al.* (1993b) evaluated prostate samples from three individuals by means of an RNase protection assay and reported that the ratio of the subtype mRNAs, α1a:α1b:α1d, was 69:3:27, similar to our result for non-BPH samples. We recognize that the ratio of interstitial to epithelial volumes may change during the progression of PBH. However, our studies evaluate poly(A)+ RNA extracted from whole prostate: further studies are required to determine any differences in mRNA expression patterns between interstitium

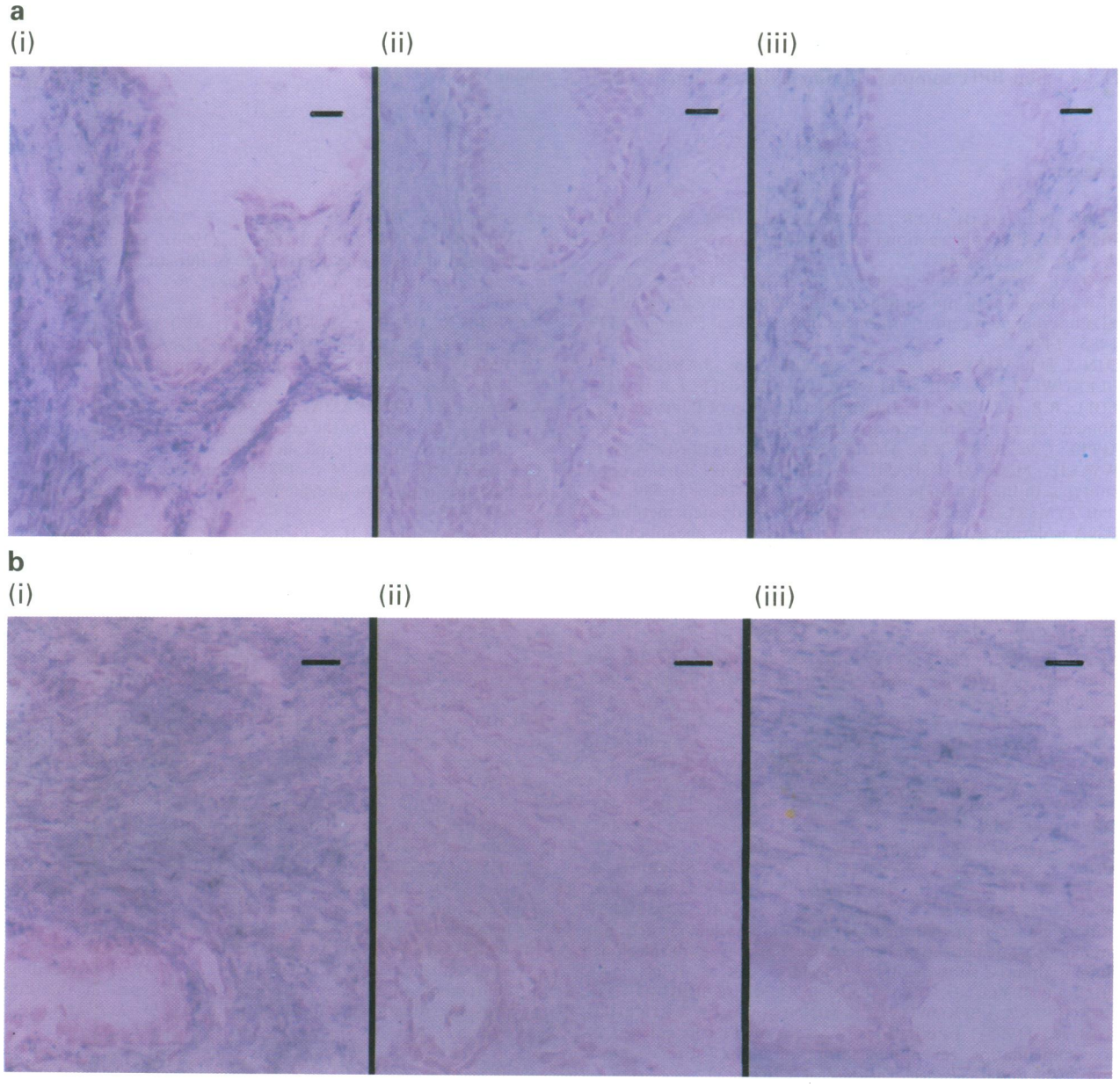

Figure 5 Microscopic views of *in situ* hybridization signals of α1-adrenoceptor subtype mRNAs in human prostatic tissue (BPH and non-BPH). (a) BPH; (b) non-BPH; (i) α1a antisense probe; (ii) α1b antisense probe; (iii) α1d antisense probe; bar, 25 μm.

and epithelium. Differences in the abundance of the α1a subtype between BPH and non-BPH tissue could affect the contraction of the prostate and might be related to the pathogenesis of the difficulty in urination. Because only low levels of α1b mRNA were detected in both BPH and non-BPH samples, this receptor subtype may not be relevant to BPH (Weinberg *et al.* (1994) have also reported low levels of α1b receptor in prostatic tissue). We suggest on the basis of our findings that prostate hypertrophy may be the result of an increase in the expression of α1-adrenoceptor mRNA, which leads to an increased expression of α1-adrenoceptors, contraction of the prostatic tissue, and obstruction of the urinary tract. Pharmacological data shows that the α1-adrenoceptor that mediates smooth muscle contraction in the human prostate is the α1a subtype (Forray *et al.*, 1994). Although the functions of the subtypes have not yet been determined, McVary *et al.* (1994) reported that adrenergic stimulation affects prostatic growth in the rat. Such an effect is not well documented for human subjects, and the involvement of the α1-adrenoceptor in such stimulated growth has yet to be studied.

We have also compared the localization of α1-adrenoceptor subtype mRNAs in human prostatic tissue by *in situ* hybridization. Our study revealed no remarkable differences in the localization of α1-adrenoceptor subtype mRNAs between BPH and non-BPH samples. *In situ* hybridization is not a quantitative technique, so we cannot accurately compare staining intensities. However, the results are in qualitative agreement with those of the RNase protection assay: α1a and α1d were clearly detected, whereas α1b staining was very faint. At present, no specific antibodies against α1-adrenoceptor subtypes are available, so *in situ* hybridization of the corresponding mRNAs may be the best guide yet available to the tissue localization of α1-adrenoceptor subtypes.

We have now confirmed that α1a is the dominant α1-adrenoceptor subtype in human prostate, both BPH and non-BPH. Furthermore, we have shown that the expression of α1-adrenoceptor mRNA in BPH samples was dramatically increased by comparison with non-BPH samples and that most of this increase was accounted for by α1a. This change in mRNA abundance may be a direct cause of the concentration of prostatic tissue that leads to obstruction of the urinary tract. Our data suggest that an α1a-selective antagonist may be of greater benefit to the BPH patient than the nonselective α1 antagonists currently in use.

This investigation was supported in part by the Human Science Foundation and a Grant-in-Aid for Scientific Research (B), No 5454432, from the Ministry of Education, Science, and Culture, Japan.

References

ABRAMS, P.H., SHAH, P.J.R., STONE, R. & CHOA, R.G. (1982). Bladder outflow obstruction treated with phenoxybenzamine. *Br. J. Urol.*, **54**, 527–530.

BRUNO, J.F., WHITTAKER, J., SONG, J. & BERELOWITZ, M. (1991). Molecular cloning and sequencing of a cDNA encoding a human α1A adrenergic receptor. *Biochem. Biophys. Res. Commun.*, **179**, 1485–1490.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B. & RUFFOLO, R.R. JR. (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.*, **46**, 121–136.

CHAPPLE, C.R., BURT, R.P., ANDERSSON, P.O., GREENGRASS, P., WYLLIE, M. & MARSHALL, I. (1994). Alpha1-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.

COTECCHIA, S., SCHWINN, D.A., RANDALL, R.R., LEFKOWITZ, R.J., CARON, M.G. & KOBILKA, B.K. (1988). Molecular cloning and expression of the cDNA for the hamster α1-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 7159–7163.

DENHARDT, D.T. (1996). A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.*, **23**, 641–646.

FOGLAR, R., SHIBATA, K., HORIE, K., HIRASAWA, A. & TSUJIMOTO, G. (1995). Use of recombinant α1-adrenoceptors to characterize subtype selectivity of drugs for the treatment of prostatic hypertrophy. *Eur. J. Pharmacol.*, **288**, 201–207.

FORD, A.P.D.W., WILLIAMS, T.J., BLUE, D.R. & CLARKE, D.E. (1994). α1-Adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.*, **15**, 167–170.

FORRAY, C., BARD, J.A., WETZEL, J.M., CHIU, G., SHAPIRO, E., TANG, R., LEPOR, H., HARTIG, P.R., WEINSHANK, R.L., BRANCHEK, T.A. & GLUCHOWSKI, C. (1994). The alpha1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human alpha1C subtype. *Mol. Pharmacol.*, **45**, 703–708.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P. & RUFFOLO, R.R. JR. (1995). International Union of Pharmacology. X. Recommendation for nomenclature of alpha1-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 α1C-adrenergic receptor. *Biochem. Biophys. Res. Commun.*, **195**, 902–909.

KAWABE, K., UENO, A., TAKIMOTO, Y., ASO, Y., KATO, H. & YM617 CLINICAL STUDY GROUP. (1990). Use of an alpha-blocker, YM617, in the treatment of benign prostatic hypertrophy. *J. Urol.*, **144**, 908–912.

KAWABE, K., MORIYAMA, N., YAMADA, S. & TANIGUCHI, N. (1994). Rationale for the use of alpha-blockers in the treatment of benign prostatic hyperplasia (BPH). *Int. J. Urol.*, **1**, 203–211.

LEE, J.J. & COSTLOW, N.A. (1987). A molecular titration assay to measure transcript prevalence levels. *Methods Enzymol.*, **152**, 633–648.

LEPOR, H., AUERBACH, S., PURAS-BAEZ, A., NARAYAN, P., SOLOWAY, M., LOWE, F., MOON, T., LEIFER, G. & MADSEN, P. (1992). A randomized, placebo-controlled multicenter study of the efficacy and safety of terazosin in the treatment of benign prostatic hyperplasia. *J. Urol.*, **148**, 1467–1474.

MCVARY, K.T., RAZZAQ, A., LEE, C., VENEGAS, M.P., RADEMAKER, A. & MCKENNA, K.E. (1994). Growth of the rat prostate gland is facilitated by the autonomic nervous system. *Biol. Reprod.*, **51**, 99–107.

MICHEL, M.C. & INSEL, P.A. (1994). Comparison of cloned and pharmacologically defined rat tissue alpha1-adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **349**, 385–395.

MINNEMAN, K.P., HAN, C. & ABEL, P.W. (1988). Comparison of α1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB4101. *Mol. Pharmacol.*, **33**, 509–514.

MORIYAMA, N., KURIOMOTO, S., HORIE, S., NASU, K., TANAKA, T., YANO, J., HIRANO, H., TSUJIMOTO, G. & KAWABE, K. (1996). Detection of α1-adrenoceptor subtypes in human hypertrophied prostate by in situ hybridization. *Histochem. J.*, (in press).

MURAMATSU, I., OSHITA, T., OHMURA, T., KIGOSHI, S., AKINO, M., GOBARA, M. & OKADA, K. (1994). Pharmacological characterization of α1-adrenoceptor subtypes in the human prostate: functional and binding studies. *Br. J. Pharmacol.*, **74**, 572–578.

PRICE, D.T., LEFKOWITZ, R.J., CARON, M.G., BERKOWITZ, D. & SCHWINN, D.A. (1993a). Localization of mRNA for three distinct α1-adrenergic receptor subtypes in human tissues: implications for human α-adrenergic physiology. *Mol. Pharmacol.*, **45,** 171–175.

PRICE, D.T., SCHWINN, D.A., LOMASNEY, J.W., ALLEN, L.F., CARO, M.G. & LEFKOWITZ, R.J. (1993b). Identification, quantification, and localization of mRNA for three distinct alpha1 adrenergic receptor subtypes in human prostate. *J. Urol.*, **150,** 546–551.

RAMARAO, C.S., DENKER, J.M.K., PEREZ, D.M., GAIVIN, R.J., RIEK, R.P. & GRAHAM, R.M. (1992). Genomic organization and expression of the human alpha-1B adrenergic receptor. *J. Biol. Chem.*, **267,** 21936–21945.

VOIGT, M.M., KISPERT, J. & CHIN, H. (1990). Sequence of a rat brain cDNA encoding an alpha-1B adrenergic receptor. *Nucl. Acids Res.*, **18,** 1053.

WEINBERG, D.H., TRIVEDI, P., TAN, C.P., MIITRA, S., PERKINS-BARROW, A., BORKOWSKI, D., STRADER, C.D. & BAYNE, M. (1994). Cloning, expression and characterization of human α adrenergic receptors α1A, α1B and α1C. *Biochem. Biophys. Res. Commun.*, **201,** 1296–1304.

(Received May 16, 1996
Accepted July 19, 1996)