British Journal of Pharmacology (1995) 115, 1475–1485

The α_{1C}-adrenoceptor in human prostate: cloning, functional expression, and localization to specific prostatic cell types

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1 Benign prostatic hyperplasia (BPH) causes urinary obstruction in aging men that frequently requires surgery to relieve the symptoms of urinary retention, nocturia, and micturition. Smooth muscle tone which contributes to the urethral constriction in the enlarged gland appears to be mediated by the α_1-adrenoceptors. In this paper, molecular and pharmacological approaches are used to establish the role played by the α_{1C}-adrenoceptor subtype in the prostate.

2 The α_1-adrenoceptor subtype(s) expressed in human prostate were investigated by use of polymerase chain reaction (PCR), Northern blot, and *in situ* hybridization. The α_{1C} subtype was found in both prostate stromal and glandular cells while α_{1B} and α_{1D} subtypes were expressed in glandular cells. High expression levels for α_{1C} were observed in prostate cancer tissues in both stroma and glandular cells.

3 Full length α_{1C}-adrenoceptor cDNA was cloned from human prostate. Stable mammalian cell lines expressing human α_{1B}-, α_{1C}-, and α_{1D}-adrenoceptors were made. Membranes prepared from these cell lines and human prostate were used to evaluate the pharmacological profiles of human α_{1B}-, α_{1C}- and α_{1D}-adrenoceptors in comparison to human prostate. Leverage plot analysis of compound affinities determined by competition for [^{125}I]-I-HEAT binding demonstrated that the α_{1C} subtype is the predominant α_1-adrenoceptor in human prostate.

4 The α_1-adrenoceptors cause smooth muscle constriction by coupling to IP_3 turnover and intracellular Ca^{2+} release. Using stable cell lines to measure IP_3 production in response to noradrenaline, α_{1C} stimulated IP_3 production most efficiently, with α_{1B} at an intermediate level, while little IP_3 above background could be detected with α_{1D}. These results supported a functional role of the α_{1C}-adrenoceptor on prostate smooth muscle constriction by noradrenaline stimulation.

Keywords: α_{1C}-Adrenoceptor; benign prostatic hyperplasia; polymerase chain reaction; Northern blot; *in situ* hybridization; radioligand binding; leverage plots

Introduction

Benign prostatic hyperplasia (BPH) is a pathological process associated with hormone imbalance and aging. For men 50–60 years old, 50–75% develop morphological BPH. Roughly one third will progress into prostatism, and 10% will eventually require surgery (Isaacs & Coffey, 1989; Schroder & Blom, 1989). Two factors contribute to urinary obstruction in BPH. First, an enlargement of the prostate gland surrounding the bladder neck without an enlargement of the prostatic capsule physically constricts the urethra. Second, the smooth muscle tone of the enlarged prostate gland provides additional constriction that is subject to nerve stimulation and is dynamic in nature (Caine, 1988). Prostate tissue contains two major cell types: stroma cells that are the smooth muscle cells, and glandular cells that are the secretory cells. Pathological studies have identified that hyperplasia of stroma cells is the major cause of prostate enlargement. A stroma to glandular cell ratio of 2:1 in normal prostate and of 5:1 in BPH were quantitated by light microscopy (Bartsch *et al.*, 1979a). Electron microscopy revealed that the quantity of cell organelles in BPH stroma cells was three times higher than that in normal prostate stroma cells, indicating activation of these cells (Bartsch *et al.*, 1979b).

Early studies indicated that stimulation of α_1-adrenoceptors by noradrenaline most likely mediated the dynamic force of prostate smooth muscle tone in BPH patients. The prostate gland is innervated with both adrenergic and cholinergic nerve fibres. Only adrenergic fibres stimulate the prostate muscle tone, while cholinergic fibres have little effect (Arver & Sjostrand, 1982). Isometric contraction experiments of prostate tissues indicated that α_1, not α_2, adrenoceptors mediated the noradrenaline stimulated prostate muscle tone (Kunisawa *et al.*, 1985). Using radioligand binding, the α_1-adrenoceptors were identified in human prostatic tissue (Lepor & Shapiro, 1984). Most importantly, clinical trials involving α_1 antagonists proved that blockade of α_1-adrenoceptors was effective in relieving BPH symptoms (Caine, 1986; Kirby, 1989; Kawabe *et al.*, 1990; Lepor, 1990; Holme *et al.*, 1994).

Identification of the α_1-adrenoceptor subtype(s) mediating adrenaline-induced prostate smooth muscle tone has important clinical implications for the pharmaceutical treatment of BPH. Approximately 70% of males with clinical BPH symptoms responded favourably to treatment with α_1 antagonists. However, the dosage was limited by adverse effects. Common adverse effects associated with α_1 antagonists are light headedness, aesthenia, nasal stuffiness, and dizziness, presumably associated with non-specific blockade of the vascular and cerebral α_1-adrenoceptors (Caine, 1986; Lepor, 1990; Holme *et al.*, 1994). Identification of the prostate α_1-adrenoceptor subtype(s) and development of subtype-specific antagonists should improve clinical management of BPH, and indirectly decrease the need for surgery. The α_1 antagonists currently available for BPH treatment were originally developed for hypertension, and are not subtype specific. It is of interest to use stable cells expressing specific human adrenoceptor subtypes to screen for subtype-specific α_1 antagonists.

With molecular biology, three α_1-adrenoceptor subtypes have been cloned from human and animal tissues; the clones were classified as α_{1B}-, α_{1C}-, and α_{1D}-adrenoceptors (Cotecchia

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et al., 1988; Schwinn *et al.*, 1990; Bruno *et al.*, 1991; Lomasney *et al.*, 1991a; Ramarao *et al.*, 1992; Hirasawa *et al.*, 1993; Forray *et al.*, 1994; Weinberg *et al.*, 1994). The α_{1C}-adrenoceptor subtype has been cloned from human prostate (Hirasawa *et al.*, 1993). By use of RNAse protection and *in situ* hybridization techniques, α_{1C} mRNA was shown to be predominant in human prostate and appeared to be localized in stromal cells (Price *et al.*, 1993). The binding affinities of the three cloned human α_1-adrenoceptors to a number of α_1-selective antagonists have been compared to the potencies of these antagonists in inhibiting α_1 agonist-stimulated human prostate muscle contraction. Again, the α_{1C} subtype correlated best to human prostate (Forray *et al.*, 1994). We have independently cloned α_{1C} from human prostate and identified α_{1C} expression in human prostate stroma cells. Contrary to previous publication, we found clear evidences of α_{1C} mRNA expression not only in the prostate stromal cells, but also in the prostate glandular cells. We compared the radioligand binding profiles of the three cloned human α_1-adrenoceptors to human prostate and reached a similar conclusion that the best correlation was between the α_{1C} subtype and human prostate. We studied the effector coupling efficiency of the human α_1-adrenoceptors. Our detailed molecular and pharmacological analysis support a functional role played by the α_{1C}-adrenoceptor in mediating BPH prostate smooth muscle constriction.

Methods

Cell lines and clones

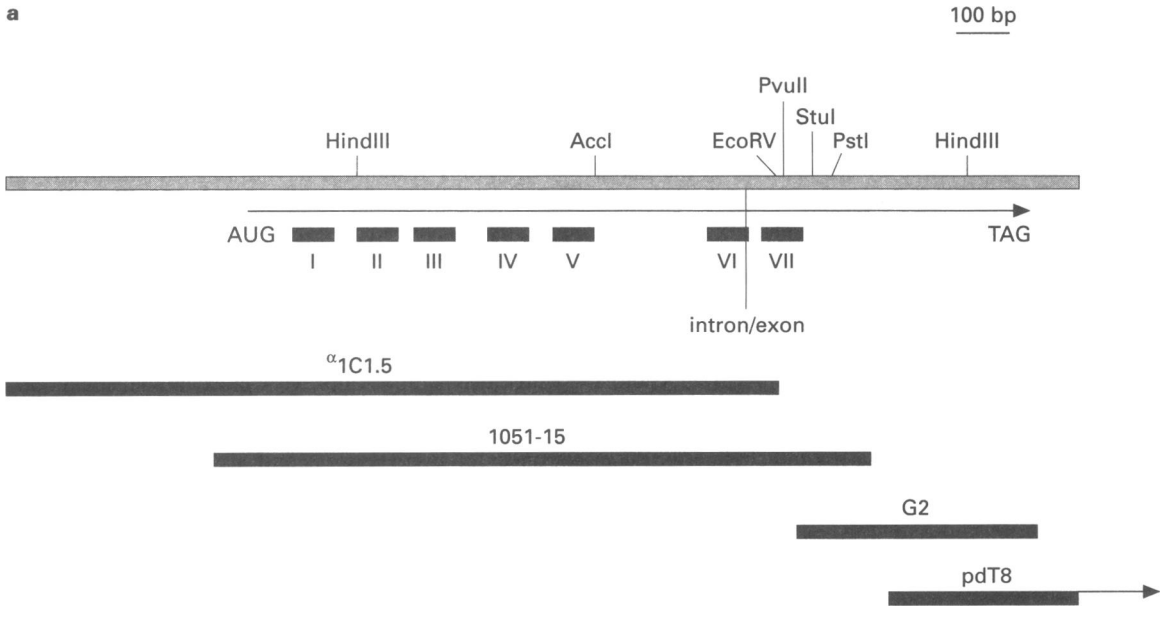
Rat-1, L-M(TK-), Cos-7, PC-3, and Du145 cell lines were from the American Type Culture Collection (ATCC). Primary stromal cells were cultured from prostate tissue obtained by radical prostatectomy. A full-length human α_{1B} cDNA clone was obtained from Dr R. Graham (Cleveland Clinical Foundation) (Ramarao *et al.*, 1992). The human α_{1D} cDNA probe was a gift from Dr J. Bruno (SUNY/Stonybrook) (Bruno *et al.*, 1991). Full-length human α_{1D} cDNA was cloned by Dr R. Buckholz of Glaxo Research Institute.

Degenerate polymerase chain reaction (PCR) and library screening

A degenerate PCR approach was used to amplify partial human α_1-adrenoceptor cDNA from λ phage DNA purified from a Clontech human prostate cDNA library (HL1051). Transmembrane III and VI sequences from hamster α_{1B}, dog α_{1B}, bovine α_{1C}, human β_2, human $\alpha_{2/C10}$, and rat 5-HT_{1c} receptors (Emorine *et al.*, 1987; Kobilka *et al.*, 1987; Cotecchia *et al.*, 1988; Julius *et al.*, 1988; Libert *et al.*, 1989; Schwinn *et al.*, 1990) were chosen to design degenerate PCR primers. Degenerate PCR primers listed below correspond to the nucleotide sequence of hamster α_{1B}-adrenoceptor (Cotecchia *et al.*, 1988): III1, 5′ GA(TC)GTICTIT(TG)CTGCACIICITCCATC 3′ (hamster α_{1B} 387 to 413); III2, 5′ CTITG(CT)IC(CT)ATCI(GC)CITIGA(CT)CGCTAC 3′ (420 to 446); VI1, 5′ CCAGCAIAIIAIGAACATICCGACIAC 3′ (935 to 909); and VI2, 5′ GAAGAAGGGIAICCAGCAIAIIAIGAA 3′ (947 to 921). PCR was performed with four primer pairs (III1 + VI1, III1 + IV2, III2 + IV1, and III2 + IV2) and with nested PCR (from primer pairs III1 + VI2 to III2 + VI1). The PCR protocol was 30 cycles of 94°C/1 min, 45°C/2 min, and 72°C/1 min, with a 72°C/10 min extension. With nested PCR the annealing temperature for the second PCR was 50°C. After amplification, Klenow DNA polymerase and 0.4 mM each of dATP, dGTP, dCTP, and dTTP were added, and the mix was incubated at 37°C for 30 min to end-fill the PCR fragments. PCR fragments at expected size range of 500 to 700 bp were purified and cloned into the SmaI site of the pBluescript KS + vector (Stratagene) and sequenced. Sequences were compared to the Genebank database using Intelligenetics software. PCR fragments from α_{1B}- and α_{1C}-adrenoceptors were used as probes for high stringency λ library screening following the procedure of Sambrook *et al.* (1989). All sequencing reactions used the dideoxynucleotide chain termination method and the enzyme sequenase (USB).

Reverse transcription-PCR (RT-PCR)

Two antisense primers were made according to bovine α_{1C}-adrenoceptor sequences (nucleotides 1518 to 1498: 5′ CCTTCTGACCTTTCCTGTCCT 3′ and 1500 to 1480: 5′ CCTTTAGACTTCCTCCCCATT 3′) (Schwinn *et al.*, 1990), and three sense primers were made from human α_{1C}-adrenoceptor sequences (Figure 1b nucleotides 1403 to 1423, 1436 to 1456, and 1455 to 1474). The template DNA was cDNA reverse transcribed from human prostate polyA$^+$ RNA. The PCR protocol was 25 cycles of 94°C/1 min, 60°C/1 min, and 72°C/2 min, hot start at 85°C and a 72°C/10 min extension at the end. Amplified PCR fragments were cloned into the EcoRV site of the pBluescript KS + vector and sequenced as described above.



b

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CGAATCATGT GCAGAATCTG AATCTTCCCC CAGCCAGGAC GAATAAGACA GCGCGGAAAA GCAGATTCTC GTAATTCTGG AATTGCATGT          90

TGCAAGGAGT CTCCTGGATC TTCGCACCCA GCTTCGGGTA GGGAGGGAGT CGGGTCCCGG CTAGGCCAGC CCGGCAGGTG GAGAGGGTCC        180

CCGGCAGCCC CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCCTTCA TGTGGCCTTC TGAGGGTTCC CAGGGCTGGC CAGGGTTGTT        270

TCCCACCCGC GCGCGCTCTC ACCCCCAGCC AAACCCACCT GGCAGGGCTC CCTCCAGCCG AGACCTTTTG ATTCCCGGCT CCCGCGCTCC        360

CGCCTCCGCG CCACGCCGGG AGGTGGCCCT GGACAGCCGG ACCTCGCCCG GCCCCGGCTG GACC ATG GTG TTT CTC TCG GGA AAT GCT    448
                                                                      MET Val Phe Leu Ser Gly Asn Ala      8

                                                                                  I
TCC GAC AGC TCC AAC TGC ACC CAA CCG CCG GCA CCG GTG AAC ATT TCC AAG GCC ATT CTG CTC GGG GTG ATC TTG GGG    526
Ser Asp Ser Ser Asn Cys Thr Gln Pro Pro Ala Pro Val Asn Ile Ser Lys Ala Ile Leu Leu Gly Val Ile Leu Gly     34

GGC CTC ATT CTT TTC GGG GTG CTG TGT AAC ATC CTA GTG ATC CTC TCC GTA GCC TGT CAC CGA CAC CTG CAC TCA GTC    604
Gly Leu Ile Leu Phe Gly Val Leu Cys Asn Ile Leu Val Ile Leu Ser Val Ala Cys His Arg His Leu His Ser Val     60

    II
ACG CAC TAC TAC ATC GTC AAC CTG GCG GTG GCC GAC CTC CTG CTC ACC TCC ACG GTG CTG CCC TTC TCC GCC ATC TTC    682
Thr His Tyr Tyr Ile Val Asn Leu Ala Val Ala Asp Leu Leu Leu Thr Ser Thr Val Leu Pro Phe Ser Ala Ile Phe     86

                                                  III
GAG GTC CTA GGC TAC TGG GCC TTC GGC AGG GTC TTC TGC AAC ATC TGG GCG GCA GTG GAT GTG CTG TGC TGC ACC GCG    760
Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg Val Phe Cys Asn Ile Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala    112

TCC ATC ATG GGC CTC TGC ATC ATC TCC ATC GAC CGC TAC ATC GGC GTG ACG TAC CCG CTG CGC TAC CCA ACC ATC GTC    838
Ser Ile MET Gly Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val Thr Tyr Pro Leu Arg Tyr Pro Thr Ile Val    138

                        IV
ACC CAG AGG AGG GGT CTC ATG GCT CTG CTC TGC GTC TGG GCA CTC TCC CTG GTC ATA TCC ATT GGA CCC CTG TTC GGC    916
Thr Gln Arg Arg Gly Leu MET Ala Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly Pro Leu Phe Gly    164

                                                                  V
TGG AGG CAG CCG GCC CCC GAG GAC GAG ACC ATC TGC CAG ATC AAC GAG GAG CCG GGC TAC GTG CTC TTC TCA GCG CTG    994
Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu    190

GGC TCC TTC TAC CTG CCT CTG GCC ATC ATC CTG GTC ATG TAC TGC CGC GTC TAC GTG GTG GCC AAG AGG GAG AGC CGG   1072
Gly Ser Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val MET Tyr Cys Arg Val Tyr Val Val Ala Lys Arg Glu Ser Arg    216

GGC CTC AAG TCT GGC CTC AAG ACC GAC AAG TCG GAC TCG GAG CAA GTG ACG CTC CGC ATC CAT CGG AAA AAC GCC CCG   1150
Gly Leu Lys Ser Gly Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His Arg Lys Asn Ala Pro    242

GCA GGA GGC AGC GGG ATG GCC AGC GCC AAG ACC AAG ACG CAC TTC TCA GTG AGG CTC CTC AAG TTC TCC CGG GAG AAG   1228
Ala Gly Gly Ser Gly MET Ala Ser Ala Lys Thr Lys Thr His Phe Ser Val Arg Leu Leu Lys Phe Ser Arg Glu Lys    268

              VI
AAA GCG GCC AAA ACG CTG GGC ATC GTG GTC GGC TGC TTC GTC CTC TGC TGG CTG CCT TTT TTC TTA GTC ATG CCC ATT   1306
Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly Cys Phe Val Leu Cys Trp Leu Pro Phe Phe Leu Val MET Pro Ile    294

                                          VII
GGG TCT TTC TTC CCT GAT TTC AAG CCC TCT GAA ACA GTT TTT AAA ATA GTA TTT TGG CTC GGA TAT CTA AAC AGC TGC   1384
Gly Ser Phe Phe Pro Asp Phe Lys Pro Ser Glu Thr Val Phe Lys Ile Val Phe Trp Leu Gly Tyr Leu Asn Ser Cys    320

ATC AAC CCC ATC ATA TAC CCA TGC TCC AGC CAA GAG TTC AAA AAG GCC TTT CAG AAT GTC TTG AGA ATC CAG TGT CTC   1462
Ile Asn Pro Ile Ile Tyr Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala Phe Gln Asn Val Leu Arg Ile Gln Cys Leu    346

C
TGC AGA AAG CAG TCT TCC AAA CAT GCC CTG GGC TAC CCC CTG CAC CCG CCC AGC CAG GCC GTG GAA GGG CAA CAC AAG   1540
Cys Arg Lys Gln Ser Ser Lys His Ala Leu Gly Tyr Pro Leu His Pro Pro Ser Gln Ala Val Glu Gly Gln His Lys    372
Arg

GAC ATG GTG CGC ATC CCC GTG GGA TCA AGA GAG ACC TTC TAC AGG ATC TCC AAG ACG GAT GGC GTT TGT GAA TGG AAA   1618
Asp MET Val Arg Ile Pro Val Gly Ser Arg Glu Thr Phe Tyr Arg Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys    398

TTT TTC TCT TCC ATG CCC CGT GGA TCT GCC AGG ATT ACA GTG TCC AAA GAC CAA TCC TCC TGT ACC ACA GCC CGG GTG   1696
Phe Phe Ser Ser MET Pro Arg Gly Ser Ala Arg Ile Thr Val Ser Lys Asp Gln Ser Ser Cys Thr Thr Ala Arg Val    424

AGA AGT AAA AGC TTT TTG CAG GTC TGC TGC TGT GTA GGG CCC TCA ACC CCC AGC CTT GAC AAG AAC CAT CAA GTT CCA   1774
Arg Ser Lys Ser Phe Leu Gln Val Cys Cys Cys Val Gly Pro Ser Thr Pro Ser Leu Asp Lys Asn His Gln Val Pro    450

                                        T                   A AGG
ACC ATT AAG GTC CAC ACC ATC TCC CTC AGT GAG AAC GGG GAG GAA GTC TAG GACAGGAAAG ATGCAGAGGA AAGGGGAATA     1855
Thr Ile Lys Val His Thr Ile Ser Leu Ser Glu Asn Gly Glu Glu Val  .                                        466

ATCTTAGGTA CCCACCCCAC TTCCTTCTCG GAAGGCCAGC TCTTCTT                                                     1902
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Figure 1 (a) Restriction map of the overlapping human α_{1C}-adrenoceptor clones. On top is a bar representing the human α_{1C} cDNA that has been sequenced repeatedly for both strands, with restriction sites, coding region, intron/exon junction, and seven transmembrane domains labelled. The heavy lines indicate the areas covered by the individual clones. (b) The compiled α_{1C} cDNA sequences and the derived protein sequences (Genebank accession number U02569). Clone α_{1C} 1.5 spans nucleotides 1 to 1378. Clone 1051-15 spans nucleotides 374 to 1543. Clone pdT8 is 1.8 Kb in length, starts at nucleotides 1585. The PCR clone G2 used for the expression cDNA construct covers nucleotides 1402 to 1828. The sequence polymorphism at nucleotide 1463 is shown above and below for DNA and protein variation, respectively. The α_{1C} cDNA construct starts at nucleotide 374 and ends at nucleotide 1828, with the 3′ bovine oligo sequence used for RT-PCR shown on top. The intron/exon junction is at 1307/1308. The seven transmembrane helices are underlined and labelled with Roman numerals I–VII. The putative 3 N-linked glycosylation sites at the amino terminus and the palmitoylation site and the PKA phosphorylation site at the carboxy terminus are in bold letters.

RNA purification, Northern blot, and cDNA library construction

The human prostate polyA$^+$ RNA used for RT-PCR and cDNA library construction was isolated from surgically removed human prostate tissue of a 63 year old man. The human liver polyA$^+$ RNA used for liver cDNA library was from a 52 year old woman with type II diabetes. For these two samples, total RNA was extracted with an acid-guanidine protocol (Chomczynski & Sacchi, 1987), and polyA$^+$ RNA was purified from total RNA using an oligo-dT cellulose column. PolyA$^+$ RNA from cultured cells (stromal, PC-3, and Du145) was isolated using an Invitrogen fast-track mRNA isolation kit. Human polyA$^+$ RNAs used for Northern blot analysis were purchased from Clontech. The RNA samples were size fractionated using a 1% formaldehyde-agarose gel and blotted to Hybond-N membrane (Amersham). Hybridization was at 42°C in 50% formamide, 5X SSC, 50 mM Tris 7.5, 0.2% SDS, 0.2 mg ml^{-1} salmon sperm DNA, and 10^6 d.p.m. ml^{-1} [α-^{32}P]dCTP random-primed probe. Blots were washed at 65°C in 2X SSC and 0.1% SDS. A Pharmacia cDNA synthesis kit and a Stratagene Gigapack II packaging extract were used for cDNA library construction.

In situ *hybridization*

Human prostate tissues were surgically removed from BPH or prostate cancer patients. Tissues were embedded in OCT compound, frozen in liquid nitrogen, and stored at –70°C. Tissue blocks were sectioned 6 μm thick at –20°C with a cryostat. The sections were collected on silane-coated glass slides, fixed with 4% paraformaldehyde, dehydrated, and stored with drying agents at –20°C. Procedures for probe preparation, hybridization, washing, emulsion-coating, and counter-staining were as described by Tseng-Crank *et al.* (1991).

Stable expression cell lines

Full-length human α_{1B}-, α_{1C}- and α_{1D}-adrenoceptor cDNAs were subcloned into pMT2′ expression vector (Ramarao *et al.*, 1992). Rat-1 and L-M(TK-) fibroblast cells were transfected with 10 μg of plasmid cDNA using a Ca^{2+}-phosphate precipitation protocol (Graham & Van der Eb, 1973). pRSV-Neo (1 μg) was co-transfected for geneticin-selection. The medium used was D-MEM, foetal bovine serum (5% for Rat-1 and 10% for L-M(TK-)), 50 units ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, and geneticin (300 μg ml^{-1} for Rat-1 and 600 μg ml^{-1} for L-M(TK-)). Geneticin-resistant colonies were tested for binding activity with (±)-β-([^{125}I]-iodo-4-hydroxyphenyl) - ethyl - aminomethyl - tetralone ([^{125}I] - I - HEAT). Clones with high binding activity were further purified by growing single cells into colonies in cloning rings to ensure culture purity. Rat-1 cells were harvested with 0.05% trypsin-EDTA, centrifuged, washed with PBS, and frozen at –70°C. L-M(TK-) cells were harvested with an enzyme-free cell dissociation solution, centrifuged, washed with PBS, and frozen at –70°C.

Membrane preparation and radioligand binding assay

The preparation of crude membrane from cells expressing human α_1-adrenoceptors was carried out at 4°C. Frozen aliquots of cells were thawed at room temperature and suspended in 10 v/w buffer (50 mM Tris, 250 mM sucrose, 1 μg ml^{-1} aprotinin, 17 μg ml^{-1} PMSF, 20 μg ml^{-1} bacitracin, 1 mM benzamidine, 10 μg ml^{-1} leupeptin and 10 μg ml^{-1} pepstatin A, pH 7.4). The cells were disrupted with 10 strokes of a dounce glass to glass homogenizer. The mixture was then centrifuged at 100,000 *g* for 30 min. The resulting pellet was washed by resuspension in 5 mM HEPES, pH 7.4 and centrifuged as above. The final pellet was resuspended in 5 mM HEPES, pH 7.4, homogenized with a glass to glass dounce homogenizer and stored at –80°C until use. Binding affinities and receptor number were constant for at least 6 months under this storage condition.

Radioligand binding reactions contained 1.0 μg membrane protein, 25 mM PIPES, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4, 65,000 c.p.m. (60 pM) of [^{125}I]-I-HEAT, and displacing ligands or vehicle (2.5 mM NaOAc/0.3% DMSO) as appropriate in a final volume of 222 μl. Reactions were performed in triplicate in 96 well polystyrene microtiter plates and were incubated for 90 min at 25°C. The reaction was terminated by rapid vacuum filtration through Whatman GF/B glass fibre filters (which had been presoaked in 0.1% BSA for 30 min) using a Brandel cell harvester (Gaithersburg, MD). The filters were washed with 4 ml ice cold 25 mM Tris-HCl (pH 7.4). Retained radioactivity was determined by gamma counting. Nonspecific binding was determined in the presence of 100 μM phentolamine and was usually $\leqslant$15% of total binding. Membrane preparation and radioligand binding experiments for human prostate tissue were as described in Goetz *et al.* (1994).

Data analysis

Data from radioligand binding experiments were fitted to single and multiple receptor site models of ligand binding implemented in the RS/1 (BBN Software Products, Cambridge MA, U.S.A. version 4.2) statistical analysis programme. A partial *F*-test was used to choose the best model. K_i values were calculated from estimated IC_{50} values by the method of Cheng & Prusoff (1973). Leverage plots (Sall, 1990) were used to aid in the graphical interpretation of the regression analyses. The advantage of leverage plots is that the effect of each predictor (subtype pK_i) and individual data point on the linear model can be easily assessed by inspection of the plots. For this study, the leverage plot is used to indentify whether the set of pK_i values for a subtype are of predictive value in a linear model to estimate binding affinity in a tissue. Methods of the calculation of the leverage for each subtype pK_i values and the interpretation of significance for the leverage plots were as described (Sall, 1990; Goetz *et al.*, 1994). Statistical analyses were done with the programme JMP (Version 2.0, SAS Institute, Cary, NC, U.S.A.).

IP_3 assay

Stable transfected cells were plated in 100 mm dishes to confluency the day before assay. Culture medium was removed and the cells rinsed twice with 37°C preincubation buffer (in mM: HEPES 20, NaCl 120, KCl 5.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, LiCl 10, glucose 11, and Na_2EDTA 0.025) and incubated at 37°C for 30 min. The buffer was removed, replaced with 37°C incubation buffer (preincubation buffer plus 3 μM ascorbic acid, 0.3% DMSO, and noradrenaline and prazosin as indicated), and incubated further (incubation time as described). To stop the reaction, dishes were placed on ice, incubation buffer was removed by suction, and 1 ml of ice cold 1 M trichloric acid was added. The cell lysate was harvested by scraping, homogenization, and centrifugation to remove debris. Trichloric acid in the cell lysate was removed by extraction with 2 volumes of 1,1,2-trichloro-1,2,2-trifluoroethane:trioctylamine (3:1). The cell extract was assayed for IP_3 concentrations with a radioreceptor assay kit from NEN (Boston, MA, U.S.A.).

Results

Cloning of human α_1-adrenoceptor cDNAs from human prostate

Under the constraint of human tissue supply, initially we used a degenerate PCR strategy to identify the α_1-adrenoceptor subtypes expressed in a human prostate cDNA library. DNA

```
                                                        1307
1088-7   CCT TTT TTC TTA GTC ATG CCC ATT Gggaaaaaaaatgaagaaa

15-2
18       CCT TTTTTTC TTA GTC ATG CCC ATT Ggtaagtcttgaacacccc
29
                                                        940
α1B      CCC TTC TTC ATC GCT CTA CCG CTT Ggtaagttggggactagca
```

Figure 2 Variation of the exon/intron junctions between the α_{1C} hippocampus clone 1088-7 and the liver clones 15-2, 18, and 29, in comparison to the α_{1B} clone isolated from hippocampus. Nucleotide number 940 for the α_{1B} exon/intron junction corresponds to a published human α_{1B} genomic sequence (Ramarao *et al.*, 1992). An extra 'T' was found in the three liver clones between nucleotides 1288 and 1289. The effect of this frameshift is not known. The exonic and intronic sequences are given in upper and lower case characters, respectively.

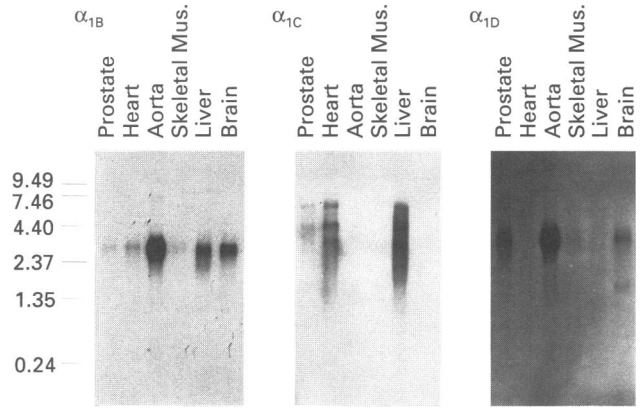

Figure 3 Northern blot analysis of human α_{1B}-, α_{1C}-, and α_{1D}-adrenoceptor mRNA expressed in various human tissues: 3 μg of polyA$^+$ RNA was used per sample. The probes used are: α_{1B}, a 670 bp XhoI/BamHI restriction fragment within the 5′ exon; α_{1C}, the α_{1C} 1.5 cDNA that cover the 5′ exon and less than 100 bp of the 3′ exon; and α_{1D}, a 940 bp XhoI fragment within the 5′ exon. Stringent hybridization and wash conditions were used. Autoradiographys shown were from a single blot. The blot was also hybridized to β-actin control probe and the result showed equal loading of polyA$^+$ RNA samples.

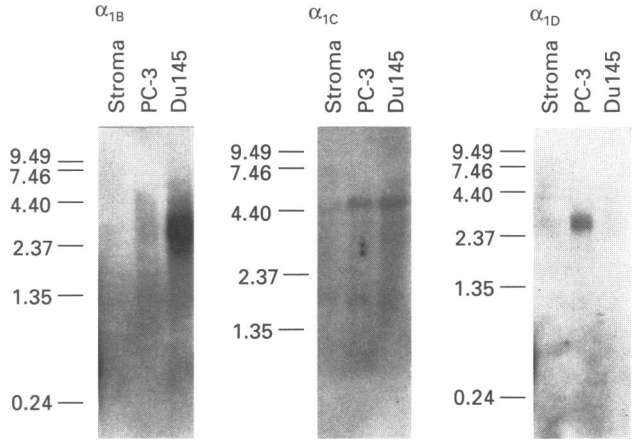

Figure 4 Northern blot analysis of human α_{1B}-, α_{1C}-, and α_{1D}-adrenoceptor mRNA expression in human prostate stroma primary cell culture and in two prostate cancer cell lines, PC-3 and Du145. α_1-Adrenoceptor and β-actin probes used were as described in Figure 3. PolyA$^+$ RNA, 5 μg per sample was used. Loading of RNA samples was checked to be correct by probing with β-actin control probe.

sequences of the PCR clones revealed the presence of α_{1B}, α_{1C}, β_2, and 5-HT$_{1a}$ receptors in human prostate. The α_{1B} and α_{1C} PCR clones were used as probes to screen the prostate cDNA library. Three redundant α_{1C} clones covering the 5′ sequences (α_{1C}1.5, Figure 1) were isolated. However, out of 5×10^6 plaques, none screened positive with the α_{1B} probe. The human prostate library was rescreened with α_{1C} 1.5 and one additional 5′ clone, 1051-15, was isolated (Figure 1).

DNA sequence comparison of the bovine and human α_{1C}-adrenoceptor clones revealed that the coding region was highly conserved while the 5′ untranslated sequences diverged sharply preceding the start codon. Assuming the same may be true for the 3′ end, bovine α_{1C} sequences at the stop codon were used in RT-PCR to amplify the human α_{1C} C-terminus from cDNA reverse transcribed from human prostate polyA$^+$ RNA. With nested PCR, fragments of correct sizes were amplified and DNA sequences of seven independent PCR clones demonstrated that the PCR fragments were amplified from α_{1C} (Figure 1).

Finally, a human prostate primary cDNA library was made and screened with a RT-PCR clone. Out of 1.5×10^6 plaques, one positive clone was isolated. This clone (pdT8) is 1.8 Kb long with 243 bp of C-terminal sequences overlapping the RT-PCR clones. The bovine primer sequence used for RT-PCR is identical to pdT8 at the protein level (Figure 1).

A cDNA clone 1051-15 and a PCR clone G2 were linked together using an unique PstI site for constructing a complete receptor coding plasmid (Figure 1a). The α_{1C} cDNA was cloned into an expression vector pSVT7 in both forward and reverse orientations. Transient expression in Cos-7 cells using the reverse orientation produced no binding activity using the α_1-adrenoceptor ligand [^{125}I]-I-HEAT. However, transient expression in Cos-7 cells using the forward orientation yielded a high level of specific binding of [^{125}I]-I-HEAT.

α_{1C} *polymorphism*

A T/C polymorphism was found in nucleotide 1463 (Figure 1b), resulting in the presence or absence of a PstI restriction enzyme site. This was first identified in RT-PCR clones. Out of seven RT-PCR clones, three had a T and four had a C. The corresponding amino acid residue 347 therefore varies, being Cys or Arg, respectively. The 1051-15 cDNA clone has a T (Cys). Human α_{1C}-adrenoceptor cDNA clones have been reported by Hirasawa *et al.* (1993) and Weinberg *et al.* (1994). Both clones have a C (Arg) in this position.

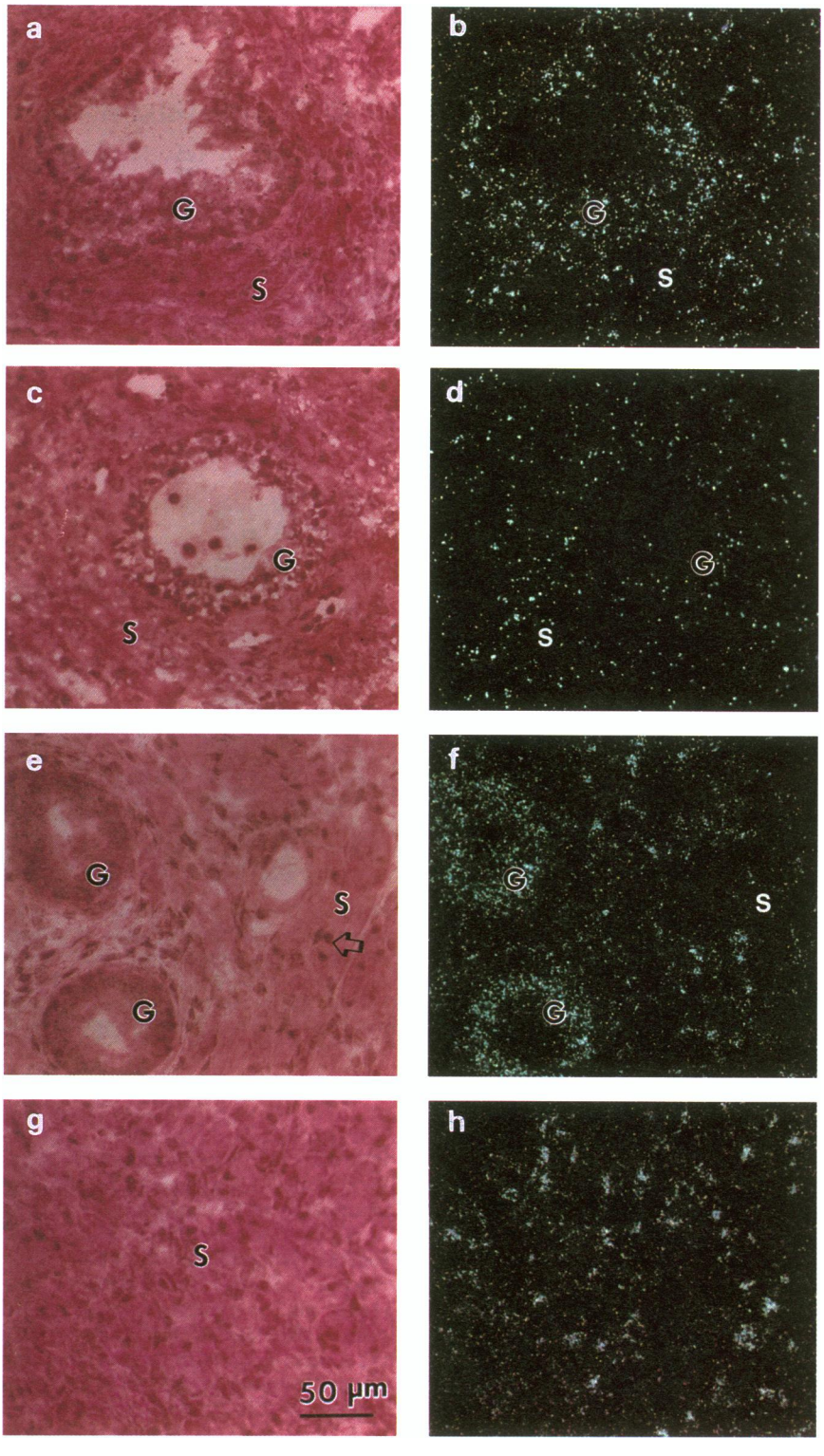

Figure 5 *In situ* hybridization of human BPH prostate tissue section, (a–d), and prostate cancer tissue section, (e–h). Panels (a), (c), (e) and (g) are bright-field pictures showing haematoxylin/eosin counter-stained prostate tissue. The epithelium glandular cells form secretory glands and are labelled G. The stroma cells surrounding the secretory glands appear elongated with a bundled morphology and are labelled S. Panels (b), (d), (f) and (h) are corresponding dark-field pictures to panels (a), (c), (e) and (g) and show silver grains resulting from hybridization to [^{35}S]-UTP-labelled RNA probe detected with photographic emulsion. Probes used are: panels (a) and (b), α_{1C} 5′ antisense probe; panels (c) and (d), α_{1C} 5′ sense probe; panels (e–h), α_{1C} 3′ antisense probe. The α_{1C} 5′ antisense and sense probes were *in vitro* transcribed from a 620 bp subclone derived from the cDNA clone α_{1C} 1.5 that covered 430 bp 5′ untranslated region and ended in transmembrane helix I. The α_{1C} 3′ antisense probe was *in vitro* transcribed from cDNA clone pdT8 that covered 243 bp of C-terminal coding region and 1.5 Kb of 3′ untranslated sequences (see Figure 1). All of the photographs were taken at 250 × magnification. The 50 μm scale bar in (g) applies to all eight panels.

Conserved intron in α_{1B} and α_{1C} genes

Additional human cDNA libraries were screened for α_{1B} and α_{1C} clones. A hippocampus library produced one clone each for α_{1B} and α_{1C} (1088-7); and three α_{1C} clones were isolated from a liver primary library (15-2, 18, and 29) (Figure 2). All five clones contain 5′ coding sequences that end in transmembrane helix VI. Apparently, 1088-7 is derived from a cripple transcript that does not contain a consensus exon/intron junction and has an in frame stop codon 19 bp after the splice site. The hippocampus α_{1B} and liver α_{1C} clones have consensus exon/intron junctions. It is noteworthy that the intron sequences immediately after the splice site are conserved between α_{1B} and α_{1C}, indicating a close evolutionary lineage for these two genes (Figure 2).

Northern analysis

A Northern blot (Figure 3) of human polyA$^+$ RNAs was sequentially probed with three human α_1-adrenoceptor cDNAs (α_{1B}, α_{1C}, and α_{1D}). The α_{1C} mRNA was at least 10 times more concentrated in liver than in prostate, with heart in between. Four bands, 6, 4, 3 and 2 Kb, were found in liver RNA, the higher 3 bands found in heart RNA, and the 6 Kb and 4 Kb bands were detected with prostate RNA. An α_{1B} mRNA of 2.6 Kb was found in descending order of concentration: aorta > > brain > liver > heart > prostate = skeletal muscle. For the α_{1D} mRNA, a 2.8 Kb band is strong in aorta, intermediate in prostate, and weak in brain. A weak α_{1D} 1.4 Kb band was reproducibly detected in brain but not in other tissues.

The prostate is composed of two major cell types, stromal and glandular cells (Bartsch *et al.*, 1979a,b). PolyA$^+$ RNAs were prepared from primary cultures of human prostate stromal cells and from two human prostate cancer cell lines PC-3 and Du145, both of prostate cancer glandular cell origin. The stromal primary cells displayed smooth muscle cell morphology of very long and spindle shaped cells of over 95% purity. Northern blots of stromal, PC-3, and Du145 polyA$^+$ RNAs were probed sequentially with human α_{1B}, α_{1C}, and α_{1D} probes (Figure 4). Both α_{1B} and α_{1D} probes hybridized to the glandular cells, but not to the stromal cells (α_{1B} to Du145 and α_{1D} to PC-3.); α_{1C}, however, hybridized to all three cells. The level of α_{1C} expression was higher in PC-3 and Du145 cells than in stromal cells. Northern analysis of cultured cells indicated only α_{1C} mRNA expression in the stroma cells. The α_{1B} and α_{1D} expression in glandular cells PC-3 and Du145 was inconsistent, possibly due to a cell line artifact that mRNA expression in these cell lines may not reflect the tissue that the cell lines were derived from.

In situ *hybridization*

In situ hybridization confirmed the Northern blot result of α_{1C}-adrenoceptor mRNA expression in both stromal and glandular cells of prostate tissue from BPH patients (Figure 5a and b). Expression in glandular cells is more apparent than in

Table 1 Ligand affinities in human prostate membranes and recombinant α_1-adrenoceptor subtypes expressed in Rat-1 fibroblast cells

	pK_i			
Compound	*Human α_{1B}*	*Human α_{1C}*	*Human α_{1D}*	*Human prostate*[a]
Prazosin	10.08 ± 0.04	9.88 ± 0.05	9.88 ± 0.16	9.59 ± 0.08
Phentolamine	7.50 ± 0.02	8.25 ± 0.03	7.64 ± 0.03	8.21 ± 0.02
(+)-Niguldipine	6.86 ± 0.08	7.76 ± 0.06	6.76 ± 0.07	9.28 ± 0.04
(−)-Niguldipine	7.09 ± 0.04	7.38 ± 0.06	6.78 ± 0.05	7.86 ± 0.09
5-CH_3-urapidil	7.46 ± 0.03	8.98 ± 0.04	7.91 ± 0.03	8.51 ± 0.15
N-CH_3-spiperone	8.36 ± 0.06	7.35 ± 0.04	7.83 ± 0.06	7.34 (n = 1)
WB-4101	8.62 ± 0.04	9.54 ± 0.03	9.33 ± 0.05	9.47 ± 0.02
Corynanthine	6.94 ± 0.06	6.95 ± 0.02	7.28 ± 0.06	6.82 ± 0.01
Indoramin	7.86 ± 0.02	8.33 ± 0.05	7.07 ± 0.08	8.17 ± 0.07
Oxymetazoline	7.13 ± 0.02	8.29 ± 0.01	7.96 ± 0.07	8.19 ± 0.02
Methoxamine	3.84 ± 0.32	4.75 ± 0.10	4.80 ± 0.14	4.55 ± 0.09
Rauwolscine	6.20 ± 0.02	5.53 ± 0.07	6.49 ± 0.04	4.93 ± 0.08

Data (in pK_i values) are expressed as mean ± standard error from at least 3 experiments. The only exception is for N-CH_3-spiperone that only one experiment has been done for human prostate membrane.
[a]The pK_i values of human prostate membrane have been used in a previous report (Goetz *et al.*, 1994). These data are included here for comparison purposes.

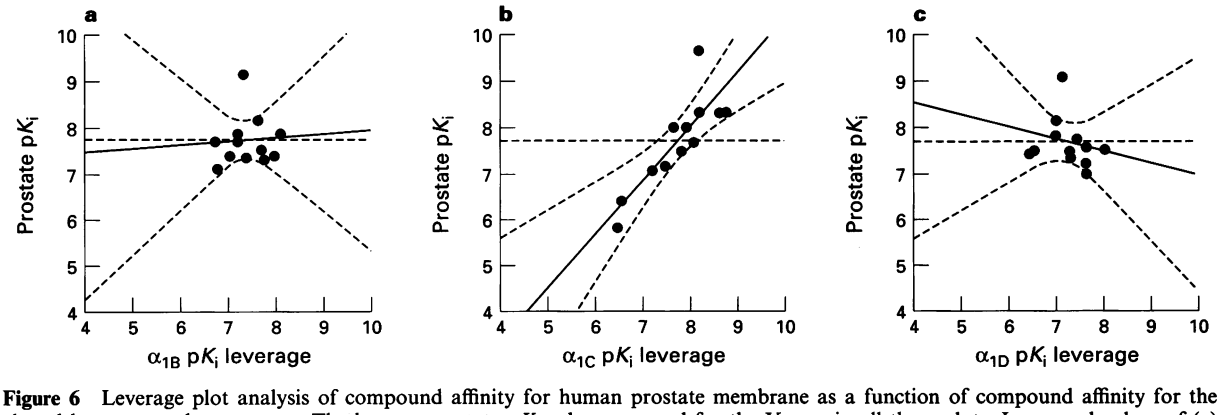

Figure 6 Leverage plot analysis of compound affinity for human prostate membrane as a function of compound affinity for the cloned human α_1-adrenoceptors. The human prostate pK_i values are used for the Y-axes in all three plots. Leveraged values of (a) human α_{1B}, (b) human α_{1C}, and (c) human α_{1D} are used for the X-axes. The pK_i values listed in Table 1 are used in these leverage plot analyses. The horizontal dotted line indicates the mean response (compound affinity for human prostate). The solid lines represent the fit of the response to the predictors (compound pK_i for cloned human α_1-adrenoceptors). Curved dotted lines represent 95% confidence intervals.

stromal cells. Expression in glandular cells was evenly distributed, but with a large variation in the signal level among the five patients tested. The expression pattern for the stromal cells was very different from the even distribution pattern of the glandular cells. Stromal cells expressing α_{1C}-adrenoceptor were scattered in the smooth muscle tissue, with only a small percentage of stromal cells displaying signal. The expression level of α_{1C} in the stromal cells appeared highly variable. No signal could be detected in 4 out of 6 patient tissues tested. Control experiments of serial sections as in Figure 5a and b probed with sense RNA probe showed no detectable signal above background (Figure 5c and d).

Robust α_{1C} expression was detected in prostate tissue sections from three prostate cancer patients (Figure 5e-h). The expression pattern of even distribution in glandular cells and scattered distribution in stroma cells was similar for both BPH and prostate cancer tissues. As in BPH prostatic tissue, in prostate cancer tissue the α_{1C} signal level in stromal cells was also more variable than in glandular cells. The difference between BPH and prostate cancer seems only in the high level of expression in prostate cancer tissue.

Binding of [^{125}I]-I-HEAT to membranes from human prostate, and cloned human α_1-adrenoceptor subtypes expressed in Rat-1 stable cell lines

Analysis of saturation binding experiments yielded a best fit of the data by a one-site model for all receptor sources examined. A number of structurally different compounds that act as antagonists or agonists of α_1-adrenoceptors were examined for

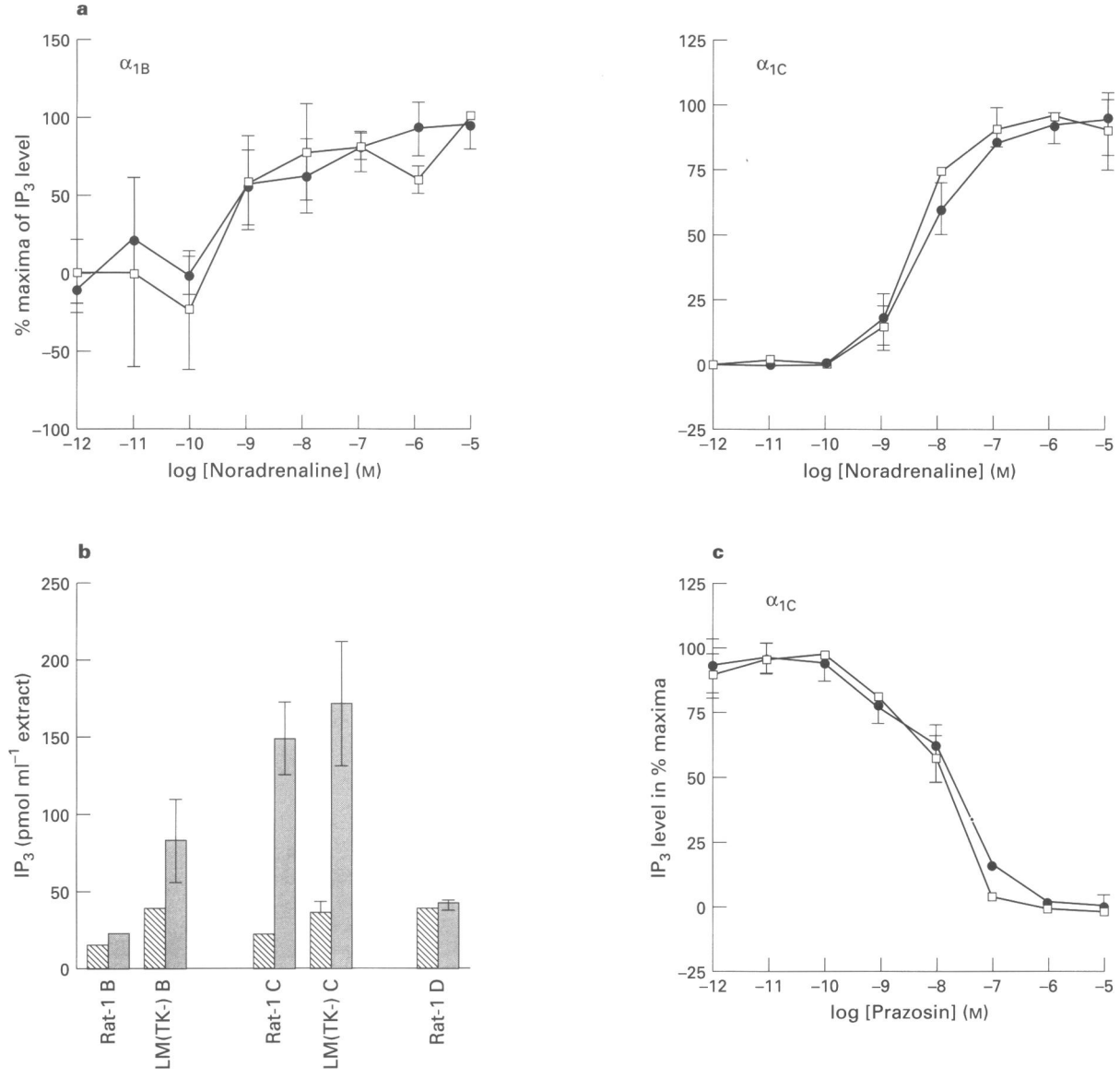

Figure 7 Coupling of α_1-adrenoceptor to phospholipase C activation as measured by IP_3 assay. (a) Dose-response curves in response to noradrenaline. The IP_3 levels were obtained as pmol ml^{-1} of extract. For each noradrenaline concentration, the IP_3 level was subtracted from the basal IP_3 level (0 M noradrenaline, measured for each experiment) to obtain the net increase in IP_3 concentration. This net IP_3 concentration was normalized by calculating as % of the maximal IP_3 concentration observed in each experiment. Each data point is the mean (with s.e.mean) value from three independent experiments. Incubation time for α_{1B} was 3 min and for α_{1C} was 2 min. The incubation time was determined by a time curve from each cell line in response to treatment with 100 μM noradrenaline. (b) Graph of direct IP_3 concentration in response to 0 M (hatched column) or 100 μM noradrenaline (stippled column) for the three α_1-adrenoceptors in Rat-1 and LM(TK-) cell lines. Noradrenaline treatment times were 3 min for α_{1B}, 2 min for α_{1C}, and 15 min for α_{1D}. (c) Dose-response curves of inhibition by prazosin. Noradrenaline at a concentration of 5 μM was included in the incubation buffer for each reaction. Incubation time was 2 min. Data were normalized as described in (a). Each point is the mean (with s.e.) of three experiments. Symbols used for (a) and (c) are: (□) Rat-1 cells; (●) L-M(TK-) cells.

their ability to inhibit [^{125}I]-I-HEAT binding. None of the compounds tested showed improvement of fit of binding to human prostate membranes by a two-site model over a one-site model. 5-CH_3-urapidil, oxymetazoline, phentolamine and indoramin had higher affinity for human prostate and α_{1C}, but lower affinity for α_{1B} and α_{1D}. (+)-Niguldipine displayed stereo-selectivity for human prostate and α_{1C}, but not for α_{1B} and α_{1D}. The α_1 antagonist, WB-4101 and the partial agonist, methoxamine, bound with higher affinity to human prostate, α_{1C}, and α_{1D}, but with lower affinity to α_{1B}. N-CH_3-spiperone had higher affinity for α_{1B} and lower affinity for α_{1C}, α_{1D}, and prostate. Several compounds, prazosin, corynanthine, and (-)-niguldipine, showed no selectivity. A summary of the data is presented in Table 1.

Leverage plot comparisons

To identify which subtype binding affinity is most predictive of human prostate, a linear model which included all three subtype pK_i values as potential predictors was fit to the prostate pK_i values. The p values were calculated with all three subtype pK_i in the linear model as the regressor which was used to build the leverage plots. For human prostate, the α_{1C} pK_i values were the most significant predictor (α_{1C}, $p = 0.0018$; α_{1B}, $p = 0.85$; and α_{1D}, $p = 0.53$). Inclusion of pK_i values from additional ligands did not augment the predicative ability of the α_{1C} pK_i.

These results were further tested by leverage plots. Leverage plot is a useful tool for graphical assessment of linear models and hypothesis testing. The leverage plots of binding in the human prostate as a function of binding in the cloned receptors (Figure 6) showed that neither the α_{1B} nor α_{1D} pK_i had a significant effect on the model's ability to predict binding in the prostate above and beyond the α_{1C} pK_i.

IP_3 production

Rat-1 and L-M(TK-) stable cell lines expressing human α_{1B}-, α_{1C}- and α_{1D}-adrenoceptors were treated with noradrenaline and tested for IP_3 production (Figure 7a and b). Activation of IP_3 formation by noradrenaline in α_{1B} and α_{1C} cell lines was concentration-dependent (Figure 7a), with EC_{50} values of 6×10^{-8} for α_{1B} and 5×10^{-7} M for α_{1C}. These EC_{50} values reflected the sensitivity of α_1-adrenoceptor subtypes to the agonist, and were consistent for the receptor subtypes regardless of the cell type used. The α_{1B} receptor is ten times more sensitive to noradrenaline than the α_{1C} receptor. Coupling efficiencies were different for the three adrenoceptor subtypes (Figure 7b). α_{1C} produced the highest IP_3 levels stimulated by noradrenaline (5–8 fold), which were consistent between the two stable cell lines. For α_{1B}, the IP_3 level in response to noradrenaline treatment was twice that of the basal level in L-M(TK-) cells, while only a 50% increase was stimulated in Rat-1 cells. For Rat-1 cells expressing α_{1D}, noradrenaline did not alter the IP_3 level after prolonged treatment (Figure 7b). Noradrenaline stimulated IP_3 production in α_{1C} cell lines was inhibited by the α_1 antagonist prazosin in a concentration-dependent manner (Figure 7c), with IC_{50} values of 1.5×10^{-8} M for Rat-1 cells and 2×10^{-8} M for LM(TK-) cells.

Discussion

α_{1C}-Adrenoceptor and BPH

Identification of the α_1-adrenoceptor subtype(s) responsible for the prostate smooth muscle tone that contribute to BPH symptoms has important clinical implications. This task has been addressed by means of molecular biology, pharmacology, and functional studies.

In this report, we identify the α_1-adrenoceptor subtypes expressed in human prostate by degenerate PCR cloning. Northern analysis, and *in situ* hybridization. The α_{1B}, α_{1C}, and α_{1D} subtypes were found in human prostate glandular cells, while α_{1C} was the only subtype identified in stromal cells, which are the smooth muscle cell type. This was first demonstrated with Northern blots using specific human prostate cell cultures (primary cultures of human BPH stromal cells and two established cultures of human prostate cancer glandular cells PC-3 and Du145); and then with *in situ* hybridization of prostate tissue sections (Figures 4 and 5). Attempts to identify the α_1-adrenoceptor subtypes by Northern analysis using human prostate tissues largely failed, due to tissue heterogeneity. Other than the fibromuscular stroma cells and the secretory glandular cells, the prostate is rich in blood vessels, nerve fibres and elastic tissue (Fawcett, 1986; Eroschenko, 1989). Tissue samples obtained by surgery may also be contaminated by ejaculatory duct, prostatic utricle, and seminal vesicle. Northern analysis of this nature provides little useful information, except that all three α_1-adrenoceptor subtypes exist in human prostate. Similar caution should also be applied to the interpretation of reports using techniques such as RT-PCR and RNAse protection assay to identify α_1-adrenoceptor subtypes in prostate and other tissues (Price *et al.*, 1993; 1994; Rokosh *et al.*, 1994).

The pharmacological binding profiles of the cloned human adrenoceptor subtypes also indicated the resemblance of α_{1C} to the human prostate membrane (Table 1). This was shown clearly with the leverage plots (Figure 7) that upon removal of the effect generated by related receptor subtypes, the binding profile of human prostate membrane became colinear only with α_{1C} and not with α_{1B} and α_{1D} adrenoceptors. These data supported a major presence of α_{1C} adrenoceptors in human prostate.

The α_1-adrenoceptors couple to a pertussis toxin-insensitive G-protein and phospholipase C, mediating noradrenaline stimulated inositol phosphate turnover and intracellular release of Ca^{2+} from endoplasmic reticulum through the IP_3-gated Ca^{2+} release channels (Lomasney *et al.*, 1991b; Garcia-Sainz, 1993). In smooth muscle cells α_1-adrenoceptors mediate noradrenaline stimulated contraction by increasing the intracellular Ca^{2+} concentration through the IP_3 pathway. An IP_3 production assay using Rat-1 and L-M(TK-) cells each stably expressing the three cloned α_1-adrenoceptor subtypes indicated different coupling efficiencies of the adrenoceptor subtypes to the IP_3 pathway (Figure 7). The α_{1C} subtype coupled to the IP_3 pathway most efficiently, followed by α_{1B}, while no significant increase in IP_3 level could be detected for α_{1D}. It was unlikely that this difference in coupling efficiency was biased by the receptor expression level, since the Rat-1 stable cell lines expressed approximately equal numbers of α_{1B}- and α_{1C}-adrenoceptors as measured by ligand saturation experiments. A recent report correlating the pharmacological profiles of the three cloned human α_1-adrenoceptor subtypes with the ability of α_1 antagonists to inhibit human prostatic muscle contraction also suggested a major role played by the α_{1C} subtype in the control of prostate smooth muscle tone (Forray *et al.*, 1994).

Taken together, three lines of experimental results, (1) the distribution of the α_1-adrenoceptor mRNA in prostate cell types, (2) the comparison of the pharmacological binding profiles of the three cloned α_1-adrenoceptors to human prostate, and (3) the coupling efficiency of the α_{1C}-adrenoceptor to the IP_3 pathway, support a functional role for the α_{1C}-adrenoceptor subtype in mediating adrenaline-stimulated prostate smooth muscle tone that is partially responsible for the BPH clinical symptom. These results indicate that a α_{1C} specific antagonist should be efficacious in relieving the clinical symptoms of BPH.

Messenger RNA size variation and instability

Human α_{1C} mRNA is highly variable in size, even when isolated from the same tissue but from different samples. For example, in Figure 3 the α_{1C} mRNA in prostate was 6 and 4 Kb, but in stroma, PC-3, and Du145 cells only 6 and 2 Kb mRNA were found instead (Figure 4). The human prostate

RNA used for RT-PCR and cDNA library construction had 6, 4 and 3 Kb bands (data not shown). In human liver, the three samples tested gave different patterns. In Figure 3, human liver had 4 bands (6, 4, 3, and 2 Kb). In another sample only the 3 higher bands were found. The human liver RNA used for primary cDNA library construction (Figure 2), however, had only a 3 Kb band (data not shown). Since all three cDNA clones isolated from this liver library contained an intron (Figure 2), the 3 Kb band is an incompletely processed transcript. α_{1B} and α_{1D} mRNA sizes, in contrast, were consistent in all the human tissues and human cell lines tested.

Interestingly, α_{1C} was the only subtype that shows RNA instability in PC-3 and Du145 cells. RNAs isolated from confluent cultures showed degradation for α_{1C} but not for α_{1B} and α_{1D} (data not shown). This observation may help explain the discrepancy of protein and RNA expression in prostate glandular cells. Autoradiography using [^{125}I]-I-HEAT binding to human prostate indicates low α_1-adrenoceptor level in glandular tissues (Kobayashi *et al.*, 1991), whereas substantial α_{1C} RNA was detected by Northern blots using cell lines and by *in situ* hybridization (Figures 4 and 5).

α_1-*Adrenoceptors in prostate cancer*

In situ hybridization and Northern blot analysis (Figures 4 and 5) provides unequivocal evidence of α_{1C} expression in prostate stroma and glandular cells in both BPH and prostate cancer tissue. Northern blot analysis (Figure 4) also suggests that the α_{1B}- and α_{1D}-adrenoceptors are expressed in the glandular cells of prostate cancer tissue. A common phenomenon for both BPH and prostate cancer is an increase in cell number. A number of seven transmembrane receptors that couple to the phospholipase C/inositol phosphate turnover pathway act like proto-oncogenes in an agonist-dependent manner, including the α_{1B}-adrenoceptor, muscarinic receptor, and 5-HT_{1c} receptor (Julius *et al.*, 1989; Allen *et al.*, 1991; Gutkind *et al.*, 1991). The finding that α_1-adrenoceptors are expressed in malignant prostate cells indicates a possible stimulatory role that α_1-adrenoceptors may play in cancer cell growth. However, it is also possible that uncontrolled cell growth increases the expression level of many genes, and heightened α_1-adrenoceptor expression in prostate cancer cells is a nonspecific effect. Whatever the case, heightened α_1-adrenoceptor expression could probably increase cell growth rate. α_1-Adrenoceptors in BPH, likewise, may also contribute to hyperplasia. In cardiac myocytes, α_1-adrenoceptors have been shown to stimulate cardiac myocyte hypertrophy (Simpson *et al.*, 1991; Ardati & Nemer, 1993; Kariya *et al.*, 1993; Knowlton *et al.*, 1993). It is possible that long-term treatment of BPH by an α_1-antagonist will not only reduce the smooth muscle tone of prostate, but also help reduce prostate hypertrophy.

We thank Drs John Gray and Joel E. Shaffer for helpful suggestions, Dr Richard Buckholz for a full-length human α_{1D}-adrenoceptor cDNA clone, and Dr John Bruno for a human α_{1D}-adrenoceptor cDNA probe. We thank G. Bruce Wisely, Sue H. Kadwell, and William C. Clay for help in receptor clone constructs and expression. We thank Timothy A. True and Holly K. King for their role in the process of the stable cell line selection.

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(Received December 8, 1994
Revised March 16, 1995
Accepted March 31, 1995)