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α_{1a}-Adrenoceptor polymorphism: pharmacological characterization and association with benign prostatic hypertrophy

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1 Two restriction fragment length polymorphisms of the human α_{1a}-adrenoceptor gene digested with PstI restriction enzyme exist; the nucleotide change causes the substitution of C residue for T at nucleotide 1441, thereby Arg492 to Cys492 transition, which might confer an additional putative palmitoylation site in the carboxy-terminal segment of the α_{1a}-adrenoceptor. In the present study, we compared their pharmacological properties and examined whether this α_{1a}-adrenoceptor polymorphism is associated with benign prostatic hypertrophy (BPH).

2 The frequency of α_{1a}-adrenoceptor polymorphism was not differently distributed between patients with benign prostatic hypertrophy (BPH) and normal subjects in Japan; thus, the relative frequencies of the C and T alleles were 0.90 : 0.10 in normal male subjects ($n=45$) and 0.87 : 0.13 in BPH patients ($n=222$), respectively. However, the frequency distribution of this polymorphism was significantly different between the Japanese and U.S. populations; thus, C and T alleles were 0.34 and 0.66 in U.S. populations.

3 Utilizing Chinese hamster ovary (CHO) cells stably expressing the two polymorphic α_{1a}-adrenoceptors (Arg492 and Cys492), we compared their binding affinity and signal transduction. Radioligand binding studies with 2-[β-(4-hydroxy-3[^{125}I]-iodophenyl) ethylamino-methyl]tetralone ([^{125}I]-HEAT) showed no marked difference in the antagonist or agonist binding affinities between the two receptors. Also, both receptors were found to be coupled to the calcium signaling, and the concentration-cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) response relationships for noradrenaline were similar for the two polymorphic receptors. Furthermore, the receptor-mediated $[Ca^{2+}]_i$ response was markedly desensitized after a 2 h exposure of phenylephrine (10 μM), and the extent of the desensitization was not significantly different between the two receptors.

4 In summary, the results showed that the two α_{1a}-adrenoceptors generated by genetic polymorphism have similar pharmacological characteristics, and the receptor-mediated $[Ca^{2+}]_i$ response can be desensitized in a similar manner. The study did not provide any evidence to support the hypothesis that α_{1a}-adrenoceptor gene polymorphism is associated with BPH.

Keywords: Benign prostatic hypertrophy; α_{1a}-adrenoceptor; restriction fragment length polymorphisms; polymorphism; palmitoylation; polymerase chain reaction; radioligand binding study; cytosolic Ca^{2+} concentrations

Introduction

Benign prostatic hypertrophy (BPH) is an age-related and progressive neoplastic condition of the prostate gland. An epidemiological survey has suggested relationships of race, nationality, sociocultural variables, specific serum condition (eg. high level of serum cholesterol or phospholipid), geriatric disease, such as coronary heart disease, cerebrovascular disease, hypertension, diabetes mellitus to the onset of BPH (Rotkin, 1983). Also, the genetic susceptibility of BPH has been reported in recent years (Sanda *et al.*, 1994). However, the molecular pathogenesis of this disorder is still poorly understood.

Although surgical treatment (transurethral resection of the prostate) has been widely used as an effective treatment of BPH, medical therapy is of more importance. Of the agents used for BPH treatment, selective α_1-adrenoceptor antagonists have received increasing attention, and the α_1-adrenoceptor is implicated in the pathophysiology of the bladder outlet obstruction caused by BPH by controlling prostatic smooth muscle tone (Caine *et al.*, 1975). α_1-Adrenoceptors belong to the superfamily of G protein-coupled receptors with seven transmembrane domains, and comprise a heterogeneous family. Molecular biological studies have identified three subtypes of α_1-adrenoceptors (α_{1a}, α_{1b}, α_{1d}) (Lomasney *et al.*, 1991; Ramarao *et al.*, 1992); Hirasawa *et al.*, 1993). Among subtypes, human prostate has been shown to express predominantly α_{1a}-adrenoceptors, and these mediate the contractile response, indicating that the α_{1a}-adrenoceptor may play an important role in the pathogenesis and treatment of BPH (Lepor *et al.*, 1993; Price *et al.*, 1993; Forray *et al.*, 1994).

Recent studies with recombinant receptor proteins have localized certain functional domains within specific regions of the receptor sequence. In particular, the third cytoplasmic loop region of G protein-coupled receptors has been shown to determine specificity for a particular G protein and mutations in the segment are found to lead the constitutive activation of the receptor/signal transduction, which eventually results in the cellular mitotic stimulation (Cotecchia *et al.*, 1990; Allen *et al.*, 1991; Kjelsberg *et al.*, 1992). Also, lipid modification (such as palmitoylation) of the receptor protein in the cytoplasmic carboxy-terminal tails appears to play a key role in receptor localization and function (O'Dowd *et al.*, 1988; Moffett *et al.*, 1993; Moench *et al.*, 1994). In those regions of G protein-

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coupled receptors, a number of human diseases have now been mapped and found to be due to either deletions, mutations or polymorphism (Alonso *et al.*, 1993; Shenker *et al.*, 1993; Kosugi *et al.*, 1994).

We have recently observed that two restriction fragment length polymorphisms (RFLPs) of the α_{1a}-adrenoceptor gene exist in man. The nucleotide change causes the substitution of C residue for T at nucleotide 1441, thereby Arg492 to Cys492 transition. Since Cys490 is postulated to be a palmitoylation site, the polymorphism results in an additional putative palmitoylation site in the carboxy-terminal segment of the α_{1a}-adrenoceptor. Hence, in the present study we have examined whether PstI-polymorphism of the α_{1a}-adrenoceptor may be associated with BPH patients and might be a pathogenic factor. Since palmitoylation in the intracellular carboxy-terminal tails of the G-protein-coupled receptor plays a key role in their cellular localization and function (O'Dowd *et al.*, 1988; Moffett *et al.*, 1993; Moench *et al.*, 1994), we further examined whether the two polymorphic receptors have different functions and regulation. Utilizing Chinese hamster ovary (CHO) cells stably expressing each α_{1a}-adrenoceptor (Arg492 and Cys492), we compared their pharmacological properties. Additionally, as described above, mutations in the third cytoplasmic loop have been shown to induce the constitutive activation of the receptor/signal transduction, thereby resulting in agonist-independent hyperfunction and eventually tumourigenicity. The 'oncomutant' effects are inhibited by the α_1-adrenoceptor antagonist prazosin, reminiscent of the BPH clinical picture (Cotecchia *et al.*, 1990; Allen *et al.*, 1991; Kjelsberg *et al.*, 1992); hence we also looked for a possible mutation in the third intracellular loop of the α_{1a}-adrenoceptor gene.

Throughout this paper, we have used the standardized nomenclature system for α_1-adrenoceptor subtypes recently recommended by the IUPHAR Committee on the Classification of Adrenoceptors (Hieble *et al.*, 1995). In this system, the cloned subtypes are designed in lower case letters as α_{1a}, α_{1b} and α_{1d} which correspond to the clones previously defined as α_{1c}, α_{1b} and α_{1a} (or $\alpha_{1a/d}$ and α_{1d}), respectively. The corresponding pharmacological subtypes are designated by upper case letters and are defined as α_{1A}, α_{1B} and α_{1D}, respectively.

Methods

Subjects

Two hundred and twenty two unrelated Japanese patients with clinically defined BPH (mean age 72, rang 51–90) were enrolled in this study from the Department of Urology, the University of Tokyo and affiliated hospitals. All had clinical symptoms for more than 2 years. After written informed consents had been obtained, blood samples were collected for α_{1a}-adrenoceptor gene analysis. Forty-five unrelated, normal male subjects, who had no known medical illness or family history of BPH, hypertension, or cardiac or endocrine disorders and were taking no medications, acted as controls.

DNA amplification and genotyping

Blood (5 ml) from each patient or control subject was drawn in sodium citrate and the lymphocytes were isolated by Ficoll gradients. Genomic DNA was prepared by standard procedures. To analyse the presence of PstI-polymorphism in the α_{1a}-adrenoceptor gene, amplification of a 502 bp (1417 through 1918) region encompassing position 1441 of the α_{1a}-adrenoceptor subtype gene was performed by polymerase chain reaction (PCR) with 100 ng of genomic DNA and 0.625 μmol of each oligonucleotide primer (P1; 5'-ATGCTCCAGCCAAGAGTTCA-3', and P2; 5'-TCCAAGAAGAGCTGGCCTTC-3') in 25 μl. The PCR amplification profiles consisted of denaturation at 94°C for 1 min, primer annealing at 55°C for 30 s, and extension at 72°C for 1 min, for 30 cycles. Negative controls without any template and positive controls of different PstI-polymorphism (cDNA) were routinely included in PCR amplifications with each primer set. Ten microlitres of the PCR product were digested with 10 unit of restriction enzyme PstI (TaKaRa, Kyoto, Japan) in a total volume of 20 μl. Digested DNA was separated by electrophoresis on an 5% polyacrylamide gel at 150 V for 30 min. Bands were visualized by ethidium bromide staining.

Cloning and expression of the human α_{1a}-adrenoceptors

Cloning of the human α_{1a}-adrenoceptor gene (Arg492) was performed as described previously (Hirasawa *et al.*, 1993), and polymorphic α_{1a}-adrenoceptor (Cys492) gene was also cloned at the same time. The 2.1 kb- full-length coding region, including 436 bp of 5' untranslated sequence and 468 bp of 3' untranslated sequence, was ligated into the EcoRI site of the eukaryotic expression vector pSVK3 containing the neomycin-resistance gene of pMAM-neo (pSVK3neo). Stable expression of each polymorphic human α_{1a}-adrenoceptor gene was performed as described previously (Horie *et al.*, 1994). Briefly, stable cell lines were obtained by transfection of the pSVK3neo containing each type of human α_{1a}-adrenoceptor cDNA construct (Arg492 and Cys492) into CHO-K1 cells, by use of the Lipofectin technique as described previously (Horie *et al.*, 1994). Cells were grown as monolayers in Ham's F-12 medium (Gibco, Grand Island, U.S.A.) containing L-glutamine supplemented with 10% foetal bovine serum, penicillin (100 units ml^{-1}) and streptomycin (100 μg ml^{-1}). Stable clones were then selected for resistance to G418 (600 μg ml^{-1}) as described previously.

Membrane preparation and [^{125}I]-HEAT binding

Subconfluent 150 mm plates of transfected cells were washed twice with 10 ml of phosphate buffered saline (PBS, composition (mM); NaCl 139, KCl 2.7, Na_2HPO_4 8.8, KH_2PO_4 1.48, pH 7.5), and harvested by scraping. Cells were pelleted by centrifugation at 500 × *g* for 5 min, washed, and the pellet was homogenized in 2 ml of ice-cold buffer A (sucrose 250 mM, Tris HCl 5 mM, $MgCl_2$ 1 mM, pH 7.4) and centrifuged at 1,000 × *g* at 4°C for 10 min to remove nuclei. The supernatant was then centrifuged at 35,000 × *g* for 20 min at 4°C and the pellet homogenized, and was frozen at −80°C until assay. The protein concentration was measured by the bicinchoninic acid protein assay kit (PIERCE, Rockford, U.S.A.).

[^{125}I]-HEAT binding

Radioligand binding studies were performed as described previously (Shibata *et al.*, 1995). Briefly, in the radioligand binding assay buffer B (Tris HCl 50 mM, $MgCl_2$ 10 mM, EGTA 10 mM, pH 7.4) was used in the incubation medium. Measurement of specific [^{125}I]-HEAT binding was performed by incubating 0.1 ml of membrane preparation (~10 μg of protein) with [^{125}I]-HEAT (2,200 Ci $mmol^{-1}$) in a final volume of 0.25 ml buffer B for 60 min at 25°C in the presence or absence of competing drugs. The incubation was terminated by adding ice-cold buffer B and immediately filtering through Whatmann GF/C glass-fibre filters with a Brandel cell harvester (Model-30, Gaithersburg, U.S.A.). Each filter was collected and the radioactivity measured. Binding assays were always performed in duplicate. For competition curve analysis, each assay contained about 70 pM [^{125}I]-HEAT. At this concentration nonspecific binding, defined as binding displaced by 10 μM phentolamine, represented less than 10% of total binding. In some experiments, the incubation was performed in buffer B containing 200 μM Gpp(NH)p to examine the effects of guanine nucleotides on agonist binding affinity.

Measurement of $[Ca^{2+}]_i$

The transfected cells at 50% confluency in 100 mm culture dishes were trypsinized and washed twice with a buffered salt

solution (BSS, composition (mM): NaCl 140, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.25, Na_2HPO_4 1, HEPES 5, glucose 11, pH 7.4), and incubated in the buffer containing 4 μM fura-2/AM for 30 min at 25°C. The cells were then washed twice and resuspended in BSS buffer without the dye. Mobilization of $[Ca^{2+}]_i$ evoked by various concentrations of agonists was monitored by a JASCO CAF-110 fluorescence spectrophotometer (Nihon Bunkoh, Tokyo, Japan) with dual excitation at 340 nm/380 nm and emission at 500 nm (Horie *et al.*, 1994). Agonists induced an acute $[Ca^{2+}]_i$ increase in the transfected cells that was followed by lower plateau $[Ca^{2+}]_i$ levels. The peak $[Ca^{2+}]_i$ values from the initial transients were used to generate the dose-response curves.

$[Ca^{2+}]_i$ was calculated based on the following formula (Grynkiewicz *et al.*, 1985): $[Ca^{2+}]_i = K_D\ S_{f380/b380}\ (R-R_{min})/(R_{max}-R)$ where K_D is 225 nM in the cytosolic environment, $S_{f380/b380}$ is the ratio of the intensities of the free and bound dye forms at 380 nm, R is the fluorescence ratio (340 nm/380 nm) of the intracellular fura-2, and R_{min} and R_{max} are the minimal and maximal fluorescence ratios, respectively. Calibration of the fluorescence levels was performed for every aliquot by equilibrating intracellular and extracellular Ca^{2+} with 5 μl of 10% Triton-X 100 followed by addition of 5 μl of 300 nM EGTA/3 M Tris buffer (pH 9.0).

When concentration-response curves were being constructed, an individual batch of cells from the same cell line was examined by administration of an individual dose of agonist, but not by the method of stepwise cumulative addition. In order to minimize the effect of increasing basal levels of $[Ca^{2+}]_i$ when estimating the elevations of $[Ca^{2+}]_i$ the measurements were performed in the ascending order of concentration of agonists for the first series, and in the descending order for the second series, then the results from those two series in one experiment were averaged. $[Ca^{2+}]_i$ measurements were completed within one hour after loading of the cells, during that time the change in baseline $[Ca^{2+}]_i$ was less than 40 nM, and the responsiveness to noradrenaline was not noticeably altered.

Screening for mutations in the third intracellular loop of the α_{1a}-adrenoceptor

After informed consent had been obtained, freshly enucleated prostate tissues were obtained from eight patients with open prostatectomy, frozen in liquid nitrogen, and stored at −80°C. Reverse transcriptase-polymerase chain reaction (RT-PCR) assay was performed as described previously (Hirasawa *et al.*, 1993). Briefly, total cellular RNA was extracted from enucleated prostate tissue by the caesium chloride gradient method. RNA 10 μg was treated with RNase free DNaseI, then reverse transcribed, and cDNA was prepared. Amplification by PCR with primer (P3; 5'-CTCTGCATCATCTCCATCGACCGCTAC-3', and P4; 5'-ACGAGGAGCCGGGCTACGTGCTCTT-3') yields a fragment of 205 bp, which encodes the third intracellular loop of α_{1a}-adrenoceptor. Fragments of PCR products were gel purified, inserted into pBluescript II KS(+) (Stratagene, La Jolla, CA, U.S.A.), and subcloned clones were analysed by the ABI 373A DNA Sequencer (Applied Biosystems, Inc., Foster City, CA, U.S.A.) by use of a fluorescent dideoxy method (Prober *et al.*, 1987).

Statistical analysis

A two-way analysis of variance with 99% confidence limits was performed, followed by Student's t test on continuous variables, and categorical variables were compared by Chi-square analysis. Computer analyses with LIGAND (Munson & Rodbard, 1980) were used to evaluate dissociation constant and receptor density. In $[Ca^{2+}]_i$ response experiments, the EC_{50} value was determined with a sigmoidal function by analytical software SigmaPlot (Jandel Scientific., San Rafael, U.S.A.). Values are expressed as means ± s.e.mean.

Materials

Sources of drugs were as follows: [^{125}I]-HEAT (2-[β-(4-hydroxy-3-[^{125}I]-iodophenyl)ethylamino-methyl]tetralone) (Du Pont - New England Nuclear, Boston, U.S.A.); phenylephrine, (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (+)-adrenaline bitrate, Gpp(NH)p (5'guanylylimidodiphosphate) (Sigma, St. Louis, U.S.A.); WB4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4benzodioxane), 5-methylurapidil (Research Biochemicals Inc., Natick, U.S.A.); phentolamine HCl (Ciba-Geigy, Summit, U.S.A.); prazosin HCl (Pfizer, Brooklyn, U.S.A.); yohimbine HCl (Wako Pure Chemical Industries, Ltd., Osaka, Japan); fura-2/AM (fura-2 tetrakis (acetoxymethyl)ester) (Dojindo, Kumamoto, Japan); TritonX-100 (polyoxyethylene(10) octhylphenyl ether) (Wako, Osaka, Japan). All other chemicals were of reagent grade. The CHO-K1 cell line was obtained from American Type Culture Collection (Rockville, U.S.A.). The expression vectors pSVK3 and pMAM-neo were from Pharmacia (Uppsala, Sweden) and Clontech (Palo Alto, U.S.A.), respectively.

Results

Frequency of PstI-polymorphism

Figure 1 shows the presence of PstI-polymorphism for the α_{1a}-adrenoceptor gene from several Japanese subjects. As summarized in Table 1A, the relative frequencies of the C and T alleles were 0.90 : 0.10 in normal male subjects and 0.87 : 0.13 in BPH patients, respectively. The frequency of PstI-poly-

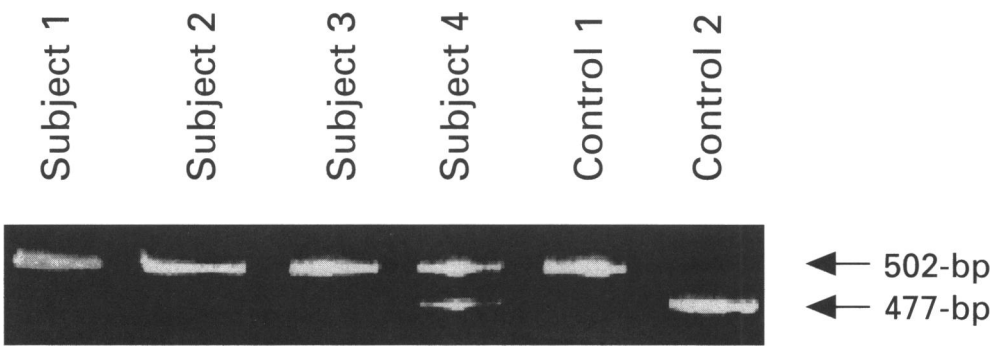

Figure 1 Restriction enzyme analysis of DNA from normal Japanese subjects. 502-bp PCR products encompassing position 1441 were prepared from four normal Japanese subjects as described in Methods, and digested with PstI. The C-T transition mutation creates an PstI site that, if digested, results in fragments of 25- and 477-bp. There were no PstI sites in Subjects 1–3, only Subject 4 was heteroplasmic for the PstI-polymorphism. Control 1; α_{1a}-adrenoceptor (Arg492) cDNA was used as template. Control 2; α_{1a}-adrenoceptor (Cys492) cDNA was used as template.

Table 1 Comparisons of frequency distribution of PstI-polymorphism of α_{1a}-adrenoceptor between (A) normal and BPH patients in Japan and (B) Japanese and the U.S.A. population

	Genotype			*Allele frequencies*	
Population	*C/C*	*C/T*	*T/T*	*C*	*T*
A					
Normal ($n=45$)	0.80	0.20	0.00	0.90	0.10
BPH ($n=222$)	0.75	0.25	0.00	0.87	0.13
				$\chi^2=0.01$ ($P>0.05$)	
B					
Japanese ($n=45$)	0.80	0.20	0.00	0.90	0.10
*U.S. population ($n=83$)	0.10	0.46	0.43	0.34	0.66
				$\chi^2=72.0$ ($P<0.01$)	

(A) DNA amplification and genotyping were performed as described in Methods. Values were compared by Chi-square analysis, and all P values are two-tailed; $n=$ number of specimens. (B) Values were compared by Chi-square analysis, and all P values are two-tailed; $n=$ number of specimens. *Data are from Hoehe *et al.* (1992).

morphism was not differently distributed between BPH patients and normal Japanese subjects. Interestingly, however, as summarized in Table 1B, the frequency distribution of this polymorphism was significantly different between the Japanese and U.S. populations (Hoehe *et al.*, 1992).

Binding studies with two polymorphic α_{1a}-adrenoceptors

We further examined whether the two polymorphic receptors have different pharmacological characters. We transfected and isolated CHO cells stably expressing each α_{1a}-adrenoceptor (Arg492 and Cys492), and compared their pharmacological properties. Membrane preparations from these CHO cells showed saturable binding of [^{125}I]-HEAT; B_{max} and K_D values for α_{1a}-adrenoceptor (Arg492) and α_{1a}-adrenoceptor (Cys492) were 1.3 ± 0.2 and 1.1 ± 0.1 pmol mg^{-1} protein ($n=4$ each), and 110 ± 20 and 95 ± 10 pM ($n=4$ each), respectively. The K_i values of α_1-adrenoceptor agonists and antagonists at each polymorphic α_{1a}-adrenoceptor are shown in Table 2. Consistent with their identity as α_1-adrenoceptors, both polymorphic α_{1a}- adrenoceptors showed a markedly low affinity for the α_2-selective antagonist yohimbine, whereas a high affinity for prazosin. α_1-Adrenoceptor subtype-selective antagonists WB-4101 and 5-methylurapidil were found to have relatively high affinity at both α_{1a}-adrenoceptors. The results indicate that both receptors have similar pharmacological characteristics to the α_{1a}-adrenoceptor, but show no marked differences in their agonist and antagonist binding affinities.

The possible effect of polymorphism on the affinity states with respect to ternary complex formation with G proteins was also tested by adding guanine nucleotides in the radioligand binding assays (Terman *et al.*, 1987). The apparent K_i for noradrenaline at both receptors was significantly ($P<0.05$) lowered when 200 μM Gpp(NH)p was added to the incubation buffer; however, the change induced by Gpp(NH)p was not significantly different between the two polymorphic receptors (Table 3).

Table 2 Pharmacological profile of two polymorphic α_{1a}-adrenoceptors

	K_i (nM)	
	**α_{1a}-adrenoceptor*	*α_{1a}-adrenoceptor*
Drugs	*(Arg492)*	*(Cys492)*
Agonists		
(−)-Adrenaline	600±250	400±35
(+)-Adrenaline	8,100±560	7,600±600
(−)-Noradrenaline	1,100±190	1,400±71
Phenylephrine	4,400±200	5,500±180
Antagonists		
Prazosin	0.18±0.01	0.17±0.01
Phentolamine	2.5±0.1	3.0±2.0
Yohimbine	400±50	520±50
5-Methylurapidil	0.89±0.08	0.65±0.03
WB-4101	0.20±0.03	0.30±0.02

CHO cell membranes stably expressing each polymorphic α_{1a}-adrenoceptor were incubated with [^{125}I]-HEAT, in the absence or presence of increasing concentrations of various agonists and antagonists. Each value represents the mean± s.e.mean from at least three individual experiments performed in duplicate. At least ten concentrations of each ligand were tested, and the points were chosen to be the linear portion of the displacement curve. K_i values were generated by the iterative curve-fitting programme LIGAND. For all drugs examined Hill slopes were not significantly different from unity. *Some of the data are from Horie *et al.* (1995).

$[Ca^{2+}]_i$ measurements in the two polymorphic α_{1a}-adrenoceptors

Next, the noradrenaline-induced $[Ca^{2+}]_i$ responses and the effect of agonist exposure (desensitization) were compared by constructing concentration-$[Ca^{2+}]_i$ response curves for noradrenaline in CHO cells stably expressing the α_{1a}-adrenoceptor (Arg492) and α_{1a}-adrenoceptor (Cys492) cells (Figure 2 and Table 4). Noradrenaline (1 μM) caused rapid increases in $[Ca^{2+}]_i$, consisting of quick transient peaks and more sustained

Table 3 Effect of Gpp(NH)p on noradrenaline inhibition of specific [^{125}I]-HEAT binding to two polymorphic α_{1a}-adrenoceptors

	K_i (nM) *Noradrenaline*	
	GppNHp (−)	*GppNHp (+)*
α_{1a}-Adrenoceptor (Arg492)	1,100±190	2,000±160[a]
α_{1a}-Adrenoceptor (Cys492)	1,400±71	2,600±94[a]

CHO cell membranes stably expressing α_{1a}-adrenoceptor (Arg492) or α_{1a}-adrenoceptor (Cys492) were incubated with [^{125}I]-HEAT, in the absence or presence of increasing concentrations of various agonists and antagonists. Each value represents the mean±s.e.mean from at least three individual experiments performed in duplicate. At least ten concentrations of each ligand were tested, and the points were chosen to be the linear portion of the displacement curve. K_i values were generated by the iterative curve-fitting programme LIGAND (Munson & Rodbard, 1980). For all drugs examined Hill slopes were not significantly different from unity. [a]Significantly different compared to without GppNHp ($P<0.05$).

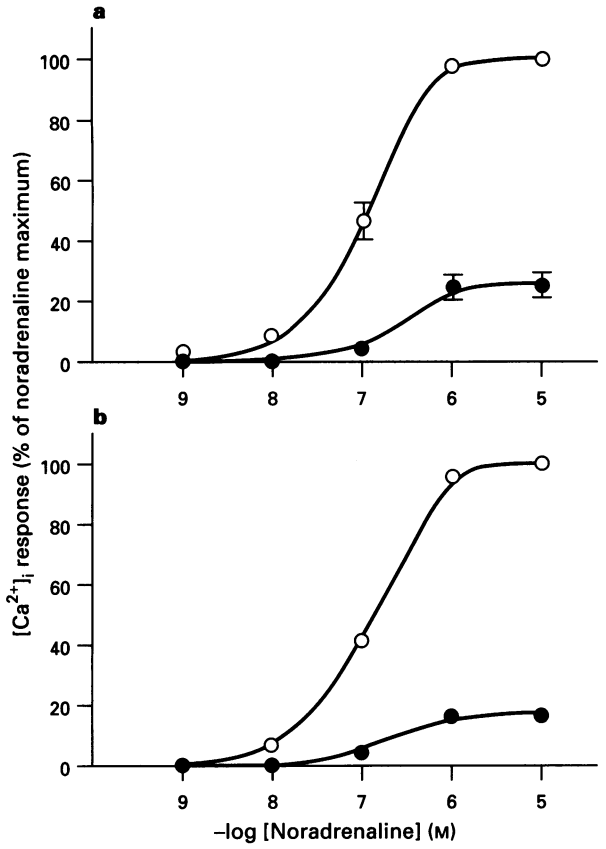

Figure 2 Desensitization of noradrenaline-induced $[Ca^{2+}]_i$ response in CHO cells stably expressing each polymorphic α_{1a}-adrenoceptor; (a) α_{1a}-adrenoceptor (Arg492), (b) α_{1a}-adrenoceptor (Cys492). Concentration-$[Ca^{2+}]_i$ response curves for noradrenaline were constructed before (○) and after 2 h phenylephrine treatment (●) as described in Methods. All responses are expressed as percentages of the maximum response induced by noradrenaline (10 μM in each cell line. Values represent the mean±s.e.mean from four different experiments performed in duplicate.

components in both CHO cells (data not shown). The maximum $[Ca^{2+}]_i$ values induced by noradrenaline (10 μM) were 1,130±103 nM in CHO cells stably expressing α_{1a}-adrenoceptor (Arg492) and 1,280±67 nM in CHO cells stably expressing α_{1a}-adrenoceptor (Cys492), respectively ($n=4$ each). As shown in Figure 2, the maximum response exerted by noradrenaline (E_{max}) and EC_{50} value of concentration-$[Ca^{2+}]_i$ response curves for noradrenaline determined were similar for the two polymorphic receptors. Also, after exposure to phenylephrine (10 μM) for 2 h, the extent of the desensitization process was compared (Figure 2 and Table 4). The maximum $[Ca^{2+}]_i$ response induced by noradrenaline (10 μM) was significantly ($P<0.01$) lowered compared to control in both cells; the maximum $[Ca^{2+}]_i$ responses of noradrenaline were 328±42 nM and 243±26 nM in CHO cells stably expressing α_{1a}-adrenoceptor (Arg492) and α_{1a}-adrenoceptor (Cys492), respectively ($n=4$ each), and the EC_{50} values were 300±24 nM and 220±19 nM, respectively ($n=4$ each). Thus, the noradrenaline-induced $[Ca^{2+}]_i$ responses and the extent of the desensitization process after agonist exposure were similar for the two polymorphic receptors.

Screening for mutation

Additionally, we examined BPH patients for a possible mutation in the third cytoplasmic loop of this receptor gene. No mutation in the third cytoplasmic loop of the α_{1a}-adrenoceptor gene was observed in eight BPH patients.

Table 4 Desensitization of noradrenaline-induced $[Ca^{2+}]_i$ response in CHO cells stably expressing each polymorphic α_{1a}-adrenoceptor

Group	E_{max} (%)	$-log\ EC_{50}$
α_{1a}-Adrenoceptor (Arg492)		
Control	100	6.95±0.07
Phenylephrine-treated	29±4[a]	6.52±0.04[a]
α_{1a}-Adrenoceptor (Cys492)		
Control	100	6.86±0.03
Phenylephrine-treated	19±2[a]	6.66±0.04[a]

$-log\ EC_{50}$ values were determined from noradrenaline concentration-response curves. E_{max} for noradrenaline was calculated as the maximum response produced, divided by the maximal response produced by noradrenaline. The basal $[Ca^{2+}]_i$ levels were 74±5 nM and 87±7 nM in α_{1a}-adrenoceptor (Arg492) and α_{1a}-adrenoceptor (Cys492) cells, respectively ($n=4$ each). The maximal responses produced by noradrenaline were 1,130±103 nM and 1,280±67 nM in α_{1a}-adrenoceptor (Arg492) and α_{1a}-adrenoceptor (Cys492) cells, respectively ($n=4$ each). Each value is the mean± s.e.mean from four individual experiments performed in duplicate. [a]Significantly different compared to the control group ($P<0.01$).

Discussion

As indicated in the Introduction, a number of human diseases have now been mapped and found to be due to either deletions, mutations or polymorphism of G protein-coupled receptors (Alonso *et al.*, 1993; Shenker *et al.*, 1993; Kosugi *et al.*, 1994). Our present analysis showed that human α_{1a}-adrenoceptor polymorphism was not closely associated with the onset of BPH; however, the frequency distribution of this polymorphism was found to be significantly different between the Japanese and U.S. populations. Utilizing CHO cells stably expressing the two polymorphic α_{1a}-adrenoceptors, we found that their pharmacological properties are similar and that both receptors are coupled to calcium signaling. Furthermore, the two polymorphic receptors are desensitized to a similar extent when exposed to the agonist. The results showed that the two α_{1a}-adrenoceptors generated by genetic polymorphism distributed markedly different among the races; however, both α_{1a}-adrenoceptors have similar pharmacological characteristics, and can be regulated in a similar manner. Additionally, we observed no mutation in the third cytoplasmic loop of the α_{1a}-adrenoceptor gene in BPH patients. Taken together, the results of present study do not provide any evidence to support the hypothesis that α_{1a}-adrenoceptor gene polymorphism is associated with BPH.

In the present study, we found that the α_{1a}-adrenoceptors polymorphism was not closely associated with the onset of BPH. On the other hand, however, the frequency distribution of this polymorphism was markedly different between the Japanese and U.S. populations (Hoehe *et al.*, 1992). We, therefore, examined whether the two polymorphic receptors may differ in their pharmacological properties by utilizing CHO cells stably expressing each α_{1a}-adrenoceptor (Arg492 and Cys492). Since Cys490 has been postulated to be a palmitoylation site, the polymorphism can result in an additional putative palmitoylation site in the carboxy-terminal segment of the α_{1a}-adrenoceptor, which appears to play a key role in receptor localization and function (O'Dowd *et al.*, 1988; Moffett *et al.*, 1993; Moench *et al.*, 1994). However, radioligand binding studies with [^{125}I]-HEAT showed that both receptors had similar binding characteristics, and a series of functional studies showed that the polymorphic receptors were coupled to the $[Ca^{2+}]_i$ signaling which can be desensitized in a similar manner. Taken together with the results obtained from recombinant cells, our study indicates that the two polymorphic receptors may behave as a similar pharmacotherapeutic target.

In summary, the present study showed that the two α_{1a}-adrenoceptors generated by genetic polymorphism, while having a markedly different ethnic distribution, have similar pharmacological characteristics, and can be regulated (desensitized) in a similar manner. Our epidemiological and *in vitro* studies do not provide any evidence to support an association between α_{1a}-adrenoceptor gene polymorphism and BPH.

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