Truncated isoforms inhibit [³H]prazosin binding and cellular trafficking of native human α_{1A} -adrenoceptors

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We have identified from human liver eight α_{1A} -adrenoceptor (α_{1A} -AR) splice variants that were also expressed in human heart, prostate and hippocampus. Three of these α_{1A} -AR isoforms (α_{1A-1} -AR, α_{1A-2b} -AR and α_{1A-3a} -AR) gave rise to receptors with seven transmembrane domains (7TM α_{1A} -AR). The other five (α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3c} -AR, α_{1A-5} -AR and α_{1A-6} -AR) led to truncated receptors lacking transmembrane domain VII (6TM α_{1A} -AR). The 7TM α_{1A} -AR isoforms transiently expressed in COS-7 cells bound [³H]prazosin with high affinity (K_d 0.2 nM) and mediated a noradrenaline (norepinephrine)-induced increase in cytoplasmic free Ca^{2+} concentration, whereas the 6TM α_{1A} -AR isoforms were incapable of ligand binding and signal transduction. Immunocytochemical studies with N-terminal epitope-tagged α_{1A} -AR isoforms showed that the 7TM α_{1A} -AR isoforms were present both at the cell surface and in intracellular com-

partments, whereas the 6TM α_{1A} -AR isoforms were exclusively localized within the cell. Interestingly, in co-transfected cells, each truncated α_{1A} -AR isoform inhibited [³H]prazosin binding and cell-surface trafficking of the co-expressed 'original' 7TM α_{1A-1} -AR. However, there was no modification of either the [³H]prazosin-binding affinity or the pharmacological properties of α_{1A-1} -AR. Immunoblotting experiments revealed that co-expression of the α_{1A-1} -AR with 6TM α_{1A} -AR isoforms did not impair α_{1A-1} -AR expression. Therefore the expression in human tissues of many truncated isoforms constitutes a new regulation pathway of biological properties of α_{1A} -AR.

Key words: coexpression, heterodimerization, receptor dimer, regulation of G protein-coupled receptors.

INTRODUCTION

α_1 -Adrenoceptors (α_1 -AR isoforms) are members of the superfamily of seven-transmembrane-domain (7TM) G-protein-coupled receptors that bind the endogenous catecholamines adrenaline (epinephrine) and noradrenaline (norepinephrine) [1]. Three α_1 -AR subtypes (α_{1A} , α_{1B} and α_{1D} ; for new IUPHAR α_1 -AR nomenclature see [2]) have been cloned, expressed in several cells and characterized pharmacologically [3–5]. The α_1 -AR isoforms mediate, via $G_q\alpha$ activation of phospholipase C β , various sympathetic nervous system responses such as smooth-muscle contraction, myocardial inotropy and hepatic glucose metabolism [1].

Clinically, the α_{1A} -AR subtype was postulated to mediate smooth-muscle contraction in human prostate, suggesting a role for this receptor in the bladder outlet obstruction observed in patients with benign prostate hypertrophy [6,7]. α_{1A} -AR also seems implicated in the development of myocardial hypertrophy [8]. An increase in α_{1A} mRNA level and protein expression has been observed in these two pathologies [9,10]. Despite the importance of α_{1A} -AR in physiopathological states, few studies have examined the regulatory mechanisms of the expression and biological function of α_{1A} -AR.

Unlike the α_{1B} -AR and α_{1D} -AR subtypes, which are expressed as a unique receptor form, four isoforms of α_{1A} -AR (α_{1A-1} , α_{1A-2} , α_{1A-3} and α_{1A-4}) have been isolated from a human prostate cDNA library [11,12]. Although α_{1A-2} , α_{1A-3} and α_{1A-4} -AR differ from the 'original' α_{1A-1} -AR [3] in length and sequence in the C-terminal region, these four α_{1A} -AR isoforms were indistinguishable with regard to their pharmacological profiles and their abilities to

mediate noradrenaline-induced cytoplasmic Ca^{2+} release [3,11,12]. Non-functional truncated isoforms have been also observed in human prostate and hippocampus [12,13] but were not extensively studied. The physiological significance of α_{1A} -AR splice variants is currently unknown.

The expression of non-functional truncated isoforms (V_{2nr} and D_{3nr}), which was correlated with the loss of the wild-type receptor, was observed for human vasopressin V_2 receptor in kidney from patients with X-linked nephrogenic diabetes insipidus [14] and for human dopamine D_3 receptor in cerebral cortex from patients with chronic schizophrenia respectively [15–17]. In the latter, the loss of D_3 mRNA results from an increased frequency of D_{3nr} alternative splicing of D_3 pre-mRNA [17]. In addition to this accumulation of truncated D_{3nr} mRNA, oligomerization occurs between this truncated form and the wild-type D_3 receptor [18]. For V_2 receptor, Zhu and Wess [19] demonstrated by co-expression in COS-7 cells that the formation of heterodimers between truncated mutants and wild-type V_2 receptors led to an important decrease in agonist binding, signal transduction and cell-surface trafficking of the full-length V_2 receptor. These results revealed two methods of regulation of the G-protein-coupled receptor: (1) via the modification of alternative splicing that changes the receptor mRNA level and (2) via the interactions between truncated receptor isoforms that inactivate the biological function of the receptor. It is thus crucial to determine whether α_{1A} -AR isoforms could form heterodimers and whether such interactions might be modified in pathological situations (benign prostate hypertrophy and myocardial hypertrophy).

In the present study, eight α_{1A} -AR splice variants have been isolated from human liver. Their characterization demonstrates

Abbreviations used: AR, adrenergic receptor (adrenoceptor); [Ca^{2+}]_i, cytoplasmic free Ca^{2+} concentration; RT-PCR, reverse-transcriptase-mediated PCR; TM, transmembrane domain.

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the existence of four new α_{1A} -AR isoforms. Co-expression in COS-7 cells of α_{1A} -AR isoforms revealed that only truncated isoforms showed modified [3 H]prazosin binding and cell-surface trafficking from that of the 'original' α_{1A-1} -AR.

EXPERIMENTAL

cDNA library screening and reverse-transcriptase-mediated PCR (RT-PCR)

Recombinant clones (2×10^6) of human liver λ gt11 library (Clontech) were screened with a 1.4 kbp PCR fragment corresponding to the complete coding sequence of human α_{1A} -AR [3] by a standard procedure [20]. cDNA species for α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3b} -AR and α_{1A-3c} -AR were isolated by RT-PCR from human liver mRNA (Clontech) by using a 5' sense primer (nt 432–452 of human α_{1A-1} -AR [3]) and two specific 3'-anti-sense primers, respectively nt 2044–2067 of human α_{1A-2} -AR [11] and nt 1942–1963 of human α_{1A-3} -AR [11]. Liver total RNA (2 μ g) was reverse-transcribed with oligo(dT)_{12–18} and N6 oligonucleotides in accordance with the first-strand cDNA synthesis protocol from Pharmacia Biotech. PCR reactions were performed in 100 μ l containing 10 mM Tris/HCl, pH 8.3, 1.5 mM MgCl₂, 0.2 mM dNTP, 2 μ l of the single-stranded cDNA preparation, 0.3 μ M of each primer and 2 units of Amplitaq Gold polymerase (Perkin–Elmer Cetus) with a 35-cycle programme of 94 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min, a hot start at 94 °C for 9 min and a final extension at 72 °C for 8 min. Cloned cDNA and PCR products were subcloned into pUC18 (Biolabs) and pGEMT-easy (Promega) respectively. Nucleotide sequence analysis on both DNA strands was performed on a ABI377 DNA sequencer (Applied Biosystems).

Tissue expression

The mRNA species of human tissues were purchased from Clontech. First-strand cDNA synthesis was performed from 250 ng of mRNA and amplified as described above. PCR primers were designed for all α_{1A} -AR isoforms: the forward primer (5'-AAGAAAGCGGCCAAACGCTGG-3'; position 1238–1259 of α_{1A-1} -AR [3]) was common to all isoforms and reverse primers were specific to α_{1A} -AR isoforms [3'-GTCACTCTTGCCCTCCTTCAGATC-5' (position 1813–1837 [3]) for α_{1A-1} -AR, 3'-CCTACTTTAGAACGAGTCTTCGAG-5' (position 2044–2067 [11]) for α_{1A-2a} -AR, α_{1A-2b} -AR and α_{1A-2c} -AR, 3'-CCTTAC-TAGTACTTACGTTTCC-5' (position 1942–1963 [11]) for α_{1A-3a} -AR, α_{1A-3b} -AR and α_{1A-3c} -AR, 3'-GGGTGAAACGGAC-TGATAACAG-5' (position 1994–2015) for α_{1A-5} -AR and 3'-CACGAGACCTTCTACTTTGTT-5' (position 1613–1636) for α_{1A-6} -AR]. mRNA not reverse-transcribed was used as a negative control.

Plasmid construction and flag epitope tagging

PCR fragments of complete coding regions of all isolated α_{1A} -AR isoforms were inserted between *Eco*RI and *Xba*I sites of the pSR α expression vector [21]. The M5 flag epitope (sequence DYKDDDDK) was introduced into these α_{1A} receptor expression constructs at the N-terminus between the first methionine residue and the second residue of α_{1A} -AR isoforms.

Immunofluorescence microscopy

COS-7 cells (A.T.C.C., Manassas, VA, U.S.A.) were grown overnight on poly-(D-lysine)-coated glass coverslips in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum. The pSR α /M5 α_{1A} -AR vectors were then transfected into COS-7

cells with Lipofectamine (Life Technologies) in accordance with the manufacturer's protocol. At 48 h after transfection, cell nuclei were stained with 33258-Hoechst dye (Sigma) and the cells were fixed in 4% (w/v) paraformaldehyde/PBS for 15 min at 25 °C, then permeabilized in OptiMEM medium containing 0.4% (v/v) Triton X-100 and 10% (v/v) goat serum (Sigma) for 30 min. Epitope-tagged α_{1A} -AR isoforms were detected by using a mouse monoclonal antibody anti-Flag M5 (Kodak Eastman) diluted to 5 μ g/ml in OptiMEM medium. After incubation for 1 h at 37 °C, coverslips were extensively washed in PBS buffer and incubated with goat Cy3[™] conjugate anti-mouse F(ab')₂ fragment IgG (Jackson Immuno Research Laboratories) diluted 1:600 in PBS for 20 min at 25 °C. Alternatively, cells were stained without being fixed or permeabilized and under the conditions described above. Immunofluorescent staining was examined with a conventional inverted epifluorescence Leica microscope (100 $\times$ / 10 $\times$ objective).

Membrane and whole-cell [3 H]prazosin binding assay

Transfected COS-7 cells were scraped and lysed with a Polytron homogenizer (Brinkman) in 20 ml of ice-cold binding buffer [50 mM Tris/HCl/1 mM EDTA/10 mM MgCl₂ (pH 7.5) supplemented with Complete[™] protease inhibitor cocktail (Boehringer)]. Lysate was centrifuged at 50000 *g* for 30 min at 4 °C; the resulting pellet was homogenized in 1 ml of binding buffer and the protein concentration was measured with a bicinchoninic acid protein assay kit (Pierce) with BSA as standard. The binding assay was performed by incubation of membrane proteins (1–2 μ g) for 1 h at 25 °C with [3 H]prazosin (specific radioactivity 75 Ci/mmol; Amersham) in a final volume of 0.5 ml. Incubation was stopped by the addition of ice-cold 50 mM Tris/HCl, pH 7.4, and immediate filtering through Whatman GF/B glass fibre filters presoaked in 0.1% polyethylenimine with a Brandel cell harvester. Filters were washed twice and radioactivity was measured. Non-specific binding was determined in the presence of 10 μ M phentolamine and was usually not more than 10% of total binding. To determine the subcellular distribution of [3 H]prazosin-binding sites, radioligand binding was performed on whole cells in a manner similar to that described by Eason and Liggett [22]. Transfected cells were resuspended in ice-cold Ham's F12 medium, then incubated with increasing concentrations (0.02–3 nM) of [3 H]prazosin in the absence or the presence of the lipophilic competitor phentolamine (10 μ M) or the hydrophilic competitor adrenaline (100 μ M) for 4 h at 4 °C. In intact cells, adrenaline, owing to its hydrophilic properties, is unable to penetrate the plasma membrane, especially at 4 °C. Surface-accessible binding was thus defined as the binding displaced by 100 μ M adrenaline. Total specific binding was defined as the binding displaced by 10 μ M phentolamine. The difference between surface-accessible and total binding represents the intracellular specific binding. Saturation and displacement curves were analysed by non-linear regression analysis with the In Plot program (GRAPHPAD Software). The statistical significance of differences between mean data was evaluated by the use of the Dunnett test. The difference was taken to be significant when *P* < 0.05. The drugs were purchased from Sigma and Research Biochemicals.

Ca²⁺ mobilization assay

Transfected COS-7 cells were detached by incubation in PBS without Ca²⁺ and Mg²⁺ for 10 min at 37 °C, centrifuged at 1000 *g* for 5 min and incubated at 10⁶ cells/ml in Hanks medium containing 0.1% BSA and 5 μ M fura-2 acetoxymethyl ester for 30 min at 37 °C. Noradrenaline (10 μ M)-induced cytoplasmic

Ca^{2+} mobilization was monitored with a Hitachi F2000 fluorescence spectrophotometer with dual excitation at 340 and 380 nm, and emission at 510 nm. Calibration of the fluorescence signals for the calculation of cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was performed by the addition of a final concentration of 0.1 % (v/v) Triton X-100 (maximal signal) followed by the addition of a final concentration of 10 mM EGTA (minimal signal).

Immunoblotting

Membrane proteins of transfected COS-7 cells were resolved by SDS/PAGE [9 % (w/v) gel] [23] and electrotransferred to nitrocellulose filters. The blot was incubated for 2 h at 25 °C in blocking PBS buffer containing 10 % (w/v) skimmed milk (Difco) and 0.1 % (v/v) Tween 20, then overnight at 4 °C with mouse monoclonal anti-Flag M5 (1 $\mu\text{g}/\text{ml}$; Kodak) in blocking buffer. Immunoblots were washed three times with washing PBS buffer containing 0.5 % BSA and 1 % (v/v) Tween 20, then incubated for 1 h at 25 °C in blocking buffer with polyclonal horseradish-peroxidase-conjugated goat anti-mouse antibody (Promega) diluted 1:3500. After three washes, peroxidase activity was revealed by enhanced chemiluminescence reagent and exposure to Hyperfilm in accordance with the manufacturer's instructions (Amersham).

RESULTS

Cloning of human α_{1A} -AR cDNA species from human liver

Screening of human liver cDNA library with a probe corresponding to the complete coding sequence of human α_{1A-1} -AR [3] and RT-PCR amplification of liver mRNA with primers specific for α_{1A-2} -AR and α_{1A-3} -AR enabled us to identify nine splice variants of α_{1A} -AR named α_{1A-1} -AR, α_{1A-2a} -AR, α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3a} -AR, α_{1A-3b} -AR, α_{1A-3c} -AR, α_{1A-5} -AR and α_{1A-6} -AR. Our nomenclature follows that used for the α_{1A} -AR

	294	TM VII	353
α_{1A-1}	IGSEFFDFKP	SETVFKIVFW LGYLNSCINP	IIYPCSSQEF KKAFQNVLR
α_{1A-2a}	IGSEFFDFKP	SETVFKIVFW LGYLNSCINP	IIYPCSSQEF KKAFQNVLR
α_{1A-2b}	IGTYILKYDV	IFWRKGLSVC TRLRERKEIK	N.....
α_{1A-2c}	IDEVSLCHQA	GVQWHDLSGL QPPPGGKRF	SCLSLPSSWD YRDVPPGRRH
α_{1A-3a}	IGSEFFDFKP	SETVFKIVFW LGYLNSCINP	IIYPCSSQEF KKAFQNVLR
α_{1A-3b}	IGTYILKYDV	IFWRKGLSVC TRLRERKEIK	N.....
α_{1A-3c}	IGTHTHDMKP	ASRPRLLSLL PKEGEHETHH	WSCDPLSLES TPGAQEPCLT
α_{1A-5}	IGKS.....		LGFTSLSSIH
α_{1A-6}	IDETEAAQGG	KNDSPSFQKP VHAAVLGLE	VMEKENLEGV SRKDTGVW
	354		413
α_{1A-1}	HALGYTLHPP	SQAVEGQHKD MYRIPVGSRE	TFYRISKTDG VCEWKFFSSM
α_{1A-2a}	HALGYTLHPP	SQAVEGQHKD MYRIPVGSRE	TFYRISKTDG VCEWKFFSSM
α_{1A-2b}	HALGYTLHPP	SQAVEGQHKD MYRIPVGSRE	TFYRISKTDG VCEWKFFSSM
α_{1A-2c}	TGFHHVGGDD	LDLLTS.....	
α_{1A-3a}	HALGYTLHPP	SQAVEGQHKD MYRIPVGSRE	TFYRISKTDG VCEWKFFSSM
α_{1A-3b}	HALGYTLHPP	SQAVEGQHKD MYRIPVGSRE	TFYRISKTDG VCEWKFFSSM
α_{1A-3c}	LTHAQIQHVT	VTDTGKTVT.....	
α_{1A-5}			
α_{1A-6}			
	414		475
α_{1A-1}	KDQSSCTTARV	RSKSFQVCC CVGPSTPSLD	KNHQVPTIKV HTISLSENSE
α_{1A-2a}	KDQSSCTTART	KSRSVTRLEC SGMLAHCLN	RLPGSRDSPA SASQAAGTTG
α_{1A-2b}			MCHQADATRS
α_{1A-2c}			
α_{1A-3a}	KDQSSCTTARG	HTPMT.....	
α_{1A-3b}			
α_{1A-3c}			
α_{1A-5}			
α_{1A-6}			

Figure 1 Deduced C-terminal amino acid sequences of α_{1A} -AR isoforms isolated from human liver

Deduced sequences after their 294th residue are presented in single-letter code. The residues in TM7 are indicated in bold. The corresponding amino acid sequence differences from the published α_{1A-2} -AR sequence [11] are underlined. A deletion of a guanosine residue at position 1827 in the α_{1A-2a} -AR sequence [11] resulted in a frame shift after the 464th residue and introduced a stop codon after the 475th residue.

Figure 2 Distribution of mRNA encoding α_{1A} -AR isoforms in human tissues

mRNA (250 ng) of human liver, heart, prostate and hippocampus was amplified by RT-PCR between a common 5' sense primer and a 3' anti-sense primer specific to each isoform, as described in the Experimental section. After 30 cycles of PCR for α_{1A-1} -AR and 40 cycles of PCR for other α_{1A} -AR isoforms, PCR products were analysed in agarose gel stained by ethidium bromide. The length of PCR fragments was estimated by molecular mass markers (Φ X174/*Hae*III) and is indicated in base pairs at the right.

isoforms cloned previously [11,12]. The α_{1A-1} -AR, α_{1A-2a} -AR and α_{1A-3a} -AR cDNA species corresponded to the three isoforms previously identified from human prostate cDNA library by Hirasawa et al. [3,11]. We did not identify the splice variant α_{1A-4} -AR cloned by Chang et al. [12]. The α_{1A-2a} -AR, α_{1A-2b} -AR and α_{1A-2c} -AR cDNA species presented identical sequences at the 5' and 3' ends but diverged between nt 1320 and 1705 (α_{1A-2a} -AR [11]). An identical alternative splicing was observed for the α_{1A-3a} -AR, α_{1A-3b} -AR and α_{1A-3c} -AR cDNA species. The α_{1A-2b} -AR and α_{1A-3b} -AR cDNA species encoded the same α_{1A} receptor (Figure 1). The sizes of the eight isolated α_{1A} -AR isoforms ranged from 297 to 475 residues. The primary sequences of these α_{1A} -AR isoforms were identical before the 294th residue and then diverged. Deduced C-terminal amino acid sequences of the eight α_{1A} -AR isoforms are shown in Figure 1. Hydrophathy analysis [24] of protein sequences revealed the presence of seven hydrophobic clusters of 20–25 residues for the α_{1A-1} -AR, α_{1A-2a} -AR and α_{1A-3a} -AR isoforms and showed the loss of TM7 for the α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3b} -AR, α_{1A-5} -AR and α_{1A-6} -AR isoforms.

Tissue expression

The expression of mRNA encoding the isolated α_{1A} -AR isoforms was investigated in the human heart, liver, prostate and hippocampus by RT-PCR with a common 5' sense primer and isoform-specific 3' anti-sense primers. The eight mRNA species encoding the α_{1A} -AR isoforms were amplified from the four tissues (Figure 2). With the same 5' and 3' primers, we observed

Table 1 Pharmacological profiles of human α_1 -AR isoforms transiently transfected in COS-7 cells and noradrenaline-induced $[Ca^{2+}]_i$

Affinities of drugs for α_1 -AR isoforms transiently transfected in COS-7 cells are shown. Results are means $\pm$ S.E.M. ($n = 3$) for radioligand displacement experiments in cell membrane homogenates with $[^3H]$ prazosin and 10 μM phentolamine for non-specific binding. For all compounds examined, Hill coefficients were not significantly different from unity. Abbreviation: n.d., not determined. Noradrenaline (10 μM)-induced $[Ca^{2+}]_i$ was determined from fura-2-loaded transfected COS-7 cells, as described in the Experimental section. $[Ca^{2+}]_i$ are transient peak values and are means $\pm$ S.E.M. ($n = 4$).

	K_i (nM)				
	α_{1A-1} -AR	α_{1A-2a} -AR	α_{1A-3a} -AR	α_{1A-1} -AR + α_{1A-3a} -AR	α_{1A-1} -AR + α_{1A-3c} -AR
Agonists					
(-)-Noradrenaline	1697 $\pm$ 948	1131 $\pm$ 470	1861 $\pm$ 1053	n.d.	n.d.
(-)-Adrenaline	876 $\pm$ 350	691 $\pm$ 130	692 $\pm$ 55	1083 $\pm$ 70	996 $\pm$ 63
Methoxamine	8259 $\pm$ 2362	6945 $\pm$ 793	7544 $\pm$ 1730	n.d.	n.d.
Oxymetazoline	8.5 $\pm$ 7.7	11.6 $\pm$ 2.5	11.8 $\pm$ 2.9	19.0 $\pm$ 2.5	20.3 $\pm$ 2.1
Antagonists					
Prazosin	0.29 $\pm$ 0.09	0.30 $\pm$ 0.08	0.26 $\pm$ 0.02	0.57 $\pm$ 0.09	0.58 $\pm$ 0.10
WB4101	0.27 $\pm$ 0.10	0.36 $\pm$ 0.14	0.70 $\pm$ 0.29	0.59 $\pm$ 0.10	0.50 $\pm$ 0.10
5-Methylurapidil	1.52 $\pm$ 0.28	1.30 $\pm$ 0.47	1.46 $\pm$ 0.54	2.81 $\pm$ 0.34	2.49 $\pm$ 0.44
Phentolamine	2.51 $\pm$ 0.46	2.79 $\pm$ 0.56	3.26 $\pm$ 0.57	n.d.	n.d.
Sipiperone	11.5 $\pm$ 5.5	12.6 $\pm$ 9.3	11.8 $\pm$ 6.8	n.d.	n.d.
BMJ7378	417 $\pm$ 223	304 $\pm$ 86	221 $\pm$ 97	n.d.	n.d.
$[Ca^{2+}]_i$ (nM)					
	α_{1A-1} -AR	α_{1A-2a} -AR	α_{1A-3a} -AR	α_{1A-1} -AR + α_{1A-3a} -AR	α_{1A-1} -AR + α_{1A-3c} -AR
	73 $\pm$ 3	60 $\pm$ 5	65 $\pm$ 5	76 $\pm$ 3	64 $\pm$ 3

Figure 3 Subcellular localization of epitope-tagged α_{1A-1} -AR and α_{1A-3c} -AR isoforms

Immunofluorescence studies were performed with transfected COS-7 cells grown on coverslips as described in the Experimental section. Cells expressing α_{1A-1} -AR (**A**, **B**) and truncated α_{1A-3c} -AR (**C**, **D**) were probed with the mouse monoclonal antibody M5 directed against the N-terminal epitope tag present in these recombinant receptors. Experiments were carried out with non-fixed and non-permeabilized (**A**, **C**) and fixed and permeabilized (**B**, **D**) cells. The fluorescence images were obtained by using Cy3TM conjugated goat anti-mouse IgG secondary antibody. COS-7 cell nuclei were stained with 33258-Hoechst dye. Magnification $\times 800$. Each picture is representative of five independent experiments.

Table 2 Effect of co-transfection of α_{1A} -AR isoforms on the expression level of α_{1A-1} -AR in COS-7 cells and on noradrenaline-induced $[Ca^{2+}]_i$

Saturation binding parameters were determined with [3H]prazosin (0.02–3 nM) by using whole COS-7 cells co-expressing α_{1A-1} -AR and another α_{1A} -AR isoform [human interleukin 4 receptor (hIL-4R) serves as a control], in Ham F12 medium for 4 h at 4 °C. Total receptors were detected as [3H]prazosin binding, with 10 μ M phentolamine for non-specific binding. Plasma membrane receptors were defined as [3H]prazosin binding displaced by 100 μ M (–)-adrenaline. Intracellular receptors were defined as the difference between the two binding values. Results are means $\pm$ S.E.M. for at least four experiments performed in duplicate. Noradrenaline-induced $[Ca^{2+}]_i$ was determined from fura-2-loaded co-transfected COS-7 cells, as described in the Experimental section. $[Ca^{2+}]_i$ are transient peak values and are means $\pm$ S.E.M. ($n = 4$). * $P \leq 0.05$ compared with α_{1A-1} -AR alone. Values in parentheses are percentages of α_{1A-1} -AR alone.

Receptor isoforms	B_{max} (fmol per 10^6 cells)			$[Ca^{2+}]_i$ (nM)
	Total receptors	Plasma membrane receptors	Intracellular receptors	
α_{1A-1} -AR + α_{1A-1} -AR†	3235 $\pm$ 369 (104)	2974 $\pm$ 412 (103)	261 $\pm$ 54 (113)	75 $\pm$ 3
α_{1A-1} -AR + α_{1A-2a} -AR‡	2937 $\pm$ 290 (97)	2707 $\pm$ 271 (94)	230 $\pm$ 10 (100)	74 $\pm$ 4
α_{1A-1} -AR + α_{1A-3a} -AR‡	3301 $\pm$ 565 (105)	3026 $\pm$ 529 (105)	275 $\pm$ 52 (119)	76 $\pm$ 3
α_{1A-1} -AR§	3100 $\pm$ 517 (100)	2870 $\pm$ 432 (100)	230 $\pm$ 40 (100)	73 $\pm$ 3
α_{1A-1} -AR + hIL-4R‡	2913 $\pm$ 237 (94)	2695 $\pm$ 235 (94)	218 $\pm$ 18 (95)	70 $\pm$ 5
α_{1A-1} -AR + α_{1A-3b} -AR‡	1598 $\pm$ 434* (51)	1487 $\pm$ 435* (52)	111 $\pm$ 21* (48)	65 $\pm$ 5
α_{1A-1} -AR + α_{1A-3c} -AR‡	1563 $\pm$ 249* (50)	1434 $\pm$ 238* (50)	129 $\pm$ 16* (56)	64 $\pm$ 3
α_{1A-1} -AR + α_{1A-6} -AR‡	1395 $\pm$ 147* (45)	1289 $\pm$ 152* (45)	106 $\pm$ 10* (46)	66 $\pm$ 6

† 2 μ g of α_{1A-1} -AR cDNA was used to transfect 10^6 COS-7 cells.

‡ 1 μ g of α_{1A-1} -AR cDNA and 1 μ g of cDNA of the other α_{1A} -AR isoform or hIL-4R were used to transfect 10^6 COS-7 cells.

§ 1 μ g of α_{1A-1} -AR cDNA was used to transfect 10^6 COS-7 cells.

that the mRNA encoding α_{1A-2a} -AR was more abundantly expressed than those encoding the truncated α_{1A-2b} -AR and α_{1A-2c} -AR (Figure 2); the α_{1A-3a} -AR mRNA seemed to be more abundant than those encoding the α_{1A-3b} -AR and α_{1A-3c} -AR isoforms (Figure 2). Although our PCR experiments were not quantitative, the mRNA encoding α_{1A-6} -AR seemed to be expressed more in liver than in the other tissues analysed (Figure 2).

Radioligand binding and agonist-induced $[Ca^{2+}]_i$ increase

To examine the pharmacological and biochemical properties of the isolated α_{1A} -AR isoforms, PCR fragments corresponding to the coding region of each α_{1A} -AR isoform were inserted into the pSR α expression vector and used to transfect COS-7 cells transiently. Membranes from COS-7 cells transfected with α_{1A-1} -AR, α_{1A-2a} -AR or α_{1A-3a} -AR were able to bind [3H]prazosin in a saturable manner with high affinity (K_d 0.22 $\pm$ 0.03 nM). The expression levels of α_{1A-1} -AR, α_{1A-2a} -AR and α_{1A-3a} -AR were respectively 14, 13 and 15 pmol/mg of protein. COS-7 cells transfected either with pSR α alone or 6TM- α_{1A} -AR isoforms (α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3b} -AR, α_{1A-3c} -AR, α_{1A-5} -AR and α_{1A-6} -AR) exhibited no specific binding. Binding of [3H]prazosin to cell membranes expressing α_{1A-1} -AR, α_{1A-2a} -AR or α_{1A-3a} -AR was inhibited with a similar rank order of potency by adrenergic agonists or antagonists (Table 1). In COS-7 cells expressing the isoforms α_{1A-1} -AR, α_{1A-2a} -AR or α_{1A-3a} -AR, noradrenaline elicited a rapid and transient increase in $[Ca^{2+}]_i$; maximal stimulation was achieved with 10 μ M noradrenaline (results not shown). The noradrenaline (10 μ M)-induced $[Ca^{2+}]_i$ response was similar in potency (66 $\pm$ 6 nM) for the three isoforms (Table 1) and its amplitude was 10-fold higher than that measured in COS-7 cells transfected with the vector alone. In contrast, noradrenaline (10 μ M) did not increase $[Ca^{2+}]_i$ in COS-7 cells transfected with 6TM- α_{1A} -AR isoforms.

Subcellular localization

The subcellular localization of α_{1A} -AR isoforms was studied with N-terminal M5 epitope-tagged recombinant α_{1A} -AR isoforms

transiently expressed in COS-7 cells. The tagged receptors were detected with anti-M5-specific antibodies and Cy3TM conjugate anti-mouse IgG antibodies. No fluorescent signal was detected from COS-7 cells transfected with the vector alone (results not shown). In non-fixed and non-permeabilized transfected COS-7 cells, the fluorescent signal was detected at the surface of cells expressing the M5-tagged 7TM- α_{1A-1} -AR isoform (Figure 3A), whereas no signal was observed in cells expressing the M5-tagged 6TM- α_{1A-3c} -AR isoform (Figure 3C). Furthermore, when cells expressing either the M5-tagged α_{1A-1} -AR or M5-tagged α_{1A-3c} -AR isoform were fixed and permeabilized, a strong red fluorescence was observed in the perinuclear region (Figures 3B and 3D). Finally, we observed that M5-tagged α_{1A-2a} -AR and α_{1A-3a} -AR isoforms were localized at the cell surface and in intracellular compartments, as was α_{1A-1} -AR, whereas the α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3b} -AR, α_{1A-5} -AR and α_{1A-6} -AR isoforms were stocked exclusively in the perinuclear compartment, as was α_{1A-3c} -AR (results not shown). The subcellular distribution of [3H]prazosin-binding sites were determined by using radioligand binding in intact transfected COS-7 cells and unlabelled competitors phentolamine (lipophilic antagonist) and adrenaline (hydrophilic agonist) to delineate the total and plasma membrane binding sites respectively. Intracellular binding sites were determined as the difference of the two binding values. The results showed that measurable binding sites were about 10-fold more abundant at the cell surface (2870 $\pm$ 432 fmol per 10^6 cells) than within intracellular compartments (230 $\pm$ 40 fmol per 10^6 cells) (Table 2).

Co-expression of α_{1A} -AR isoforms

To examine whether co-expressed α_{1A} -AR isoforms interfered with the ability of 'original' α_{1A-1} -AR to bind [3H]prazosin, intact co-transfected COS-7 cells were tested for their capacity to bind the radioligand. These studies showed that the co-transfection of α_{1A-1} -AR (1 μ g of cDNA per 10^6 cells) along with the α_{1A-2a} -AR or α_{1A-3a} -AR isoforms (1 μ g of cDNA per 10^6 cells; B_{max} 2937–3301 fmol per 10^6 cells) in COS-7 cells did not alter [3H]prazosin binding in comparison with COS-7 cells expressing α_{1A-1} -AR alone (2 μ g of cDNA per 10^6 cells; B_{max} 3235 fmol per

Figure 4 Cell-surface expression of M5 epitope-tagged α_{1A-1} -AR in COS-7 co-expressed with other α_{1A} -AR isoforms

COS-7 cells were co-transfected with plasmid DNA encoding the N-terminal M5 epitope-tagged α_{1A-1} -AR (1 μ g of cDNA per 10^6 cells) and α_{1A-2a} -AR, α_{1A-3a} -AR, α_{1A-3b} -AR, α_{1A-3c} -AR or α_{1A-6} -AR (1 μ g of cDNA per 10^6 cells). The M5-tagged α_{1A-1} -AR present on the cell surface was revealed by immunostaining in non-fixed and non-permeabilized cells, as described in the Experimental section. For each transfection experiment, the proportion of immunofluorescent cells as a percentage of the number of total cells was determined by staining of cell nuclei with 33258-Hoechst dye. Results are means $\pm$ S.E.M. for three independent experiments performed in duplicate; approx. 600 cells were analysed for each transfection experiment.

10^6 cells) (Table 2). In contrast, COS-7 cells transiently co-transfected with α_{1A-1} -AR (1 μ g of cDNA per 10^6 cells) and a six-TM α_{1A-3b} -AR, α_{1A-3c} -AR or α_{1A-6} -AR isoform (1 μ g of cDNA per 10^6 cells) showed a 50% decrease in total [3 H]prazosin-binding sites ($B_{\max}$ 1395–1598 fmol/ 10^6 cells) in comparison with COS-7 cells transfected with α_{1A-1} -AR alone (1 μ g of cDNA per 10^6 cells; $B_{\max}$ 3100 fmol per 10^6 cells) (Table 2). The equilibrium dissociation constants were not modified (K_d 0.42 ± 0.02 nM)

and a Scatchard analysis revealed the presence of a single high-affinity [3 H]prazosin-binding site (results not shown). Furthermore, experiments with either phentolamine or (–)adrenaline for non-specific binding showed that intracellular and cell-surface [3 H]prazosin-binding sites were both decreased by approx. 50% (Table 2). This inhibition was specific for truncated α_{1A} -AR isoforms because the co-expression of α_{1A-1} -AR with the human interleukin 4 receptor did not modify [3 H]prazosin binding properties ($B_{\max}$ 2913 fmol per 10^6 cells) (Table 2). Even if the number of [3 H]prazosin-binding sites was significantly decreased (50%) in cells transfected with both α_{1A-1} -AR and α_{1A-3c} -AR, the pharmacological profile remained identical with that obtained with COS-7 cells expressing α_{1A-1} -AR alone (Table 1). The co-expression of α_{1A-1} -AR (1 μ g of cDNA per 10^6 cells) with the α_{1A-3b} -AR, α_{1A-3c} -AR or α_{1A-6} -AR isoforms (1 μ g of cDNA per 10^6 cells) did not induce a statistically significant decrease in noradrenaline-mediated [Ca^{2+}] $_i$ response ([Ca^{2+}] $_i$ 65 ± 5 nM) in comparison with COS-7 cells transfected with α_{1A-1} -AR alone ([Ca^{2+}] $_i$ 73 ± 3 nM) (Table 2).

Our next goal was to determine whether truncated α_{1A} -AR isoforms were able to interfere with the cell-surface localization of the co-expressed M5-tagged α_{1A-1} -AR. For this purpose we determined the percentage of immunostained co-transfected cells that were non-fixed and non-permeabilized. Strikingly, co-expression of M5-tagged α_{1A-1} -AR with the truncated non-tagged α_{1A-3b} -AR, α_{1A-3c} -AR or α_{1A-6} -AR isoforms led to a strong decrease (approx. 56%) in the number of immunofluorescent cells in comparison with COS-7 cells expressing M5-tagged α_{1A-1} -AR alone (Figure 4). No decrease in the number of immunofluorescent cells was observed when M5-tagged α_{1A-1} -AR was co-transfected with the α_{1A-2a} -AR or α_{1A-3a} -AR isoforms (Figure 4). We also observed that 5% of the cells co-expressing untagged α_{1A-1} -AR and M5-tagged 6TM α_{1A} -AR (α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3c} -AR, α_{1A-5} -AR or α_{1A-6} -AR) presented a fluorescent signal at the cell surface (1000 cells analysed for each isoform; results not shown).

Figure 5 Immunoblotting of human α_{1A} -AR isoforms expressed in COS-7 cells

Membrane proteins (10 μ g) from COS-7 cells expressing each epitope-tagged α_{1A} -AR isoform (A) or from COS-7 cells co-expressing M5 epitope-tagged α_{1A-1} -AR and untagged α_{1A} -AR isoforms (B) were separated by SDS/PAGE and transferred to nitrocellulose filters, as described in the Experimental section. Filters were incubated with anti-M5 antibody. Detection of primary antibody binding was performed with a specific horseradish-peroxidase-linked secondary antibody followed by enhanced-chemiluminescence-directed exposure to Hyperfilm. The blots were calibrated with Rainbow molecular mass markers (Amersham), as indicated at the left. The estimated molecular masses of the α_{1A} -AR isoforms are indicated at the right. Each blot is representative of three independent experiments.

Western blot analysis

Western blot analysis of SDS-treated membrane fractions of COS-7 cells transfected with tagged α_{1A} -AR with the use of an anti-M5 antibody revealed immunoreactive polypeptides corresponding to lower (35–42 kDa) as well as intermediate (55–70 kDa) and higher (110–140 and 165–210 kDa) molecular mass species (Figure 5A). All these detected proteins seemed to be related to M5 epitope-tagged α_{1A} -AR because no polypeptide was detected when cells were transfected with the vector alone (Figure 5A). Furthermore, when cells were transfected with various α_{1A} -AR isoforms, including α_{1A-1} -AR (466 residues), α_{1A-2a} -AR (475 residues), α_{1A-3a} -AR (429 residues), α_{1A-3c} -AR (372 residues) and α_{1A-6} -AR (342 residues), the pattern of detected polypeptides followed the change in calculated molecular mass based on amino acid compositions. SDS/PAGE and autoradiography of photolabelled membranes of COS-7 cells expressing α_{1A-1} -AR with [125 I]iodoarylazidoprazosin revealed a single major polypeptide of 65 kDa (results not shown). Therefore the intermediate peptides (55–70 kDa) might be monomeric glycosylated α_{1A} -AR, with slightly higher molecular masses than those expected by calculation for each α_{1A} -AR. The lower-molecular-mass species (30–42 kDa) might result from the degradation of the cytoplasmic tail of intermediate polypeptides. The high-molecular-mass polypeptides (110–140 and 165–210 kDa) were 2-fold or 3-fold the intermediate one (50–70 kDa), suggesting that they might be homodimers or homotrimers of α_{1A} -AR isoforms respectively. Quantification by densitometry of autoradiography obtained with the COS-7 cells expressing M5 epitope-tagged α_{1A-1} -AR along with an untagged 7TM α_{1A} -AR (α_{1A-2a} -AR or α_{1A-3a} -AR) or 6TM α_{1A} -AR (α_{1A-3b} -AR, α_{1A-3c} -AR or α_{1A-6} -AR) isoform showed that the four protein bands (40, 65, 130 and 195 kDa) had similar intensities to those revealed in COS-7 cells expressing M5-tagged α_{1A-1} -AR alone (Figure 5B). Therefore co-transfected 7TM α_{1A} -AR or 6TM α_{1A} -AR isoforms did not modify M5-tagged α_{1A-1} -AR protein expression and did not disrupt the oligomerization of α_{1A-1} -AR.

DISCUSSION

α_{1A} -AR, which is the predominant α_1 -AR in prostate and heart, seems to be important in benign prostatic hyperplasia and cardiac hypertrophy [6–8]. However, the regulation of the expression and function of α_{1A} -AR in these pathologies remains unknown. In the present study we have cloned and characterized several splice variants of α_{1A} -AR. With the use of co-expression experiments in COS-7 cells, we have analysed the interactions between these α_{1A} -AR isoforms and the 'original' α_{1A-1} receptor to determine whether the splice variants could modify the biological properties of α_{1A-1} -AR.

Although its structure is not yet completely known, the human gene for α_{1A} -AR differs from other human AR genes by the existence of several introns [11,12,25]. Our present study, together with those of Hirasawa et al. [11] and Chang et al. [12], revealed that at least 12 α_{1A} -AR isoforms existed, deriving from a complex alternative splicing of the gene for α_{1A} -AR. It is interesting to note that all isolated cDNA species diverged after nt 1320 (Figure 1), which corresponded to the junction of TM6 and the third extracellular loop, a location at which Razik et al. [25] demonstrated the presence of a large intron (more than 9 kb). Four of the twelve cloned α_{1A} -AR isoforms (α_{1A-1} -AR, α_{1A-2a} -AR, α_{1A-3a} -AR and α_{1A-4} -AR) presented a G-protein-coupled receptor structure and differed only in their C-terminal regions (Figure 1). The role of the different C-terminal sequences remains to be elucidated. The eight other isoforms lack TM7 and their C-

terminal tails are located extracellularly (Figure 1). In the present study, the functional analysis of the truncated six-TM α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3c} -AR, α_{1A-5} -AR and α_{1A-6} -AR showed that these receptors were unable to bind the α_1 -antagonist [3 H]prazosin and did not respond to adrenaline by an increase in [Ca^{2+}]_i.

To study the tissue distribution of the mRNA encoding the different isolated isoforms, RT-PCR analysis was performed on human tissues that were known for their high expression of α_{1A} -AR transcripts. All nine mRNA species encoding α_{1A-1} -AR, α_{1A-2a} -AR, α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3a} -AR, α_{1A-3b} -AR, α_{1A-3c} -AR, α_{1A-5} -AR and α_{1A-6} -AR were co-expressed in human liver, heart, prostate and hippocampus, thus displaying no tissue specificity (Figure 2). The mRNA of the α_{1A} -AR isoforms containing seven TMs (α_{1A-1} -AR, α_{1A-2a} -AR and α_{1A-3a} -AR) seemed to be more abundant than the truncated isoforms lacking TM7. This latter observation is in accord with the results for the human V_2 vasopressin [26] and mouse neuropeptide Y- Y_1 receptors [27]; the truncated isoforms represented approx. 15 % of total receptors.

The existence of interactions between the α_{1A} -AR isoforms was examined by transient co-expression in COS-7 cells. Co-expression of the 'original' α_{1A-1} -AR with one of the seven other isolated isoforms demonstrated that the α_{1A-2a} -AR and α_{1A-3a} -AR isoforms, which possess seven TMs, modified neither the expression level nor the pharmacological properties of α_{1A-1} -AR (Table 1). In contrast, co-expression of truncated isoforms (α_{1A-2b} -AR, α_{1A-2c} -AR, α_{1A-3c} -AR, α_{1A-5} -AR or α_{1A-6} -AR) with α_{1A-1} -AR induced a 50 % decrease in [3 H]prazosin binding to α_{1A-1} -AR without modifying its pharmacological properties (Tables 1 and 2). Western blot analysis showed that this decrease was not linked to a diminution of the expression of α_{1A-1} -AR in co-transfected cells (Figure 5B). This decrease in binding was specific for the truncated isoforms because no decrease was observed when α_{1A-2a} -AR, α_{1A-3a} -AR or interleukin 4 receptor was co-expressed with α_{1A-1} -AR (Table 2). A 50 % decrease in agonist binding has been described for the V_2 vasopressin and gonadotropin-releasing hormone receptors when wild-type receptors were co-expressed with truncated isoforms lacking TM6 and TM7 [19,27]. For both receptors, this decrease in binding sites was related to a decrease in cell-surface trafficking and in signal transduction of the wild-type receptor [19,28].

Subcellular localization of α_{1A-1} -AR examined by immunocytochemistry revealed a high concentration of α_{1A-1} -AR isoforms in the perinuclear region (Figure 3B). This subcellular distribution of α_{1A-1} -AR differed clearly from the distribution of α_{1B} -AR, which was massively expressed at the cell surface [29,30]. A subcellular localization similar to that of α_{1A-1} -AR has been observed for α_{2C} -AR [31] but the physiological and functional relevance of such a large intracellular receptor pool remains unknown. However, similarly to α_{2C} -AR [22], binding experiments using intact cells revealed that most measurable [3 H]prazosin-binding sites were located at the cell surface (92 ± 9 % of binding sites). The result was similar to those obtained with HEK-293 cells expressing bovine α_{1A} -AR (87 ± 7 % of [125 I]-BE2254-binding sites at the cell surface) by Theroux et al. [32]. Most of the α_{1A} -AR in intracellular storage might therefore have an unfavourable conformation for recognition of the radioligand. Despite the small amount of cell-surface α_{1A-1} -AR isoforms, we could observe by immunocytochemistry that co-expression of M5-tagged α_{1A-1} -AR with truncated untagged α_{1A} -AR isoforms profoundly inhibited (by approx. 56 %) the appearance of α_{1A-1} -AR at the plasma membrane (Figure 4). These results suggested that the impaired insertion of α_{1A-1} -AR into the plasma membrane might be the molecular mechanism underlying the specific

negative effect of the co-expressed truncated α_{1A} -AR isoforms. The unexpected decrease in the number of intracellular [3 H]-prazosin-binding sites, which was not associated with a variation in the amount of receptor proteins (as measured by Western blots), supports the hypothesis of direct interactions between α_{1A} -AR isoforms. These molecular interactions probably lead to a modification of the conformation of the α_{1A-1} receptors that would account for the loss of [3 H]prazosin-binding sites. In that perspective, the loss of binding sites observed at surface of the co-transfected cells, which could be explained by impaired trafficking of the receptors, could also be the result of an alteration of the receptor conformation. Both molecular mechanisms could be the consequence of heterodimer formation between α_{1A-1} -AR and the truncated isoforms. Surprisingly, the loss of binding sites both in the intracellular pool and at the cell surface observed in co-expression experiments was accompanied by a very slight decrease in the noradrenaline-stimulated [Ca^{2+}]_i response (Table 2), suggesting that, in our experimental conditions, only a small portion of α_{1A} -AR was required at the cell surface to obtain a maximal increase in [Ca^{2+}]_i or that the interaction between α_{1A-1} -AR and the truncated isoforms did not strongly alter this transduction pathway. For the V_2 and gonadotropin-releasing hormone receptors, an excess (15-fold) of splice variant cDNA in comparison with the wild type was necessary to observe a decrease of 50% in agonist-induced cAMP accumulation and phosphoinositide hydrolysis respectively. However, one could note that such an excess of splice variant might not be observed under physiological conditions. Recently, Berts et al. [33] suggested that Ca^{2+} release and protein kinase C activation were neither necessary nor sufficient for the α_{1A} -AR-mediated activation of mitogenic responses in PC12 cells. It would therefore be interesting to analyse the possibility that truncated α_{1A} -AR isoforms could modulate more specifically this new transduction pathway of α_{1A-1} -AR.

Negative regulation of wild-type receptors by their mutant truncated counterparts, reported for the V_2 receptors, was correlated with the formation of heterodimers between wild-type and mutant receptors [19]. However, the proportion of heterodimers compared with the total amount of oligomers displayed in immunoblotting experiments was particularly small to account entirely for the negative activity of mutant receptors on wild-type V_2 receptors, thus suggesting a possible lack of stability of such heterodimers under SDS/PAGE conditions. Although no heterodimers between the full-length α_{1A-1} -AR and the truncated α_{1A} -AR isoforms were found by immunoblotting, a large number of pieces of evidence suggested the existence of direct interactions between α_{1A-1} -AR and the truncated isoforms. A small proportion (5%) of cells co-expressing truncated M5-tagged α_{1A-3c} -AR, which was unable to traffic to the plasma membrane when expressed alone, and untagged α_{1A-1} receptors exhibited a fluorescent signal at the cell surface. This modification in the subcellular localization of the α_{1A-3c} -AR isoform when co-expressed with full-length α_{1A-1} -AR supported the hypothesis of an interaction between truncated isoforms and the full-length receptor, interaction that would interfere with the proper intracellular trafficking of two receptors and would allow cell-surface trafficking of the α_{1A-3c} -AR. In addition, Western blot analysis revealed that all isolated α_{1A} -AR isoforms expressed in COS-7 cells were present as glycosylated monomeric, dimeric, trimeric and tetrameric forms (Figure 5A). Such oligomeric structures resistant to SDS, heat and reducing agents have been observed for the human β_2 -adrenergic receptor [34]. Hebert et al. [34] demonstrated that Gly-276, Gly-280 and Leu-284 residues, which belong to TM6 of the β_2 -adrenergic receptor, had a crucial role in the dimerization of this receptor. Interestingly, the cytoplasmic

end of TM6 of α_{1A} -AR (274 LGIVVGCFL 283) shared 60% identity with the human β_2 -adrenergic receptor (275 LGIIMGTF-TL 284) and the positions of leucine and glycine residues were preserved. Hebert et al. [35] also showed that a peptide based on the sequence of TM6 of the β_2 -adrenergic receptor was able to disrupt dimerization and adenylate cyclase activation of the β_2 receptor. The same kind of biochemical event occurred with the dopamine D_1 receptor, for which the D_1 TM6 peptide might interact with native TMs to alter the three-dimensional conformation of the D_1 receptor and modify its ligand and signal transduction properties without disrupting the homodimer [36]. Our results suggest that the truncated α_{1A} -AR isoforms isolated in our study could interact directly with functional α_{1A-1} -AR oligomers, possibly via TM6. Similarly to the dopamine D_1 receptor, this interaction would modify the binding capacity and intracellular trafficking of α_{1A-1} -AR without either the production of SDS-stable heterodimers or the disruption of the α_{1A-1} -AR oligomers.

In summary, we present for the first time the existence of interactions between α_{1A-1} -AR and other α_{1A} -AR isoforms. The existence of a large number of truncated α_{1A} -AR isoforms that could interfere with α_{1A-1} -AR suggests that these truncated isoforms might have a role in some pathologies involving α_{1A} -AR, such as benign prostate hyperplasia and myocardial hypertrophy. These interactions might constitute a novel mechanism regulating the biological properties of α_{1A} -AR that does not exist for the human α_{1B} -AR and α_{1D} -AR isoforms, thus opening new avenues for the pharmacologist.

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