The Impact of $\alpha_1$-Adrenoceptors Up-Regulation Accompanied by the Impairment of $\beta$-Adrenergic Vasodilatation in Hypertension

Eduardo Oliver, Daniel Martí, Fermí Montó, Nicla Flacco, Lucrecia Moreno, Domingo Barettoni, M. Dolores Ivirona, and Pilar D’OCon

Departmento de Farmacología, Facultad de Farmacia, Universitat de València, Valencia, Spain (E.O., F.M., N.F., M.D.I., P.D.); Instituto de Biomedicina de Valencia, Valencia, Spain (D.M., D.B.); and Departamento de Ciencias Biomédicas, Universidad Cardenal Herrera-CEU, Valencia, Spain (D.M., L.M.)

Received September 12, 2008; accepted December 4, 2008

ABSTRACT

In human and animal hypertension models, increased activity of G-protein-coupled receptor kinase (GRK) 2 determines a generalized decrease of $\beta$-adrenergic vasodilatation. We analyzed the possibility of differential changes in the expression and functionality of $\alpha_{1A}$, $\alpha_{1D}$, $\beta_1$, $\beta_2$, and $\beta_3$-ARs also being involved in the process. We combined the quantification of mRNA levels with immunoblotting and functional studies in aortas of young and adult spontaneously hypertensive rats (SHRs) and their controls (Wistar Kyoto). We found the expression and function of $\beta_1$-adrenoceptors in young prehypertensive SHRs to be higher, whereas a generalized increase in the expression of the six adrenoceptors and GRK2 was observed in aortas of adult hypertensive SHRs. $\alpha_{1D}^-$ and $\beta_3^+$-Adrenoceptors, the subtypes that are more resistant to GRK2-mediated internalization and mostly expressed in rat aorta, exhibited an increased functional role in hypertensive animals, showing two hemodynamic consequences: 1) an increased sensitivity to the vasoconstrictor stimulus accompanied by a decreased sensitivity to the vasodilator stimulus ($\alpha_1^-\text{ARs}$ are the most sensitive to agonists, and $\beta_3^-$ARs are the least sensitive to agonists); and 2) a slower recovery of the basal tone after adrenergic stimulus removal because of the kinetic characteristic of the $\alpha_{1D}$ subtype. These functional changes might be involved in the greater sympathetic vasoconstrictor tone observed in hypertension.

Although there is growing evidence that essential hypertension is related to the overactivity of the sympathetic nervous system, the exact causes are still poorly understood. The adrenergic-dependent increase in vascular resistance could reflect an imbalance between vasoconstrictor and vasodilator mechanisms related to changes in both the expression and function of $\alpha_1$-adrenoceptors (ARs), which mediate vasoconstriction, $\beta$-ARs, which mediate vasodilatation, and/or changes in G-protein-coupled receptor kinases (GRKs), the key regulators of the $\beta$-ARs (Feldman and Gros, 2006; Penela et al., 2006).

This work was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología [Grant SAF2004-01541]; Generalitat Valenciana [Grants GV2004-8-085, GRUPOS05-038]; and Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias [Grant FIS PI070509]; and by the Spanish Ministry of Education and Science [Fellowships AP-2004-3536 and AP-2005-5076]. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.108.146043.

ABBREVIATIONS: AR, adrenoceptor or adrenergic receptor; GRK, G-protein-coupled receptor kinase; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto; SBP, systolic blood pressure; RT, reverse transcription; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ct, threshold cycle; SHS861A, ethyl (7S)-7-[(2R)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydro-2H-1-benzopyran-2-ol; BMY 7378, 8-[2-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride; 5-methylurapidil, 5-methyl-$\beta$-[3-[4-[2-methoxyphenyl]-1-piperazinyl]propyl]amino]-1,3-dimethyluracil); phenylephrine, (R)-(−)-1-(3-hydroxyphenyl)-2-methylaminooethyl hydrochloride; prazosin, 1-(4-amino-6,7-dimethoxy-2-quinoxalinyl)-4-(2-furanylcarbonyl)piperazine hydrochloride.
2002a,b; Ziani et al., 2002; D'Ocon, 2003; Lyssand et al., 2008), but the mechanism responsible for this increase has not been elucidated previously.

We believed that analyzing the expression of the different ARs involved in the control of blood pressure in vessels, and also GRK2, the kinase that regulates their activity, could lead to an accurate picture of the sympathetic changes related to the hypertensive state. Therefore, in this study, we combined a relative quantification of mRNA levels for GRK2, and the $\alpha_1A$, $\alpha_1B$, $\alpha_1D$, $\beta_1$, $\beta_2$, and $\beta_3$ARs with the determination of the protein expression by Western blot in thoracic aortas obtained from spontaneously hypertensive rats (SHRs). Then, we compared the results with their respective controls [Wistar Kyoto (WKY) rats]. To determine whether the changes in the expression accompany the hypertensive state, we performed our study in two different groups of animals, young rats in a prehypertensive state, and hypertensive adult rats. The data presented herein demonstrate that the GRK2 and ARs expressions are up-regulated in hypertensive animals. Therefore, functional studies were also performed to analyze the consequences of these changes in the control of the vascular tone.

Materials and Methods

Normotensive (WKY) rats and SHRs (6 and 16 weeks old) were used (Harlan, Indianapolis, IN) and housed under a 12-h light/dark cycle at 22°C and 60% humidity. Systolic blood pressure (SBP) and heart rate were measured from the tail of unanesthetized rats with a plethysmographic method (NIPREM 645; Cibertec, Madrid, Spain). An average of six readings was recorded for each animal. Thoracic aortas were obtained as described previously (Gisbert et al., 2003a).

Real-Time Quantitative RT-PCR. Total RNA was obtained and the RT reaction was performed as described previously (Marti et al., 2005). mRNAs encoding the three $\alpha_1$-adrenoceptors ($\alpha_1A$, $\alpha_1B$, and $\alpha_1D$), the three $\beta$-adrenoceptors ($\beta_1$, $\beta_2$, and $\beta_3$), GRK2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard, were quantified by TaqMan real-time RT-PCR with a GeneAmp 5700 sequence-detection system (Applied Biosystems, Foster City, CA). We analyzed (in duplicate reactions) a 10-fold dilution of the RT reaction of each sample using the TaqMan Gene Expression Assays (Applied Biosystems).

The eight specific primer-probes were $\alpha_1A$-AR (Rn00567876_m1), $\alpha_1B$-AR (Rn01471343_m1), $\alpha_1D$-AR (Rn00577931_m1), $\beta_1$-AR (Rn00824536_s1), $\beta_2$-AR (Rn00566056_s1), $\beta_3$-AR (Rn00565939_m1), GRK2 (Rn00562822_m1), and GAPDH (Rn99999916_s1) (Applied Biosystems). Real-time PCR reactions were done in 25 µl with TaqMan Universal PCR Master Mix (Applied Biosystems), including 5 µl of diluted RT reaction, and 1.25 µl of 20× TaqMan Gene Expression Assay Mix (250 nM for the probe and 900 nM for each primer). cDNA was amplified following the manufacturer's conditions: one initial hold step at 95°C for 10 min, a second step with 40 cycles, 15 s at 95°C (denaturation), and 1 min at 60°C (annealing/extension). The targets and reference (GAPDH) were amplified in parallel reactions. A minimum of three samples from three different animals were analyzed for each condition.

The threshold cycle (Ct) values obtained for each gene were referenced to GAPDH and converted to the linear form using the term $2^{-\Delta\Delta C_t}$ as a value directly proportional to the copy number of mRNA. GAPDH levels increased in 16-week-old animals. To compare the mRNA levels of the target genes between strains, the expression was also assessed using the comparative ($2^{-\Delta C_t}$) method, but in this case, the value obtained for each gene in the WKY animals was used as a reference (Livak and Schmittgen, 2001).

Western Blot. To obtain total proteins, the frozen aortas were ground to powder in a mortar and homogenized with a Microson XL 2000 Ultrasonic Liquid Processor in ice-cooled lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1.5 mM MgCl₂, 0.1% SDS, 1 mM EDTA, 100 mM NaF, 1% Triton, 1% sodium deoxycholate) containing protease inhibitor cocktail (Complete; Roche Applied Science, Indianapolis, IN). This was centrifuged at 16,000g for 15 min at 4°C. The protein concentration was determined by the Bradford (1976) method (Bio-Rad, Hercules, CA).

Protein extracts (50 µg for GRK2 and 150 µg for adrenoreceptors) were loaded onto 10% SDS-polyacrylamide gels according to Laemmli (1970), and electrophoresed proteins were transferred to polyvinylidene difluoride membranes 2 h at 375 mA, using a liquid Mini Trans-Blot Electrophoretic Transfer Cell system (Bio-Rad). Membranes were blocked in 6% nonfat dried milk in phosphate-buffered saline containing 0.1% Tween 20 for 1 h at room temperature with gentle agitation. Membranes were washed and then incubated with goat polyclonal antibody against $\alpha_1A$-AR (sc-1477, 1:100), $\alpha_1B$-AR (sc-1476, 1:100), $\alpha_1D$-AR (sc-1475, 1:250), $\beta_2$-AR (sc-1473, 1:100), rabbit polyclonal antibody against $\beta_2$-AR (sc-568, 1:100), $\beta_3$-AR (sc-9042, 1:250), and GRK2 (sc-562, 1:250) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and with rabbit Anti-Actin (A2066, 1:2000; Sigma-Aldrich, St. Louis, MO) as a loading control diluted in blocking solution at 4°C overnight. Membranes were then washed three times, incubated with rabbit anti-goat IgG horse-radish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at 1:2500 or with donkey anti-rabbit IgG horse-radish peroxidase conjugated (GE Healthcare, Chalfont St. Giles, UK) at 1:2500 or 1:3000 for 50 min at room temperature and washed extensively before incubation with enhanced chemiluminescence Western blotting detection reagent (GE Healthcare). Membranes were immediately documented and quantified with an Autochemi BiolImaging System using the Labworks 4.6 capture software (Ultra-Violet Products Ltd., Cambridge, UK).

Functional Study in Isolated Organ Bath. Rings of fresh rat aorta were mounted in an organ bath containing Krebs’ solution gassed with 95% O₂ and 5% CO₂. The presence of a functional endothelium was confirmed as described previously (Marti et al., 2005).

Addition of cumulative doses of Phe (10⁻⁹ to 10⁻⁶ M) was carried out until a maximal response was reached (Eₘₐₓ). The concentration [−log (molar)], needed to produce 50% of contraction (pEC₅₀), was obtained from a nonlinear regression plot (GraphPad Software Inc., San Diego, CA). After agonist removal from the tissue bath, we also analyzed the kinetics of tissue relaxation. For this purpose, the washing procedure was carried out with a total replacement of the bathing solution by three repeated washes within the first 30 s and by two other repeated washes every 5 min in all cases.

Relaxation-response curves to β-AR agonists [isoprenaline (10⁻¹⁰ to 10⁻⁴ M) and SR58611A (10⁻¹⁰ to 10⁻⁴ M)] or α₁-AR antagonists [BMY 7378 (10⁻⁹ to 10⁻⁶ M) or SR5415 (10⁻⁹ to 10⁻⁶ M)] were performed by adding cumulative concentrations to vessels in which sustained contractions had been induced by a maximal concentration of phenylephrine (10⁻⁴ M). The concentration [−log (molar)] needed to either produce 50% relaxation (pEC₅₀) or to inhibit contractility was determined by applying the Hill equation (Hill, 1902).

| SBP (mm Hg) | Heart rate (beats/min) |
|------------|------------------------|-----------------|----------------|------------------|
| Young Rats | Adult Rats |
| WKY | SHR | WKY | SHR |
| 120 ± 3 | 129 ± 12 | 140 ± 2 | 197 ± 4*** |
| 274 ± 28 | 292 ± 69 | 311 ± 10 | 374 ± 7*** |

*** P < 0.001 vs. respective WKY.
50% of the maximal contractile response (pIC$_{50}$) was obtained from a nonlinear regression plot, and the data of the mean curve were fitted to one- or two-site models (GraphPad Software Inc.), as described previously (Marti et al., 2005).

**Data Analysis.** The results are presented as the mean ± S.E.M. for $n$ determinations obtained from different animals. A statistical analysis was performed by two-way analysis of variance, followed by the Student’s t test for unpaired samples (GraphPad Software Inc.). Significance was defined as $P < 0.05$.

**Drugs.** The following drugs were obtained from Sigma-Aldrich: phenylephrine, prazosin, BMY 7378, and 5-methylurapidil. SR58611A was a generous gift from sanofi-aventis (Bridgewater, NJ). Other reagents were of analytical grade.

**Results**

**Hemodynamic Constants.** SBP and the heart rate of WKY rats and SHRs are summarized in Table 1. No significant differences were found between WKY rats and SHRs in the group of young animals (6 weeks old). However, in the adult SHRs (16 weeks old), the hypertensive state in SHRs was confirmed by hemodynamic constants.

![Graph](image_url)

**Fig. 1.** mRNA levels of the $\alpha_{1A}$-, $\alpha_{1B}$-, $\alpha_{1D}$-, $\beta_1$-, $\beta_2$-, and $\beta_3$-adrenoceptors and GRK2 in aortas of young (white bars) and adult (striped bars) WKY rats. Values were expressed as $2^{-\Delta Ct}$, using GAPDH as a housekeeping gene, and are the mean ± S.E.M. of $n = 4$ to 6 different animals.

![Graph](image_url)

**Fig. 2.** Comparative analysis of the expression of the $\alpha_{1A}$-, $\alpha_{1B}$-, $\alpha_{1D}$-, $\beta_1$-, $\beta_2$-, and $\beta_3$-adrenoceptors and GRK2 in aortas of young prehypertensive SHRs (black bars) and their controls (WKY; white bars). Graphs show mRNA levels expressed as $2^{-\Delta Ct}$ using GAPDH as a housekeeping gene (mean ± S.E.M. of $n = 4$–6 different animals) (A); protein expression measured by densitometric analysis and expressed as the ratio to $\beta$-actin (B); and immunoblotting representative of three different experiments (C).
Analysis of the Expression of ARs and GRK2 in Aortas and Changes with Hypertension. mRNA for the \( \alpha_{1A}, \alpha_{1D}, \alpha_{2A}, \alpha_{2D}, \beta_1, \) and \( \beta_2 \)-ARs and GRK2 was present in the rat aorta from young and adult WKY rats. No significant differences were found with age, and the highest level of expression corresponds to the \( \alpha_{1D} \) and \( \beta_2 \)-ARs (Fig. 1).

Figure 2 shows that the mRNA levels of the three \( \alpha_1 \)-ARs tested were similar between strains in young animals. A nonsignificant increase in \( \beta_1 \)-ARs at the mRNA levels, which was more evident in Western blots, was observed in SHRs (Fig. 2B). The other slight changes observed in the mRNA expression were not corroborated by immunoblotting (data not shown).

The Ct of GAPDH was the same between strains in young animals, but, as Fig. 3 shows, the level of expression of GAPDH was significantly higher in the rat aorta from adult SHRs in relation to WKY rats. This increase in GAPDH could be related to the vascular remodeling characteristic of hypertension (Xin et al., 1997). To avoid any misinterpretation of the changes in the expression of the target genes, their values were not assessed in relation to GAPDH. Instead, in this case, the expression level of each gene in WKY animals was used as a reference, and an increase in the mRNA expression of the six ARs and GRK2 was observed in aorta from adult SHRs in relation to WKY animals (Fig. 4A). These results correlate well to the increase in protein expression determined by immunoblotting (Fig. 4B).

Changes in the Functional Role of the ARs in Aorta from SHRs and WKY Animals. To determine the functional consequences of changes in the expression of ARs and GRK2 in aortas of young and adult WKY rats and SHRs, concentration-response curves of contraction to the selective \( \alpha_1 \)-agonist phenylephrine or relaxation to \( \alpha_1 \)-AR antagonists or \( \beta_2 \)-AR agonists were performed.

The main findings were as follows. 1) The maximal contractile response of rat aorta to phenylephrine, and to KCl, decreases in young prehypertensive and adult SHRs (Table 2), which corroborates previous results observed in the aorta but not in other vessels (Gisbert et al., 2002). pEC\textsubscript{50} to phenylephrine does not change in young SHRs versus WKY rats. However, pEC\textsubscript{50} in adult animals was significantly higher in SHRs than in WKY rats (Table 2; Fig. 5A). The difference in the potency of phenylephrine was accompanied by a significantly slower return to baseline after removal of the agonist in SHRs versus WKY rats (Fig. 5B), which was not observed after KCl removal (data not shown).

2) The \( \alpha_1 \)-AR antagonists 5-methylurapidil and BMY 7378 inhibit the sustained contraction elicited by phenylephrine in aorta in a concentration-dependent manner. As shown in Table 3 and Fig. 6, a significant increase in the potency (pIC\textsubscript{50}) of the selective \( \alpha_{1D} \) agonist BMY 7378 was seen in aortas from adult SHRs versus WKY animals confirming the major functional role of the \( \alpha_{1D} \)-ARs previously reported in aorta from adult hypertensive rats but not in young animals (previous results, Gisbert et al., 2002). However, no such change was found with the selective \( \alpha_{1A} \) antagonist 5-methylurapidil (Table 3; Fig. 6).

3) The \( \beta_2 \)-AR agonists isoprenaline and SR58611A relax the sustained contraction elicited by phenylephrine concentration-dependently. Isoprenaline showed a higher potency in young prehypertensive SHRs but a lower potency in adult SHRs compared with controls (Table 3; Fig. 7). Curves of relaxation to the selective \( \beta_2 \)-AR agonist SR58611A were biphasic and discriminated two populations of \( \beta_2 \)-adrenoceptors with a high and low potency in both young and adult animals (Table 3; Fig. 7), indicating that a mixed population of \( \beta_2 \) and \( \beta_2 \)-ARs play a functional role in the rat aorta. A significant increase in the fraction of high potency by SR58611A was found in the aorta of adult hypertensive animals, suggesting a higher functional role of \( \beta_2 \)-ARs in this strain (Table 3).

**Discussion**

The results obtained in the present study focus on two different aspects: 1) The first aspect is the majority expression and functional role of \( \alpha_{1A} \)-ARs in the aorta, corroborating previous functional evidences (Gisbert et al., 2000; Marti et al., 2005), and the unexpected results obtained with \( \beta_2 \)-ARs, higher levels of mRNA for the \( \beta_2 \)-AR, followed by \( \beta_1 \)-AR and a slight expression of \( \beta_2 \)-AR. Although a quantitative determination of mRNA for the different \( \beta_2 \)-adrenoceptors has not been systematically performed before in the rat aorta, these results contrast with classic pharmacological studies that attribute the \( \beta_2 \)-mediated relaxant response in vessels to the \( \beta_2 \)-AR (Guimaraes and Moura, 2001). Furthermore, they are in accordance with more recent evidence of a role for the \( \beta_2 \)- (Chruscienski et al., 2001) and \( \beta_3 \)-ARs in the rat aorta (Trocho et al., 1999; Rautureau et al., 2002). 2) The second aspect is changes in the expression of adrenoceptors and GRK2 related to hypertension. In the aorta of young prehypertensive SHRs, only an increase in the \( \beta_1 \)-adrenoceptor expression and function was observed. Then, this change preceded the hypertensive state and could be involved in the development of hypertension. Comparing age-matched WKY rats and SHRs, however, we observed a higher expression (mRNA and protein) of the \( \alpha_{1A}, \alpha_{1D}, \beta_1, \beta_2 \), and \( \beta_2 \)-ARs, accompanied by an up-regulation of GRK2.

The increased mRNA expression of \( \alpha_{1D} \) and \( \beta_2 \)-AR in aorta from SHRs has been described previously by Godinez-Hernández et al. (2006) and Mallem et al. (2005), respectively.
The higher protein expression of GRK2, directly related to hypertension, confirms previous evidence in both the aorta and lymphocytes of rats (Gros et al., 2000) and in lymphocytes of hypertensive patients (Gros et al., 1999). Although no changes in mRNA levels for GRK2 have been reported previously, the apparent discrepancies between our results and
It is well known that GRK2 is apparently the main factor involved in diminishing β-AR signaling in hypertension (Feldman and Gros, 2006; Penela et al., 2006). Nonetheless, β3-AR is resistant to agonist-promoted desensitization mediated by GRK2 (Rozec and Gauthier, 2006). In accordance with this, an increased expression of β3-AR justifies the higher vasorelaxant potency of isoprenaline found in prehypertensive aortas. In hypertensive aortas, the higher expression of β1- and β2-ARs could be counteracted by the increased levels of the active GRK2 found. However, β2-ARs resist the desensitization mediated by GRK2. Thus, the functional role of this subtype could be increased in relation to the other two. Our functional studies confirm this proposal; in the aorta of hypertensive rats, the lower potency of isoprenaline (which exhibits a low affinity for the β3-ARs (Strosberg, 1997), and the high potency of the selective β3 agonist SR58611A, together with an increase in the percentage of sites of high affinity for this agonist, suggest an increased functional role of β3-ARs in hypertension. From a physiopathological point of view, because vascular β3-ARs are only stimulated by higher doses of catecholamines (Strosberg, 1997), the major role of β3-ARs in hypertensive vessels determines an impaired β-mediated vasodilator mechanism that is only triggered by a higher adrenergic stimulus.

Table 2: Parameters of the concentration-response curves of contraction to phenylephrine and the maximal contraction obtained with a depolarizing solution (60 mM KCl) in the aorta isolated from young and adult WKY and SHR animals. Values were expressed as the mean ± S.E.M. of n = 4 animals.

<table>
<thead>
<tr>
<th></th>
<th>Young Rats</th>
<th>Adult Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_{max}</td>
<td>7.03 ± 1.18</td>
<td>5.46 ± 0.66</td>
</tr>
<tr>
<td>Phenyphrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_{max}</td>
<td>7.47 ± 1.09</td>
<td>4.33 ± 0.40*</td>
</tr>
<tr>
<td>pEC_{50}</td>
<td>8.38 ± 0.34</td>
<td>8.42 ± 0.17</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. WKY.
** P < 0.01 vs. WKY.
*** P < 0.001 vs. WKY.

Fig. 5. A, cumulative concentration-response curves of contraction to phenylephrine in adult rats. B, time course of the decay in the maximal contractile response to phenylephrine after removal of the agonist in adult rats. Experiments were carried out in aortas obtained from SHRs (●) and WKY rats (○). Data are the mean ± S.E.M. of four to eight experiments.
In addition, α1-AR subtypes also exhibit different phosphorylation and internalization patterns: α1D-AR (Chalothorn et al., 2002) undergoes a rapid and intense desensitization mediated by GRKs (Diviani et al., 1996; García-Sáinz et al., 2000). The α1A subtype exhibits a continuous and agonist-independent trafficking, which is also agonist- and GRK2-dependent, between the membrane and cytosol (Morrisey et al., 2004; Pediani et al., 2005). Finally, the intracellularly located α1D subtype (McCune et al., 2000; Hague et al., 2004) has a major affinity for the agonists (Minneman et al., 1994; Marti et al., 2005), does not exhibit an agonist-dependent internalization (McCune et al., 2000), and remains active even when the agonist is removed (Gisbert et al., 2000, 2002, 2003b). In accordance, the α1D subtype does not seem to be as sensitive to GRKs modulation as the α1A and α1B subtypes, and a higher increase in the expression of the GRK2 might have no functional relevance in the activity of α1D-ARs. Thus, we must expect an increased functionality of the α1D-ARs in aortas of adult SHRs, even when the GRK2 expression increases. Considering that the α1D directly regulates blood pressure via vasoconstriction (Tanoue et al., 2002a,b; Lyssand et al., 2008), the higher functionality of this subtype in hypertensive arteries could determine the increase in the mean arterial pressure observed in adult SHRs.

Present results and our previous observations in aorta and main mesenteric and small mesenteric arteries (Gisbert et al., 2002) confirm this proposal. The higher potency, exhibited only by the α1D-selective antagonist BMY 7378 and not by the selective α1A antagonist 5-methylurapidil, indicates an increased role of the α1D subtype in the sympathetic vasoconstriction of the aortas of SHRs. There were two hemodynamic consequences of the higher functionality of α1D-ARs: 1) a higher potency of phenylephrine in hypertensive aortas because of the higher affinity of this subtype for agonists (Minneman et al., 1994; Marti et al., 2005), then being the hypertensive vessels more sensitive to the contractile adrenergic stimulus; 2) a slower decay of the contractile response after removing the agonist because of the characteristic of the α1D-AR to remain active when the stimulus disappears, as the present results confirm and our previous studies describe in the aorta, main mesenteric artery, and small mesenteric arteries (Gisbert et al., 2002, 2003b; Ziani et al., 2002). Therefore, the consequences of an increased functionality of α1D-ARs, a higher sensitivity to an α1-adrenergic stimulus, together with a significantly slower decay in the contractile tone after stimulus removal, could all determine the pathological increase in the adrenergic vascular tone observed in hypertension. This is especially relevant if...
we consider that, as occurs in aorta, a similar increase in the functional role of \( \alpha_{1D} \)-ARs was shown previously by us in small mesenteric arteries from SHRs, and this increase was prevented by captopril treatment (Gisbert et al., 2002).

In conclusion, our results clearly show that an increase in the expression of \( \beta_1 \)-AR precedes the hypertensive state, whereas an increase in the expression of the \( \alpha_{1A} \), \( \alpha_{1B} \), \( \alpha_{1D} \), \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-adrenoceptors, accompanied by an increase in the expression of GRK2, occurs in the aorta when the hypertensive state appears. The higher GRK2 expression impairs the expression of GRK2, occurs in the aorta when the hypertension, and this increase was prevented by captopril treatment (Gisbert et al., 2002). Functional characterization of \( \alpha_{1A} \)-adrenoceptor subtypes, in Inverse Agonism (Ijzeran AP ed) pp 63–74, Elsevier, Leiden, The Netherlands.

### References


---

**Fig. 7.** Cumulative concentration-response curves of relaxation for the \( \beta \)-adrenoceptor agonists isoprenaline and SR58611A on the phenylephrine-induced contraction in young and adult rats. Experiments were carried out in aortas obtained from SHRs (●) and WKY rats (○). Data are the mean ± S.E.M. of four to eight experiments.


Villalobos-Molina R and Ibarra M (1996) alpha(1D)-adrenoceptors mediating contraction in arteries of normotensive and spontaneously hypertensive rats are of the alpha(1D) or alpha(1A) subtypes. Eur J Pharmacol 298:257–263.

Address correspondence to: Pilar D'Ocon, Departamento de Farmacología, Facultad de Farmacia, Universitat de València, Avda. Vicent Andrés Estelles s/n, Burjassot 46100, València, Spain. E-mail: m.pilar.docen@uv.es