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Title page

The impact of α_1-adrenoceptors up-regulation accompanied by the impairment of β-adrenergic vasodilatation in hypertension

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Abbreviations:

GRK, G-protein-coupled Receptor Kinase; SHR, Spontaneously Hypertensive Rats; WKY, Wistar Kyoto rats; AR, Adrenoceptor or Adrenergic Receptor; SBP, Systolic blood pressure; HR, Heart Rate; RT, Reverse Transcription; PCR, Polymerase Chain Reaction; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; PVDF, Polyvinyldiene fluoride; PBS, Phosphate Buffered Saline; ECL, Enhanced Chemiluminescence; Ct, Threshold Cycle.

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ABSTRACT

In human and animal hypertension models, increased activity of G-protein-coupled receptor kinase 2 (GRK2) determines a generalized decrease of β-adrenergic vasodilatation. We analyzed the possibility of differential changes in the expression and functionality of α_{1A}, α_{1B}, α_{1D}, β_1, β_2 and β_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 (SHR) and their controls (WKY). We found the expression and function of β_1 adrenoceptors in young prehypertensive SHR animals to be higher, while a generalized increase in the expression of the six adrenoceptors and GRK2 was observed in aortas of adult hypertensive SHR rats. α_{1D} and β_3 adrenoceptors, the subtypes that are more resistant to GRK2-mediated internalization, and majoritary 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 (α_{1D} ARs are the most sensitive and β_3 ARs are the least sensitive to agonists); (2) a slower recovery of the basal tone after adrenergic stimulus removal due to the kinetic characteristic of the α_{1D} subtype. These functional changes might be involved in the greater sympathetic vasoconstrictor tone observed in hypertension.

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INTRODUCTION

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 α_1 adrenoceptors (ARs) which mediate vasoconstriction, and β-ARs which mediate vasodilatation and/or changes in G-protein coupled receptor kinases (GRKs), the key regulators of the β-ARs (Penela *et al.*, 2006; Feldman and Gros, 2006).

A generalized impairment of β-adrenergic mediated vasodilatation has been shown in human hypertensive patients and also in animal models of hypertension (Borkowski *et al.*, 1992). This impairment has been related to an increase in the activity of GRKs, especially GRK2 which mediates reduced β–adrenoceptor/G-protein coupling (Feldman and Gros, 2006). However, the possibility of differential changes in both the expression and function of β_1, β_2 and β_3 ARs also being involved in this process has not been previously evaluated.

The α_1 ARs present in vessels (α_{1A} , α_{1B}, α_{1D}) not only play an essential role in the modulation of the vascular tone, but also in the regulation of blood pressure through their vasoconstrictor activity (Piascik *et al.*,1995; Villalobos-Molina and Ibarra, 1996; Noguera *et al.*,1996, Hrometz *et al.*, 1999; Gisbert *et al.*, 2000; 2003a). An increased functional role of α_{1D} ARs has been proposed as one of the changes involved in the hypertensive increase of vascular resistance (Villalobos-Molina *et al.*, 1999; Gisbert *et al.*, 2002; Tanoue et al., 2002a and 2002b; Ziani *et*

al., 2002; D'Ocon, 2003; Lyssand et al., 2008), but the mechanism responsible for this increase has not been previously elucidated.

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 α_{1A}, α_{1B}, $\alpha_{1D,}$ β_1, β_2 and β_3 ARs with the determination of the protein expression by western blot in thoracic aortas obtained from spontanously hipertensive rats (SHR). Then we compared the results with their respective controls (WKY rats). In order 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 upregulated in hypertensive animals. Therefore, functional studies were also performed to analyze the consequences of these changes in the control of the vascular tone.

METHODS

Normotensive (WKY) and SHR rats (6- and 16-week-old) were used (Harlan Interfauna Ibérica, Spain) and housed under a 12-hour light/dark cycle at 22ºC and 60% humidity. Systolic blood pressure (SBP) and heart rate (HR) were measured from the tail of unanaesthetized rats with a plethysmographic method (NIPREM 645 Cibertec, Spain). An average of six readings were recorded for each animal. Thoracic aortas were obtained as previously described (Gisbert et al., 2003b). This research work conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996), and was also approved by the Ethics Committee of the University of Valencia.

Real-time quantitative RT-PCR

Total RNA was obtained and the RT reaction was performed as previously described (Marti *et al.*, 2005). mRNAs encoding the three α_1- AR subtypes (α_{1A}, α_{1B} and α_{1D}), the three β-adrenoceptors (β_1, β_2 and β_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, USA). We analyzed (in duplicate reactions) a 10-fold dilution of the RT reaction of each sample using the TaqMan® Gene Expression Assays (Applied Biosystems, USA).

The eight specific primer-probes were α_{1A}-AR (Rn00567876_m1), α_{1B}-AR (Rn01471343_m1), α_{1D}-AR (Rn00577931_m1), β_1-AR (Rn00824536_s1), β_2-AR (Rn00560650_s1), β_3 (Rn00565393_m1), GRK2 (Rn00562822_m1) and GAPDH (Rn99999916_s1) (Applied Biosystems, USA). Real-time PCR reactions were done in 25 µL with TaqMan® Universal PCR Master Mix (Applied Biosystems, USA), including 5 µL of diluted RT reaction, and 1.25 µL of 20X TaqMan® Gene Expression Assay Mix (250 nmol/L for the probe and 900 nmol/L for each primer). cDNA was amplified following the manufacture's conditions: one initial Hold-step at 95ºC 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 Ct values obtained for each gene were referenced to GAPDH and converted to the linear form using the term $2^{-\Delta Ct}$ 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 ($2^{-\Delta Ct}$) method, but in this case the value obtained for each gene in the WKY animals was used as a reference (Livak *et al.* 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 RIPA lysis buffer (50mmol/L HEPES pH=7.5, 150mmol/L NaCl, 10% Glicerol, 1.5mmol/L $MgCl_2$, 0.1% SDS, 1mmol/L EGTA, 100mmol/L NaF, 1% Triton, 1% Sodium deoxycolate) containing protease inhibitor cocktail (Complete®, Roche Applied Science, Germany). This was centrifuged at 16,000 g for 15 min at 4ºC. The protein concentration was determined by the Bradford method (BioRad Laboratories, Inc.).

Protein extracts (50 mg for GRK2, and 150μg for adrenoceptors) were loaded onto 10% SDS-Polyacrylamide gels according to Laemmli (1970), and electrophoresed proteins were transferred to polyvinyldiene fluoride (PVDF) membranes 2 h at 375 mA, using a liquid Mini Trans-Blot® Electrophoretic Transfer Cell system (Bio-Rad Laboratories, Inc.). Membranes were blocked in 6% nonfat dried milk in phosphate buffered saline (PBS) 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 α_{1A}-AR (sc-1477, 1:100), α_{1B}-AR (sc-1476, 1:100), α_{1D}-AR (sc-1475, 1:250), β_3-AR (sc-1473, 1:100), rabbit polyclonal antibody against β_1-AR (sc-568, 1:100), β_2-AR (sc-9042, 1:250), GRK2 (sc-562, 1:250) from Santa Cruz Biotechnology, and with rabbit Anti-Actin (A2066 1:2000, Sigma-Aldrich) as a loading control diluted in blocking solution at 4ºC overnight. Membranes were then washed three times, incubated with rabbit anti-goat IgG horseradish peroxidase-conjugated secondary antibody (Jackson Immuno-Research laboratories, Inc.) at 1:2500, or with donkey anti-rabbit IgG horseradish peroxidase-conjugated (Amersham Biosciences, UK) (at 1:2500 or 1:3000) for 50 min at room temperature, and washed extensively before incubation

with ECL® western blotting detection reagent (Amersham Biosciences, UK). Membranes were immediately documented and quantified with an Autochemi™ BioImaging 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% O_2 and 5% CO_2. The presence of a functional endothelium was confirmed as previously described (Marti *et al.*, 2005).

Addition of cumulative doses of Phe (10^{-9} to 10^{-6} mol/L) was carried out until a maximal response was reached (Emax). The concentration (-log [mol/L]), needed to produce 50% of contraction (pEC_{50}), was obtained from a nonlinear regression plot (Graph Pad Software; San Diego, California, U.S.A). 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} mol/L) and SR 58611A (10^{-10} to 10^{-4} mol/L), α_1- AR antagonists BMY 7378 (10^{-9} to 10^{-5} mol/L) or to 5-methylurapidil (10^{-9} to 10^{-4} mol/L) were performed by adding cumulative concentrations to vessels in which sustained contractions had been induced by a maximal concentration of phenylephrine (10^{-6} mol/L). The concentration (-log [mol/L]) needed to either produce 50% relaxation (pEC_{50}) or

to inhibit 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 (Graph Pad Software; San Diego, California, U.S.A), as previously described (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 ANOVA followed by the Student's *t* test for unpaired samples (Graph Pad Software). Significance was defined as $P < 0.05$.

Drugs

The following drugs were obtained from Sigma (St Louis, MO, U.S.A.): phenylephrine ((*R*)-(−)-1-(3-Hydroxyphenyl)-2-methylaminoethanol hydrochloride)), prazosin(1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine hydrochloride), BMY 7378 (8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) and 5-methylurapidil (5-Methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]amino]-1,3-dimethyluracil). SR 58611A (ethyl {(7S)-7-[(2R)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphthalen-2-yloxy}acetate hydrochloride) was a generous gift off Sanofi Synthelabo (Toulouse, France). Other reagents were of analytical grade.

RESULTS

Hemodynamic constants

Systolic blood pressure (SBP) and the heart rate of WKY and SHR animals are summarized in Table 1. No significant differences were found between WKY and SHR rats in the group of young animals (6-weeks-old). In the adult SHR animals (16 weeks old) however, the hypertensive state in SHR was confirmed by hemodynamic constants

Analysis of the expression of ARs and GRK2 in aortas and changes with hypertension

mRNA for the $\alpha_{1A,}$ $\alpha_{1B,}$ α_{1D}, β_1, β_2 and β_3 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 α_{1D} and β_3 ARs (Figure 1A).

Figure 2 shows that the mRNA levels of the three α_1 ARs tested were similar between strains in young animals. A non significant increase in β_1 ARs at the mRNA levels, which was more evident in western blots, was observed in SHR (Figure 2B). The other slight changes observed in the mRNA expression were not corroborated by immunoblotting (results not shown).

The threshold cycle (Ct) of GAPDH was the same between strains in young animals but, as Figure 3 shows, the level of expression of GAPDH was significantly higher in the rat aorta from adult SHR in relation to WKY. 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 SHR in relation to WKY animals (Figure 4A). These results correlate well to the increase in protein expression determined by immunoblotting (Figure 4B).

Changes in the functional role of the ARs in aorta from SHR and WKY animals

In order to determine the functional consequences of changes in the expression of ARs and GRK2 in aortas of young and adult WKY and SHR animals, concentration–response curves of contraction to the selective α_1 agonist phenylephrine, or relaxation to α_1-AR antagonists or β-AR agonists, were performed.

The main findings were:

i. The maximal contractile response of rat aorta to phenylephrine, and to KCl, decreases in young prehypertensive and adult SHR (Table 2), which corroborates previous results observed in the aorta but not in other vessels (Gisbert *et al.*, 2002). pEC_{50} to phenylephrine does not change in young SHR vs. WKY. However, pEC_{50} in adult animals was significantly higher in SHR than in WKY (Table 2 and Figure 5A). The difference in the potency of phenylephrine was accompanied by a significantly slower return to the baseline after removal of

the agonist in SHR vs. WKY rats (Figure 5B), which was not observed after KCl removal (results not shown).

ii. The α_1-AR antagonists 5-methylurapidil and BMY 7378 inhibit the sustained contraction elicited by phenylephrine in aorta in a concentration-dependent manner. As Table 3 and Figure 6 show, a significant increase in the potency (pIC_{50}) of the selective α_{1D} antagonist BMY 7378 was seen in aortas from adult SHR vs. WKY animals confirming the major functional role of the α_{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 α_{1A} antagonist 5-methylurapidil (Table 3 and Figure 6)

iii. The β-AR agonists isoprenaline and SR58611A relax the sustained contraction elicited by phenylephrine concentration dependently. Isoprenaline showed a higher potency in young pre-hypertensive SHR, but a lower potency in adult SHR animals when compared with controls (Table 3, Figure 7). Curves of relaxation to the selective β_3-AR agonist SR58611A were biphasic, and discriminated two populations of β adrenoceptors with a high and low potency in both young and adult animals (Table 3, Figure 7), indicating that a mixed population of β_3 and β_1 /β_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 β_3 ARs in this strain (Table 3).

DISCUSSION

The results obtained in the present study focus on two different aspects:

(1) The majority expression and functional role of α_{1D}-ARs in the aorta, corroborating previous functional evidences (Gisbert *et al.*, 2000; Marti *et al.*, 2005), and the unexpected results obtained with β-ARs: higher levels of mRNA for the β_3-AR, followed by β_1-AR and a slight expression of β_2-AR. Although a quantitative determination of mRNA for the different β adrenoceptors has not been systematically performed before in the rat aorta, these results contrast with classic pharmacological studies which attribute the β-mediated relaxant response in vessels to the β_2 AR (Guimaraes and Moura, 2001). Furthermore, they are in accordance with more recent evidence of a role for the β_1- (Chruscinski *et al.*, 2001) and β_3-ARs in the rat aorta (Trochu *et al.*, 1999; Rautureau *et al.*, 2002).

(2) Changes in the expression of adrenoceptors and GRK2 related to hypertension. In the aorta of young pre-hypertensive SHR, only an increase in the β_1 adrenoceptor expression and function was observed. Then, this change preceded the hypertensive state and could be involved in the development of hypertension. When comparing age-matched WKY and SHR animals however, we observed a higher expression (mRNA and protein) of the α_{1A}, α_{1B}, α_{1D}, β_1, β_2 and β_3 ARs, accompanied by an up-regulation of GRK2.

The increased mRNA expression of α_{1D} and β_3 AR in aorta from SHR animals has been previously described by Godínez-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), as well as in lymphocytes of hypertensive patients (Gros *et al.*, 1999). Although no changes in mRNA levels for GRK2 have been previously reported, the apparent discrepancies between our results and previous ones could be explained by the different methodology used to determine mRNA levels. Previous works analyzed the GRK2-mRNA expression by northern blot analysis and normalized the GAPDH (Gros *et al.*, 1999)., We have quantified mRNA levels by real-time RT-PCR, and the results obtained in SHR have been normalized for the age-matched WKY controls, but not for the GAPDH expression since this parameter was also raised in the aorta of SHR animals.

The increased mRNA and protein expression in GRK2 in adult hypertensive animals was not observed in young pre-hypertensive rats. Therefore higher GRK2 could be an adaptive change as a consequence of (i) the increase observed in the expression of adrenoceptors and/or (ii) elevated blood pressure. The latter possibility is not supported by the additional consideration that elevated blood pressure was observed in transgenic mice with a smooth muscle cell-specific overexpression of GRK2, suggesting a more causal relationship between increased GRK2 expression and the development of hypertension (Eckhart *et al.*, 2002).

What could the hemodynamic consequences of these changes be ?

The basic hemodynamic abnormality in hypertension is increased vascular resistance. Thus, an increase in vasoconstrictor mechanisms, due to a rise in the expression and functionality of the α_1 adrenoceptors, would justify it. However, the increase in the α_1 function in vessels could be counteracted by the increase in the

β-adrenoceptors expression which mediates vasodilatatory mechanisms. In addition, the elevated GRK2 expression and the associated impairment of adrenoceptor-G-protein coupling could have main functional implications.

It is well-known that GRK2 is apparently the main factor involved in diminishing β-AR signaling in hypertension (Penela *et al.*, 2006; Feldman and Gros, 2006). Nonetheless, β_3-AR is resistant to agonist-promoted desensitization mediated by GRK2 (Rozec and Gauthier, 2006). In accordance with this,, an increased expression of β_1-ARs justify the higher vasorelaxant potency of isoprenaline found in pre-hypertensive aortas . In hypertensive aortas, the higher expression of β_1 and β_2-ARs could be counteracted by the increased levels of the active GRK2 found. However, β_3-AR 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 β_3 ARs (Strosberg, 1997), and the high potency of the selective β_3 agonist SR58611A, together with an increase in the percentage of sites of high affinity for this agonist, suggest an increased functional role of β_3-ARs in hypertension. From a physiopathological point of view, because vascular β_3-ARs are only stimulated by higher doses of catecholamines (Strosberg, 1997), the major role of β_3-ARs in hypertensive vessels determines an impaired β-mediated vasodilator mechanism which is only triggered by a higher adrenergic stimulus.

In addition, α_1-AR subtypes also exhibit different phosphorylation and internalization patterns: α_{1B}-AR, (Chalothorn *et al.*, 2002), undergoes a rapid and intense desensitization mediated by GRKs (Garcia-Sainz *et al.*, 2000, Diviani *et al.*, 1996). The α_{1A} subtype exhibits a continuous and agonist-independent trafficking, which is also agonist and GRK2-dependent, between the membrane and cytosol (Morris *et al.*, 2004; Pediani *et al.*, 2005). Finally, the intracellularly located α_{1D} subtype (McCune *et al.*, 2000; Hague *et al.*, 2004) has a major affinity for the agonists (Marti *et al.*, 2005; Minneman *et al.*, 1994), 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; 2003a). Accordingly, the α_{1D} subtype does not appear to be as sensitive to GRKs modulation as the α_{1A} and α_{1B} subtypes, and a higher increase in the expression of the GRK2 might have no functional relevance in the activity of α_{1D} ARs. Thus, we must expect an increased functionality of the α_{1D}-ARs in aortas of adult SHR animals even when the GRK2 expression increases. Considering that the α_{1D} directly regulates blood pressure via vasoconstriction (Tanoue *et al.*, 2002a; 2002b; 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 SHR rats.

Present results and our previous observations in aorta, main mesenteric and small mesenteric arteries (Gisbert et al., 2002), confirm this proposal The higher potency, exhibited only by the α_{1D} selective antagonist BMY 7378 and not by the selective α_{1A} antagonist 5-methylurapidil, indicates an increased role of the α_{1D} subtype in the sympathetic vasoconstriction of the aortas of SHR animals. There

were two hemodynamic consequences of the higher functionality of α_{1D}-ARs: (i) a higher potency of phenylephrine in hypertensive aortas due to the higher affinity of this subtype for agonists (Minneman *et al.*, 1994; Marti *et al.*, 2005) then being the hypertensive vessles more sensitive to the contractile adrenergic stimulus; (ii) a slower decay of the contractile response after removing the agonist due to the characteristic of the α_{1D}-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; 2003a; Ziani *et al.*, 2002). Therefore, the consequences of an increased functionality of α_{1D}-ARs, a higher sensitivity to an α_1-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 specially relevant if we consider that, as occurs in aorta, a similar increase in the functional role of α_{1D} ARs was previously shown by us in small mesenteric arteries from SHR animals 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 β_1 AR precedes the hypertensive state, whereas an increase in the expression of the α_{1A}, α_{1B}, α_{1D}, β_1, β_2 and β_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 vasodilator mechanisms mediated by β_1 and β_2, and counteracts the vasoconstrictor mechanisms mediated by α_{1A} and α_{1B}. However, it neither affects the vasodilator response mediated by β_3-ARs, nor the

vasoconstrictor response mediated by α_{1D} ARs, the two subtypes majoritary in rat aorta. Therefore, the functional role of these subtypes is more evident. As β_3 ARs are least sensitive and α_{1D} ARs are most sensitive to the adrenergic stimulus, the hemodynamic consequences of these changes are twofold: (i)an imbalance between sensitivity to vasoconstrictor (increased) and vasodilator (decreased) adrenergic stimulus in vessels of hypertensive animals;(ii) a slower kinetics of the disappearance of the contractile response when the adrenergic stimulus disappears owing to the major role of α_{1D} ARs. These changes might be involved in the greater sympathetic vasoconstrictor tone that is characteristic of hypertension. Moreover, they may add new perspectives to its therapeutic management, suggesting that non subtype selective α_1-AR antagonists, or selective β_1 and β_1/β_2 antagonists, might not be the best therapeutic options.

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Footnotes

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Legends for figures

Figure 1. mRNA levels of the α_{1A}, α_{1B}, α_{1D}, β_1, β_2, β_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= 4-6 different animals

Figure 2. Comparative analysis of the expression of the α_{1A}, α_{1B}, α_{1D}, β_1, β_2, β_3 adrenoceptors and GRK2 in aortas of young pre-hypertensive SHR (black bars) and their controls (WKY: white bars). Graphs shows:

(A) mRNA levels expressed as $2^{-\Delta Ct}$ using GAPDH as a housekeeping gene, (mean + s.e.m of n= 4-6 different animals)

(B) Protein expression measured by densitometric analysis and expressed as the ratio to β-actin.

(C) Immunoblotting representative of three different experiments

Figure 3. mRNA levels of GAPDH in the aorta of young and adult SHR (black bars) and WKY (white bars) rats. Values were expressed as $2^{-\Delta Ct}$, using the mean GAPDH expression from WKY animals as reference, (mean + s.e.m of n= 4-6 different animals)

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Figure 4. Comparative analysis of the expression of the α_{1A}, α_{1B}, α_{1D}, β_1, β_2, β_3 adrenoceptors and GRK2 in aortas of adult hypertensive SHR (black bars) and their normotensive controls (WKY: white bars).

(A) mRNA levels expressed as $2^{-\Delta Ct}$, using the mean expression of each gene obtained in the corresponding WKY animals as a reference, (mean + s.e.m of n= 4-6 different animals)

(B) Immunodetection of the six adrenoceptors and GRK2 (immunoblotting representative of three different experiments) and quantitative analysis using β-actin as reference (bar diagrams representing mean + s.e.m of n= 3 different animals).

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

Figure 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 SHR (black circles) and WKY (white circles) rats. Data are the mean ± s.e.m. of 4-8 experiments.

Figure 6.

Cumulative concentration-response curves of relaxation for the α_1-adrenoceptor antagonists 5-methyl urapidil and BMY 7378 on the phenylephrine-induced contraction in adult rats

Experiments were carried out in aortas obtained from SHR (black circles) and WKY (white circles) rats. Data are the mean ± s.e.m. of 4-8 experiments.

Figure 7.

Cumulative concentration-response curves of relaxation for the β-adrenoceptor agonists isoprenaline and SR58611A on the phenylephrine-induced contraction in young and adult rats

Experiments were carried out in aortas obtained from SHR (black circles) and WKY (white circles) rats. Data are the mean ± s.e.m. of 4-8 experiments.

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TABLE 1.

Systolic blood pressure (SBP) and heart rate in young (6-week-old) and adult (16-week-old) rats.

	Young rats		**Adult rats**	
	WKY	**SHR**	**WKY**	**SHR**
SBP, mmHg	120 ± 3	129 ± 12	140 ± 2	197± 4***
Heart rate, beats/min	274 ± 28	292 ± 69	311 ±10	374 ± 7***

Values were determined 24 h before the animals were sacrificed and are expressed as the mean ± S.E.M. of n = 4-8 animals

*** P < 0.001 *vs.* respective WKY

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TABLE 2.

Parameters of the concentration-response curves of contraction to phenylephrine, and the maximal contraction obtained with a depolarizing solution (KCl 60 mmol/L) in the aorta isolated from young and adult WKY and SHR animals

		Young rats		**Adult rats**	
		WKY	**SHR**	**WKY**	**SHR**
KCl	E_{max}	7.03 ± 1.18	5.46 ± 0.66	8.26 ±0.33	5.84 ± 0.62*
Phenylephrine	E_{max}	7.47 ± 1.09	4.33 ± 0.40*	7.99 ± 0.58	4.44 ± 0.67**
	pEC_{50}	8.38 ± 0.34	8.42 ± 0.17	7.70 ± 0.08	8.22 ± 0.02***

E_{max} = maximal contractile response expressed as mN

pEC_{50} = -log [phenylephrine] required to produce 50% of the maximal response

Values were expressed as the mean ± S.E.M. of n = 4 animals

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

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TABLE 3.

Parameters of the concentration-response curves of relaxation obtained by addition of α_1-AR antagonists (5 methylurapidil, BMY 7378) and β-AR agonists (isoprenaline, SR58611A), to aortas pre-contracted by phenylephrine.

		Young rats		Adult rats	
		WKY	**SHR**	**WKY**	**SHR**
5-Methylurapidil	**pIC_{50}**	N.D.	N.D.	6.81 ± 0.22	7.05 ± 0.12
BMY 7378	**pIC_{50}**	8.19 [(a)]	8.03 [(a)]	7.55 ± 0.03	8.26 ± 0.06***
Isoprenaline	**pEC_{50}**	7.32 ± 0.03	8.08 ± 0.03***	7.41 ± 0.02	7.03 ± 0.07**
SR 58611A	**pEC_{50} (1)**	7.45 ± 0.10	7.26 ± 0.09	8.41 ± 0.06	8.61 ± 0.06
	pEC_{50} (2)	4.61 ± 0.05	4.79 ± 0.04	4.63 ± 0.05	5.08 ± 0.07
	% (1)	27.4 ± 1.5	26.8 ± 1.4	35.4 ± 0.9	50.1 ± 1.4***

pIC_{50} = -log [M] required to inhibit 50% of the maximal contractile response

pEC_{50} = -log [M] required to produce 50% of the maximal relaxant response

pEC_{50} (1) and pEC_{50} (2) represents the potency of the agent tested on each subpopulation of receptors when the curve that fitted two different sites was statistically significant (see data analysis)

% (1) = fraction of receptors corresponding to the high potency site

Values were expressed as the mean ± S.E.M. of n = 4-8 animals

** $P < 0.01$ *vs.* WKY, *** $P < 0.001$ *vs.* WKY

N.D. = not determined

(a) data previously published in Gisbert et al., 2002

Figure 1

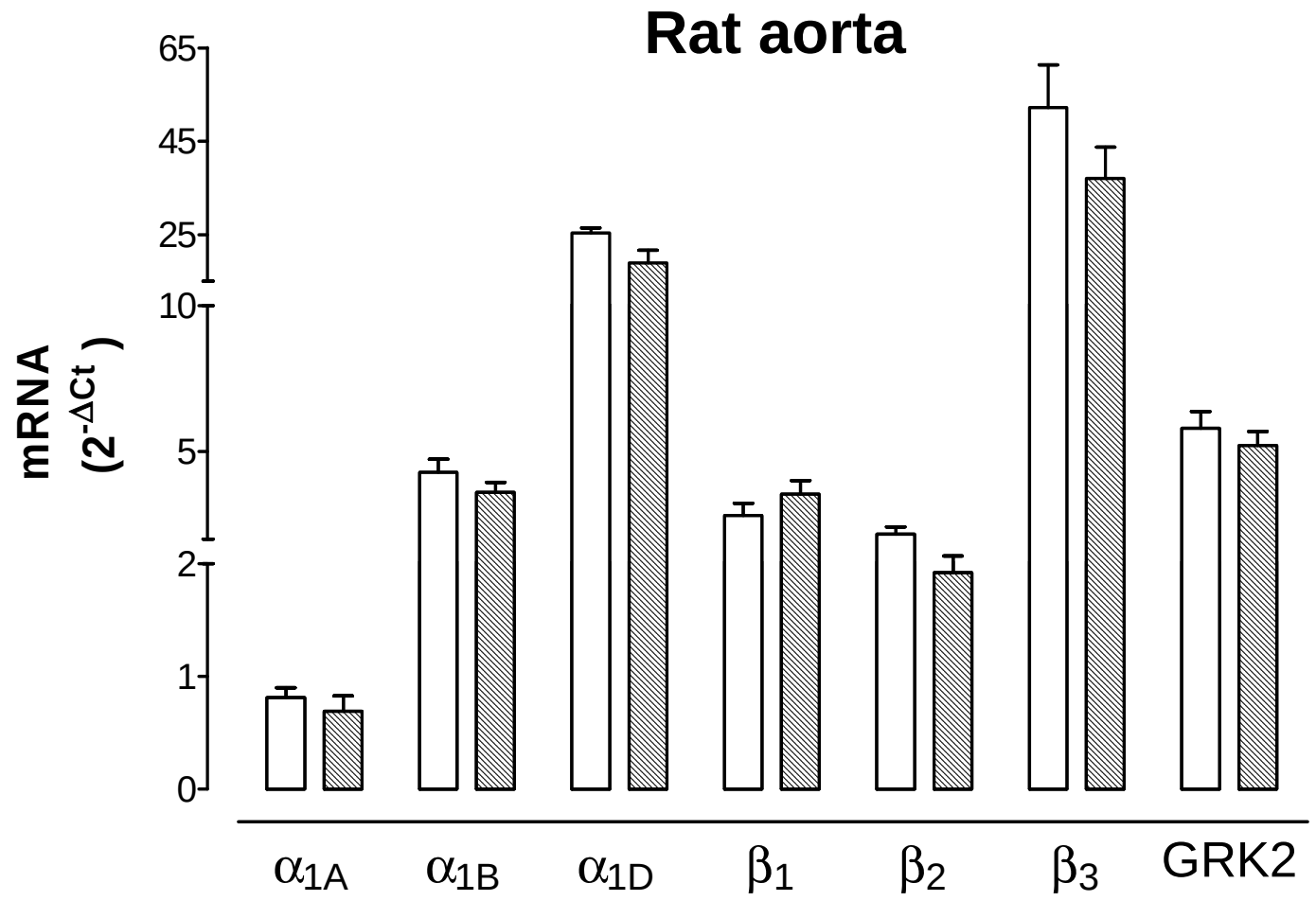
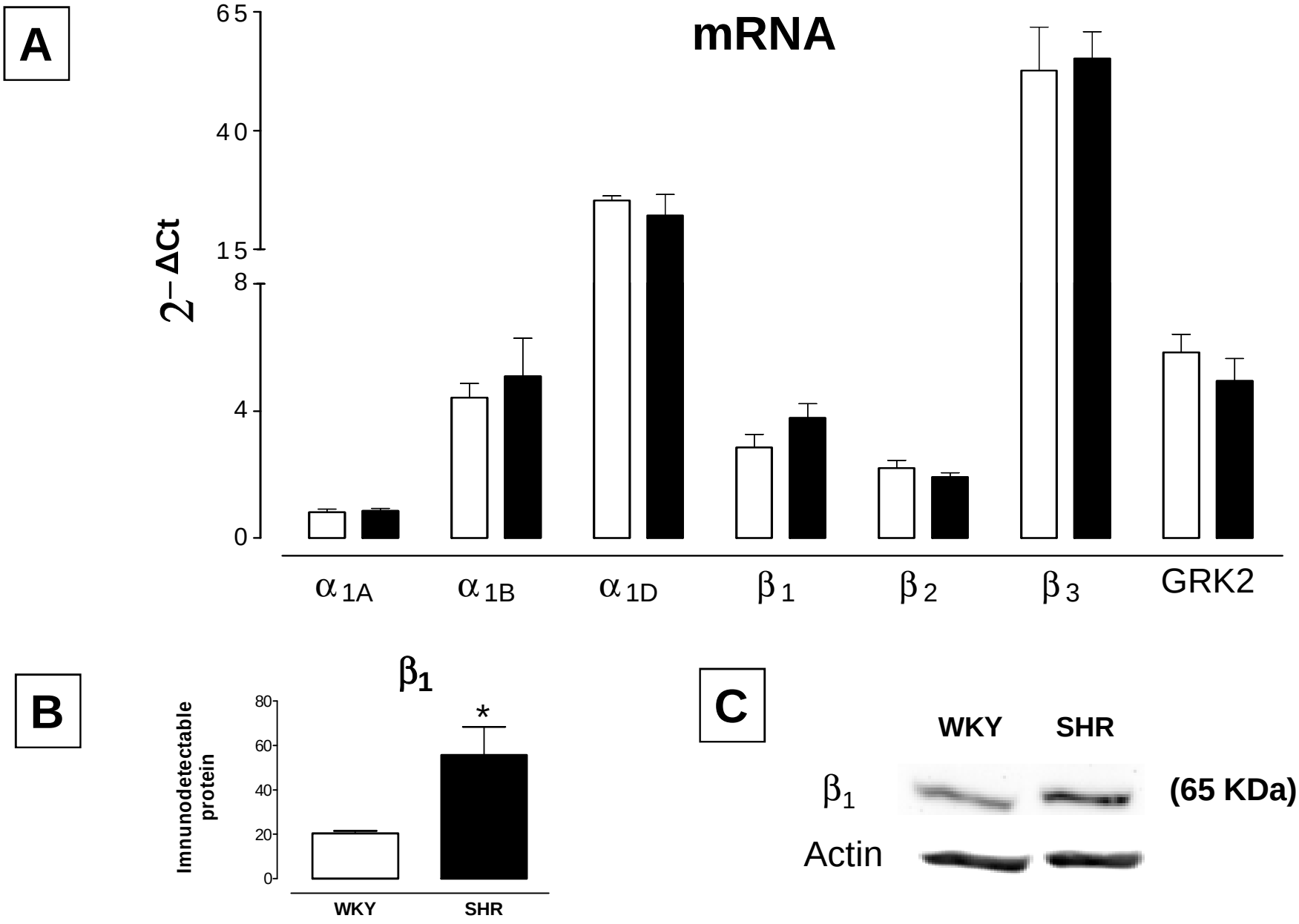


Figure 2



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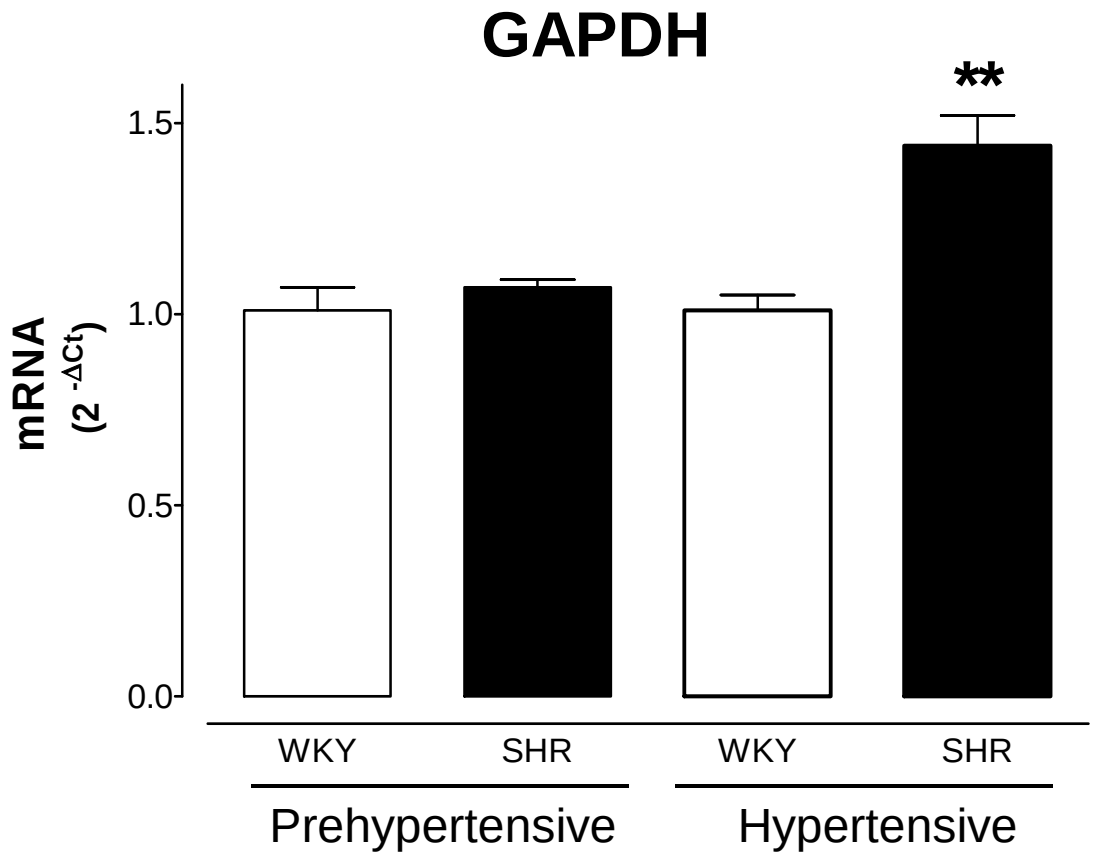
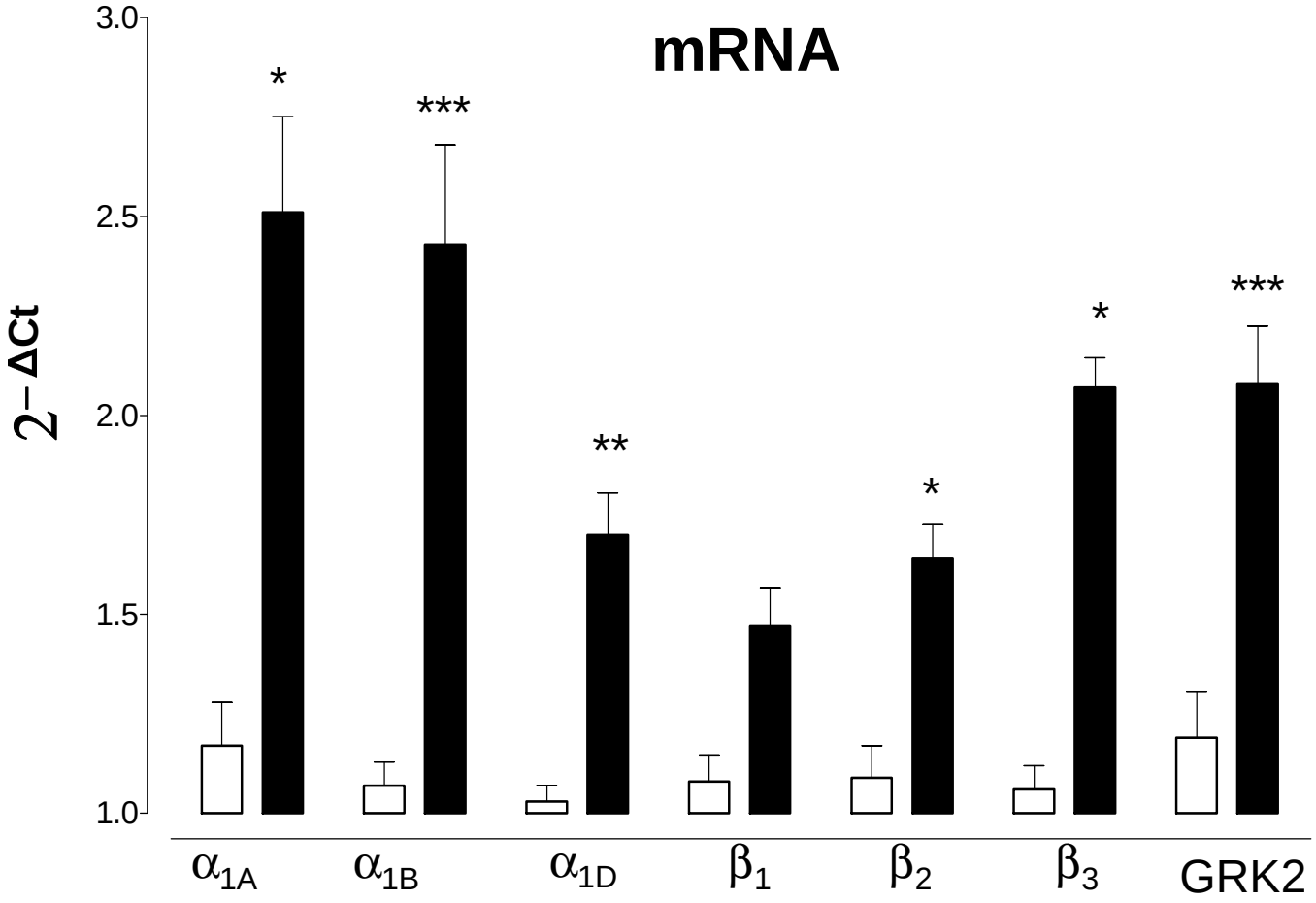


Figure 4A



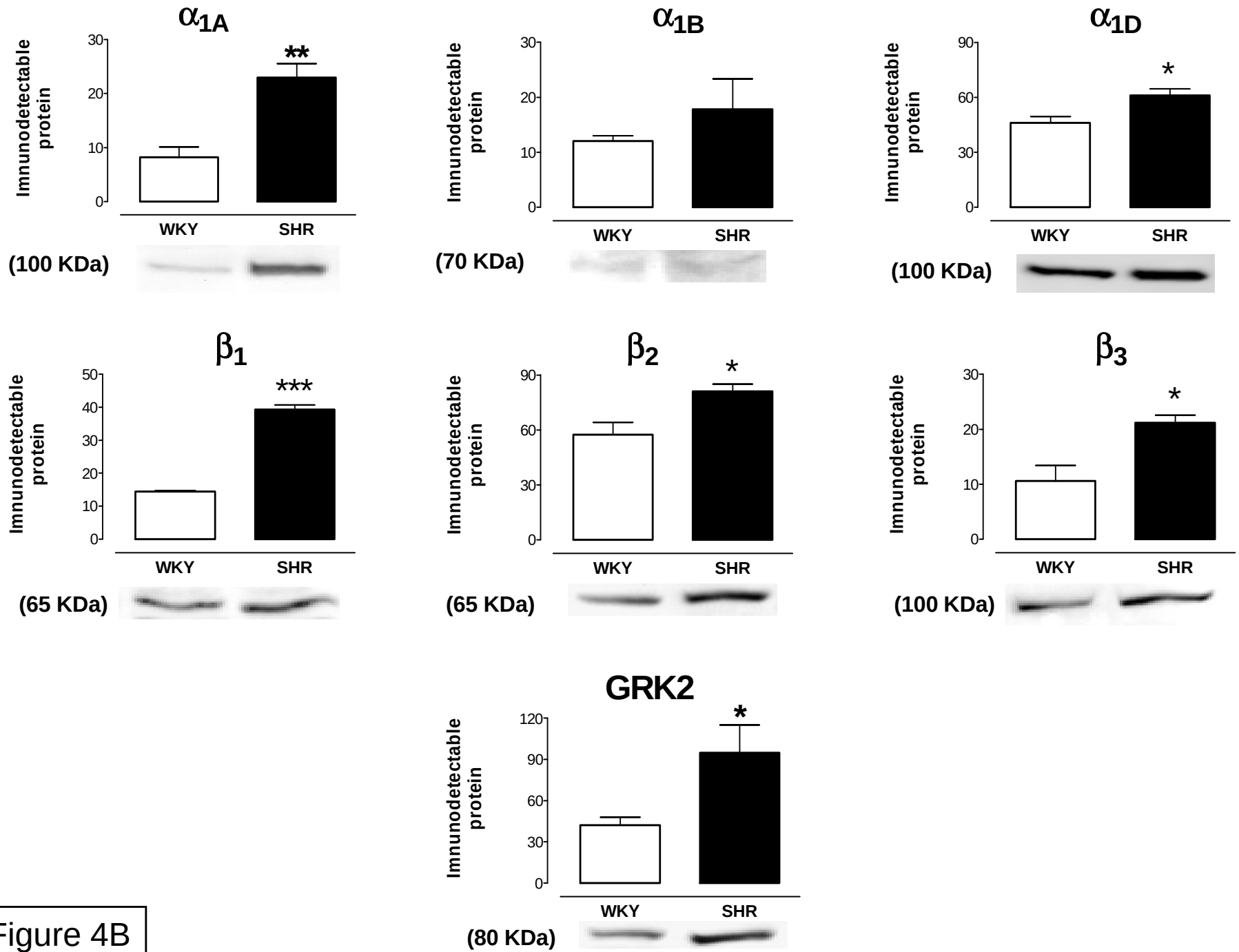
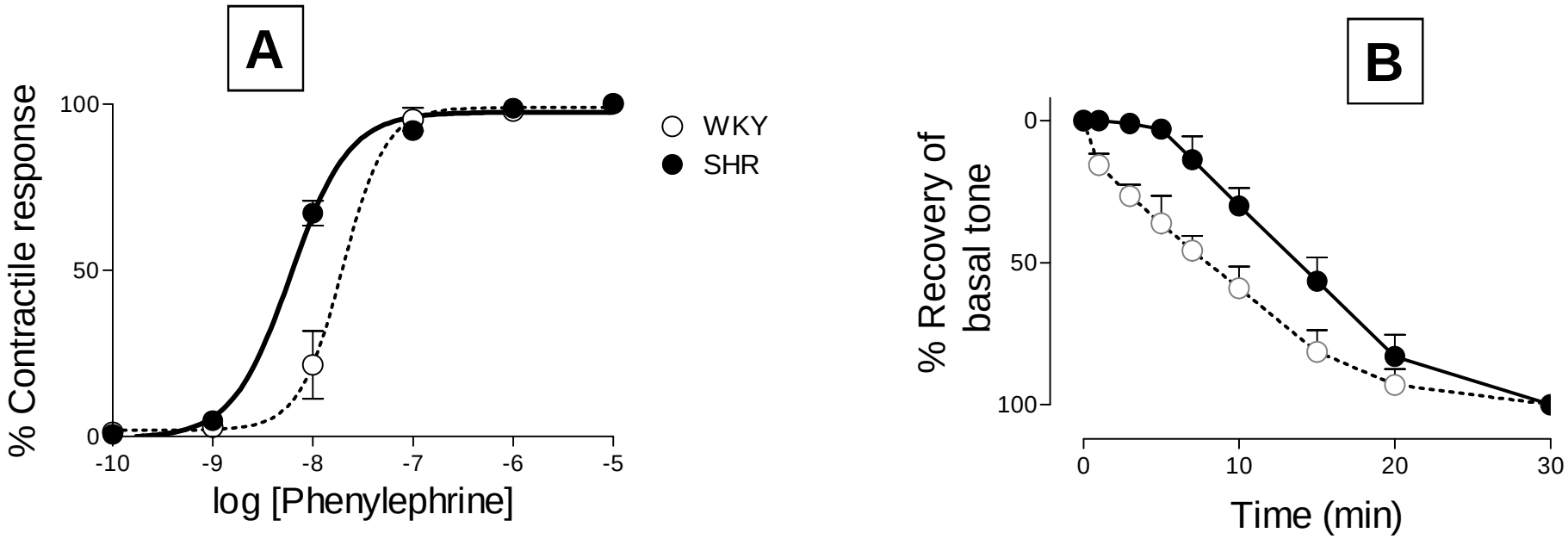


Figure 4B

Figure 5



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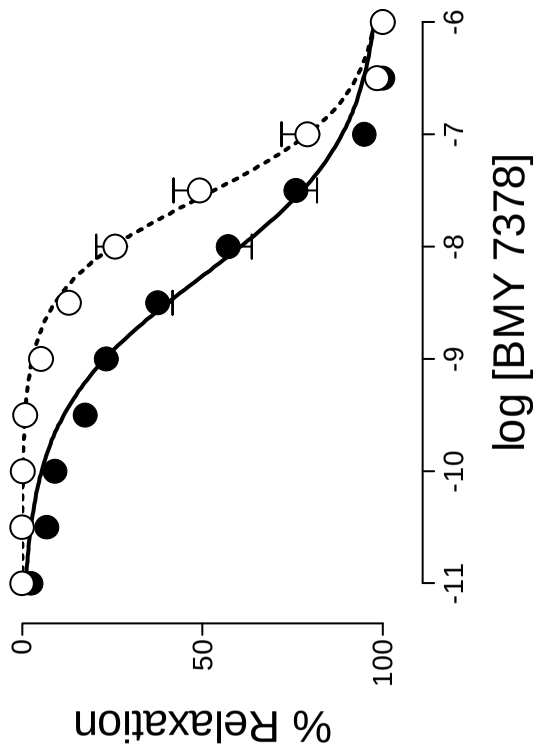

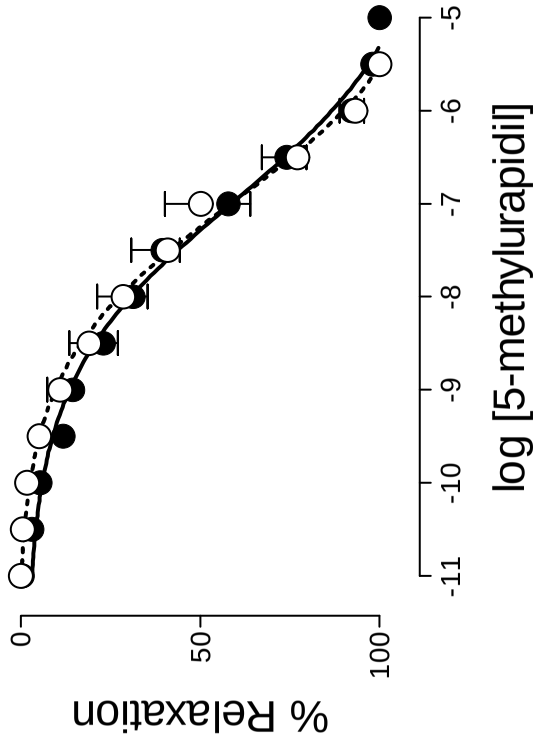

Figure 6

Figure 7