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Vascular α_{1D}-adrenoceptors are overexpressed in aorta of the aryl hydrocarbon receptor null mouse: role of increased angiotensin II

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Summary

1. The hypothesis that α_{1D}-adrenoceptors may mediate the pro-hypertensive actions of angiotensin II (Ang II) was tested in isolated aorta (α_{1D}-adrenoceptor bearing tissue) of the aryl hydrocarbon receptor null mouse ($AhR^{-/-}$), which shows increased levels of Ang II, cardiac hypertrophy and hypertension.
2. The effect of captopril (an angiotensin converting enzyme inhibitor) on both blood pressure and aortic α_{1D}-adrenoceptor expression and function in mice were determined.
3. Basal blood pressure was higher in $AhR^{-/-}$ mice, while captopril therapy decreased it to wild-type (WT) values.
4. Aortas of adult WT and $AhR^{-/-}$ mice were stimulated by phenylephrine or noradrenaline to induce contraction; the maximal effect was higher in $AhR^{-/-}$ mice, without a significant change in pEC_{50}.
5. PA_2 values for the selective α_{1D}-adrenoceptor antagonist BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazynil]ethyl]-8-azaspiro [4.5]decane-7,9-dione) were 9.19 and 8.94 for WT and $AhR^{-/-}$, respectively; while Schild slopes were not different from 1.
6. PCR experiments showed *c*. 77% increase in $AhR^{-/-}$ α_{1D}-adrenoceptors cDNA compared with WT mice; while western blot analysis demonstrated *c*. 88% increase in α_{1D}-adrenoceptor protein in $AhR^{-/-}$ mice.
7. Captopril therapy decreased α_{1D}-adrenoceptor-induced contraction and protein in $AhR^{-/-}$ mice to WT levels.
8. These data support the hypothesis that under conditions where Ang II is elevated, vascular α_{1D}-adrenoceptors are increased, and further suggest that both Ang II and vascular α_{1D}-adrenoceptors could be related in the onset of hypertension.

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Keywords

angiotensin II; α_{1D}-adrenoceptors; aryl hydrocarbon receptor null mouse; hypertension; captopril

Introduction

Angiotensin II (Ang II) plays an important role in regulating systemic arterial pressure through its synthesis by the renin–angiotensin system (RAS). Ang II is a potent vasoconstrictor that activates Ang II type 1 (AT_1) receptors on vascular smooth muscle and affects vascular and cardiac remodelling cardiac contractility and heart rate through increased sympathetic nervous system tone by promoting noradrenaline release (Weir & Dzau, 1999). Several physiological parameters regulated by the RAS, such as plasma renin activity, plasma angiotensinogen concentration (Ruiz *et al.*, 1990; Zicha & Kune, 1999) and kidney renin release (Henrich & Levi, 1991; Zicha & Kune, 1999) are known to be elevated in young spontaneously hypertensive rats (SHR), suggesting that these components might contribute to the pathogenesis of genetic hypertension. Recent evidence suggests that the AT_1 receptor signalling pathway mediates both the physiological and pathogenic pleiotropic actions of Ang II (reviewed in Hunyady & Catt, 2006).

On the other hand, a decade ago it was demonstrated that Ang II induces α_1-adrenoceptor expression, mainly the α_{1D}-subtype, in isolated rat aorta smooth muscle cells (VSMC) (Hu *et al.*,1995). Activation of the α_{1D}-adrenoceptor subtype increases protein synthesis in VSMC (Xin *et al.*, 1997). These data suggest that Ang II may facilitate aorta smooth muscle hypersensitivity and hypertrophy through α_{1D}-adrenoceptors expression.

Increasing evidence shows that vascular α_{1D}-adrenoceptors are functionally related with the genesis and/or maintenance of hypertension, they seem to be present prior to the establishment of hypertension, and it has been suggested that an increase in the population of constitutively active α_{1D}-adrenoceptor could be involved in the pathology of elevated sympathetic tone found in SHR (Villalobos-Molina & Ibarra, 1996, 1999; Villalobos-Molina *et al.*, 1999; Guimaraes & Moura, 2001; Gisbert *et al.*, 2002; García-Sáinz & Villalobos-Molina, 2004). We have found that inhibiting Ang II production with captopril decreased both the expression and function of α_{1D}-adrenoceptors in the aorta of pre-hypertensive SHR rats (Godínez-Hernández *et al.*, 2006). Taken together, these data suggest that the RAS and α_{1D}-adrenoceptors might interact with each other at the onset of hypertension. Thus, we hypothesize that under conditions of elevated levels of Ang II, vascular α_{1D}-adrenoceptors will also be increased, and may mediate some of the pro-hypertensive actions of the hormone (Villalobos-Molina & Ibarra, 2005; Godínez-Hernández *et al.*, 2006). One condition where elevated levels of AngII are observed is the aryl hydrocarbon receptor null ($AhR^{-/-}$) mouse, which exhibits cardiac hypertrophy, increased levels of endothelin-1 and high arterial blood pressure (Lund *et al.*, 2003). However, so far no explanation exists as to why the absence of the aryl hydrocarbon receptor leads to the pathology observed in these mice. As it is known that mouse aorta express α_{1D}-adrenoceptors that mediate contraction (Yamamoto & Koike, 2001; Lázaro-Suárez *et al.*, 2005), we chose this model to test our hypothesis.

Material and methods

Animals

Adult (4 months-old) $AhR^{-/-}$ (targeted mutation of aryl hydrocarbon receptor gene) and wild-type C57BL6N/129sv (WT) male mice, were maintained in a pathogen-free environment with a controlled temperature (22 ± 2 °C) at 40–60% humidity. The mice had 12/12 light/dark cycles and were given free access to food and water. Animal housing, care and all procedures were

conducted in accordance with the Mexican Regulations of Animal Care and Use (NOM-062-ZOO-1999, Ministry of Agriculture, México), and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. All protocols were approved by the institutional committee for the use of animals.

Blood pressure and captopril therapy

Wild-type and $AhR^{-/-}$ mice were used for the measurement of blood pressure, with a tail-cuff device (Letica, Panlab, Barcelona, Spain), as follows: a mouse was restrained in a plastic container (size match), a sensor and a ring containing the inflatable latex were placed in the tail; while the mouse was kept warm in the same device. The animals were trained to be inside the container with the cuff placed in the tail and the latex ring was inflated–deflated (this was done several times in a week). Then mice (3–6 animals) were tested for blood pressure measurement (three stable readings were recorded for each mouse) and the mean was used to construct a time-point.

Aryl hydrocarbon receptor null mice were treated with captopril (10 $mg^{-1}kg^{-1}day^{-1}$, in the drinking water) over 2 weeks, while blood pressure was measured. After this therapy, animals were killed and the aorta was harvested.

Contraction in isolated aorta

Animals were killed and the thoracic aorta was excised and cleaned from surrounding connective tissue. Isolated arteries were cut into rings (*c*.5 mm in length) and the endothelium removed by gently rubbing the intimal surface with a metal device, as previously described (Lázaro-Suárez *et al.*, 2005). In brief, aortic rings were placed in tissue chambers filled with 10 ml Krebs–Henseleit solution, maintained at 37 °C, pH 7.4 and bubbled with 95% O_2 containing 5% CO_2. Arterial rings were hooked to the bottom of the chamber and to a Grass FT03 force displacement transducer (Astro-Med, Inc., West Warwick, RI, USA), connected to a MP100 data-acquisition system (Biopac Systems Inc., Santa Barbara, CA, USA), to record the isometric tension developed by aortic rings. An optimal tension of 1.5 g was applied to mouse aorta then phenylephrine (Sigma-Aldrich, St Louis, MO, USA) or nor-adrenaline (supplemented with 2% ascorbic acid) concentration–response curves were constructed. All curves were constructed in the presence of rauwolscine (1×10^{-7} M) and propranolol (1×10^{-7} M), to antagonize α_2- and β-adrenoceptors, respectively. The tissue was challenged with phenylephrine or noradrenaline and washed every 30 min for 2 h. Then, reproducible cumulative concentration–response curves to agonists (1×10^{-9} to 3×10^{-5} M) were obtained for each artery. To avoid fatigue of the preparation, a recovery period of 60 min was allowed between agonist curves.

In some experiments, arteries were incubated with BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazynil]ethyl]-8-azaspiro [4.5]decane-7,9-dione), a highly selective α_{1D}-adrenoceptor antagonist, for 30 min before and during phenylephrine exposure. Schild analysis was used to obtain pA_2 and slope (*m*) values (Arunlakshana & Schild, 1959). Solutions were prepared daily.

Reverse transcription-polymerase chain reaction

Total RNA of mice aorta artery was purified by the Trizol reagent method (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. First strand cDNA was synthesized using reverse transcriptase, 1 μg of DNase-treated RNA, 1 mM dNTPs, RNase inhibitor, oligo(dT) and random hexamers as primers in a total volume of 50 μl at 42 °C. The cDNA synthesized was used as template in polymerase chain reaction. The relative abundance of α_{1D}-adrenergic receptor was estimated by normalization with 18S-ribosomal RNA, PCR amplified. For α_{1D}-adrenoceptor amplification, the sequence of primers was: forward, 5′-TGGTATCTGTGGGACCGCTACTAGG-3′, and reverse 5′-

TACACGCGGCAGTACATGACCACG-3′. PCR conditions were carried out for 27 cycles each 94 °C for 40 s, 62 °C for 40 s and 72 °C for 30 s, followed by 5 min at 72 °C, expected amplified sequence is a 158-bp fragment. For 18S-ribosomal RNA forward sequence was 5′-GGGAGCCTGAGAAACGGC-3′ and the reverse sequence was 5′-GGGTCGGGAGTGGGTAATTT-3′ the amplicon length is 67 bp; the PCR amplification was conducted for 10 cycles under identical conditions. PCR amplicons were visualized in 2% agarose gels, with SYBR green-1 stain in a FLA-5000 image analyzer (Fujifilm Medical Systems, Stamford, CT, USA). Densitometric analysis was obtained with MULTI GAUGE 3.0 software (Fujifilm).

Western blot

Protein homogenates from a pool of three thoracic aorta were obtained by mechanical disruption in a Dounce homogenizer, in RIPA buffer: 10 mM Tris–HCl pH 7.4, 1 mM EDTA, 150 mM NaCl, 0.1% Triton, 0.1% sodium dodecyl sulphate (SDS) and complete mini protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). The total protein concentration in the extracts was determined with the Folin reagent (Lowry *et al.*, 1951). For electrophoresis, 60 μg of each sample were diluted in one volume of 2× Laemlli buffer, run on 10% SDS-polyacrylamide gel and transferred to polyvinyldenofluoride membranes (Amersham Biosciences, Piscataway, NJ, USA). Membranes were blocked for 1 h at room temperature in 20 mM Tris, pH 7.4, 0.1% Tween 20 (TBS-T) containing 5% non-fat dry milk, rinsed for three times with TBS-T and then incubated overnight at 4 °C with rabbit anti α_{1D}-adrenergic receptor antisera (20, the immunogenic sequence for the utilized antibody is identical for rats and mice), in a 1:2000 dilution. After, membranes were washed with TBS-T, incubated 1 h at room temperature with 1:10000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody (Zymed Laboratories Inc., San Francisco, CA, USA) in TBS-T with 5% non-fat milk. After washing, signals were visualized by addition of chemiluminescent substrate (Pierce Biotechnology, Inc., Rockford, IL, USA) and the signal documented by autoradiography. Densitometric analysis of digitalized images was obtained with MULTI GAUGE 3.0 software (Fujifilm).

Results

Blood pressure measurements

Systolic blood pressure in WT mice was 110 ± 5 mmHg and 150 ± 3 mmHg in $AhR^{-/-}$ mice (Fig. 1). Captopril therapy decreased blood pressure in a time-dependent manner, showing a significant diminution at 24 h of treatment and reaching WT values (Fig. 1).

Contraction in isolated aorta

Noradrenaline induced contraction in aorta of both WT and $AhR^{-/-}$ mice in a concentration-dependent manner (Fig. 2). Agonist potency was similar between mice strains (pEC_{50} of 7.6 ± 0.1 *vs.* 7.4 ± 0.2 in $AhR^{-/-}$ and WT), but efficacy was different because the $AhR^{-/-}$ aorta showed a maximal effect of 1.9 ± 0.3 *vs.* 1.2 ± 0.2 g in the WT mouse ($P < 0.05$). Similar effects were obtained with the agonist phenylephrine (pEC_{50}, 7.1 ± 0.1 *vs.* 6.8 ± 0.1, E_{max}, 1.5 ± 0.2 *vs.* 0.9 ± 0.2 g in $AhR^{-/-}$ *vs.* WT, respectively, $P < 0.05$). Captopril treatment reduced the magnitude of contractions in $AhR^{-/-}$ aorta ($P < 0.05$), such that the values obtained were similar to those WT irrespective of the agonist used (NE pEC_{50}, 7.0 ± 0.1 *vs.* 6.8 ± 0.1, E_{max}, 0.9 ± 0.3 *vs.* 0.9 ± 0.2 g in $AhR^{-/-}$ + captopril *vs.* WT, respectively and phenylephrine pEC_{50}, 6.9 ± 0.1 *vs* 6.8 ± 0.1, E_{max}, 0.8 ± 0.2 *vs.* 0.9 ± 0.2 g in $AhR^{-/-}$ *vs.* WT, respectively) (Fig. 2).

pA_2 values for BMY 7378 were 8.94 ± 0.21 *vs.* 9.19 ± 0.29 for $AhR^{-/-}$ *vs.* WT, respectively (Fig. 3); whereas Schild slopes were 0.76 (95% confidence limits, −1.13 to −0.4) *vs.* −0.90

(95% confidence limits, −1.59 to −0.21) for $AhR^{-/-}$ *vs.* WT, respectively ($n = 3$ in each group), and not statistically different from 1.

Determination of α_{1D}-adrenoceptor transcript in aorta of $AhR^{-/-}$ mice

Relative abundance of the α_{1D}-adrenoceptor transcript was explored by reverse transcription of aortic total RNA and PCR amplification with specific primers designed against Adra1d cDNA sequence (NCBI accession number NM_013460).

Electrophoretic analysis of the PCR reaction exhibited a single band of the expected size (158 bp) without unspecific or ambiguous bands, reverse transcriptase negative control did not show amplification (Fig. 4a). The lower size band could be an oligomer formed by primers once the reaction cools down. The melting point analysis of this amplicon using SYBR Green showed a single amplicon with a mean temperature of *c*. 89 °C, while for -RT no amplicon was detected (data not shown). We concluded that the reaction product is specific and no amplification efficiency is affected. Quantity was corrected against 18S ribosomal RNA because it is widely referred in literature and there are no reports of in variation in the quantity of this rRNA or in the ribosome number in the hypertensive state (Fig. 4b).

Abundance of the receptor in $AhR^{-/-}$ mice was higher (*c*. 77.5% ± 12%) than in WT mice (Figs. 4b,c). These results strongly suggest that α_{1D}-adrenoceptor is overexpressed in aorta of null mice.

Determination of α_{1D}-adrenoceptor protein in aorta of $AhR^{-/-}$ mice

Western blot was conducted with a rabbit policlonal antibody directed against the carboxi termini of rat α_{1D}-adrenoceptor (García-Sáinz *et al.*, 2001). As positive control crude extracts of Rat-1 cells stably expressing rat α_{1D}-adrenoceptor were utilized (kindly donated by Dr J. A. García-Sáinz). The receptor is detectable with an apparent molecular weight of *c*. 85 kDa.

In mouse aorta homogenates, the specific *c*. 85 kDa band is present (Fig. 5a). The analysis of the receptor's relative abundance was carried out in a pool of three aorta from each group, and data corrected relative to actin as a reference protein (Fig. 5b). Aortic homogenates from $AhR^{-/-}$ mice showed *c*. 88% increase in α_{1D}-adrenoceptors compared with WT (Fig. 5c) while captopril therapy levels to that of WT mice (Fig. 5c).

Discussion

It is well recognized that the pathogenic actions of Ang II, signalling via AT_1 receptors, are involved in diseases such as hypertension. In support of this contention, therapy with angiotensin converting enzyme (ACE) inhibitors and AT_1 receptor antagonists have proven to be successful in patients and rats with diabetic nephropathy, heart failure, hypertension and left ventricular hypertrophy (Martínez & Villalobos-Molina, 2003; Ferrario *et al*., 2004). This suggests that these agents have actions other than just the control of blood pressure. In this regard, it has been reported that the knockout of the AhR gene, involved in regulation of xenobiotic metabolism, generates mice with liver alterations, elevated levels of Ang II, endothelin-1, blood pressure and also develop cardiac hypertrophy (Gonzales & Fernandez-Salguero, 1998; Lund *et al.*, 2003), the latter events decreased towards normal levels after chronic therapy (8–12 weeks) with the ACE inhibitor captopril (Lund *et al.*, 2003). Our results confirm the data reported by Lund *et al.*, in that $AhR^{-/-}$ mice are hypertensive and that captopril therapy decreases blood pressure, but over a shorter time-course (2 weeks). This result means that captopril exerts its antihypertensive action quite rapidly, as expected, and suggests that the results reported by Lund *et al.* could be observed after several days of therapy. It is known that Ang II induces the expression of α_{1D}-adrenoceptors in rat-derived VSMC (Hu *et al.*,

1995), suggesting that the tissue might become hypersensitive. It is also known that stimulation of α_1-adrenoceptors is involved in VSMC hypertrophy (Xin *et al.*, 1997). As both phenomena have been reported to occur in hypertension then, it was important to assess whether aortic arterial diameter in $AhR^{-/-}$ mice was affected by captopril treatment.

It has been shown that chronic therapy with captopril (50 mg kg^{-1} day^{-1} per 10 weeks) abolished the increase in resting tone (IRT) response, a measure of α_{1D}-adrenoceptor constitutive activity, in several arteries taken from either Wistar-Kyoto rats (WKY) or SHR rats. It was suggested that IRT was not observed because SHR rats did not become hypertensive (Gisbert *et al.*, 2002). However, recent findings in aorta of pre-hypertensive SHR exhibited elevated basal levels of α_{1D}-adrenoceptor mRNA and protein as compared with normotensive WKY rats, and that therapy with a lower dose of captopril (3 mg kg^{-1} day^{-1}) over a shorter time (1 week) decreased both expression (mRNA as well as protein) and function of aorta (α_{1D}-adrenoceptor bearing tissue) from pre-hypertensive rats (Godínez-Hernández *et al.*, 2006). These results indicated that the absence of IRT reported by Gisbert *et al.* occurred because captopril inhibited Ang II production and as a consequence, prevented the increase in α_{1D}-adrenoceptor population, which are putatively involved in the pathology of the SHR (Villalobos-Molina & Ibarra,1999, 2005; Villalobos-Molina *et al.*,1999; Gisbert *et al.*, 2002; Godínez-Hernández *et al.*, 2006). Our current results agree with the latter suggestion, i.e. the reduction in Ang II levels by captopril is associated with a decrease in α_{1D}-adrenoceptor expression and function. The fact that $AhR^{-/-}$ mouse shows the described phenotype, would allow the study of Ang II-α_{1D}-adrenoceptor cross-talk at the genomic level, an interesting but as yet unknown interrelationship that would support our hypothesis (Villalobos-Molina & Ibarra, 2005). Furthermore, we found similar results in a model where Ang II is continuously infused into rats, increasing blood pressure and contractile responses in isolated aorta (I. A. Gallardo-Ortíz, P. López-Sánchez and R. Villalobos-Molina, unpublished observations). As a corollary, Tsujimoto and coworkers showed that disruption of the α_{1D}-adrenoceptor gene resulted in hypotensive mice and concluded that these receptors are important for blood pressure control (Tanoue *et al.*, 2002). Taken together, the data suggest that Ang II and α_{1D}-adrenoceptors interact in the genesis and/or maintenance of hypertension.

On the other hand, although the mechanism by which knockout of the AhR causes an increase in vasoactive peptides, high blood pressure and cardiac hypertrophy is unknown, it is reasonable to conclude that it is associated with Ang II-dependent hypertension and vascular reactivity to adrenoceptor ligands. Our data suggests that this augmentation of vascular reactivity may be due to elevated α_{1D}-adrenoceptor expression.

Our data support the hypothesis that under conditions where Ang II is elevated, vascular α_{1D}-adrenoceptor expression is upregulated, and suggests that Ang II and vascular α_{1D}-adrenoceptors could interact at the onset of hypertension.

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References

Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. Br. J. Pharmacol 1959;14:48–58.

Ferrario C, Abdelhamed AI, Moore M. Angiotensin II antagonists in hypertension, heart failure, and diabetic nephropathy: focus on losartan. Curr. Med. Res. Opin 2004;20:279–293. [PubMed: 15025837]

García-Sáinz JA, Villalobos-Molina R. The elusive α_{1D}-adrenergic receptor: molecular and cellular characteristics and integrative roles. Eur. J. Pharmacol 2004;500:113–120. [PubMed: 15464025]

García-Sáinz JA, Vázquez-Cuevas FG, Romero-Avila MT. Phosphorylation and desensitization of α_{1D}-adrenergic receptors. Biochem. J 2001;353:603–610. [PubMed: 11171057]

Gisbert R, Ziani K, Miquel R, Noguera MA, Ivorra MD, Anselmi E, Docon P. Pathological role of a constitutively active population of α_{1D}-adrenoceptors in arteries of spontaneously hypertensive rats. Br. J. Pharmacol 2002;135:206–216. [PubMed: 11786496]

Godínez-Hernández D, Gallardo-Ortíz IA, López-Sánchez P, Villalobos-Molina R. Captopril therapy decreases the expression and function of α_{1D}-adrenoceptors in aorta of pre-hypertensive rats. Auton. Autacoid. Pharmacol 2006;26:21–29. [PubMed: 16371063]

Gonzales FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. Drug Metab. Disp 1998;26:1194–1198.

Guimaraes S, Moura D. Vascular adrenoceptors: an update. Pharmacol. Rev 2001;53:319–356. [PubMed: 11356987]

Henrich WL, Levi M. Ontogeny of renal rennin release in spontaneously hypertensive rat and Wistar–Kyoto rat. Am. J. Physiol 1991;260:F530–F535. [PubMed: 1849363]

Hu ZW, Shi XY, Okazaki M, Hoffman BB. Angiotensin II induces transcription and expression of alpha-1 adrenergic receptors in vascular smooth muscle cells. Am. J. Physiol 1995;268:H1006–H1014. [PubMed: 7900855]

Hunyady L, Catt KJ. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Mol. Endocrinol 2006;20:953–970. [PubMed: 16141358]

Lázaro-Suárez ML, Gómez-Zamudio JH, Gallardo-ortíz IA, Tanoue A, Tsujimoto G, Farias-Rodríguez VM, Villalobos-Molina R. Chloroethylclonidine reveals α_{1A}-adrenoceptors mediate contraction in aorta of α_{1D}-adrenoceptor knockout mice. Auton. Autacoid. Pharmacol 2005;25:179–183. [PubMed: 16176450]

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem 1951;193:265–275. [PubMed: 14907713]

Lund AK, Goens MB, Kanagy NL, Walker MK. Cardiac hypertrophy in aryl hydrocarbon receptor null mice is correlated with elevated angiotensin II, endothelin-1 and mean arterial blood pressure. Toxicol. Appl. Pharmacol 2003;193:177–187. [PubMed: 14644620]

Martínez AL, Villalobos-Molina R. Early and chronic captopril or losartan therapy reduces infarct size and avoids congestive heart failure after myocardial infarction in rats. Arch. Med. Res 2003;34:357–361. [PubMed: 14602500]

Ruiz P, Basso N, Cannata MA, Taquini AC. The renin–angiotensin system in different stages of spontaneous hypertension in the rat (SHR). Clin. Exp. Hypertens. A 1990;12:63–81. [PubMed: 2407392]

Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T, Sunada S, Takeo S, Tsujimoto G. The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. J. Clin. Invest 2002;109:765–775. [PubMed: 11901185]

Villalobos-Molina R, Ibarra M. α_1-Adrenoceptors that mediate contraction in arteries of normotensive and spontaneously hypertensive rats are of the α_{1D} or α_{1A} subtypes. Eur. J. Pharmacol 1996;298:257–263. [PubMed: 8846824]

Villalobos-Molina R, Ibarra M. Vascular α_{1D}-adrenoceptors: are they related to hypertension? Arch. Med. Res 1999;30:347–352. [PubMed: 10596451]

Villalobos-Molina R, Ibarra M. Increased expression and function of vascular α_{1D}-adrenoceptors may mediate the pro-hypertensive effects of angiotensin II. Mol. Interv 2005;5:340–342. [PubMed: 16394249]

Villalobos-Molina R, López-Guerrero JJ, Ibarra M. Functional evidence of α_{1D}-adrenoceptors in the vasculature of young and adult spontaneously hypertensive rats. Br. J. Pharmacol 1999;126:1534–1536. [PubMed: 10323583]

Weir MR, Dzau VJ. The renin–angiotensin-aldosterone system: a specific target for hypertension management. Am. J. Hypertens 1999;12:205S–213S. [PubMed: 10619573]

Xin X, Yang N, Eckhart AD, Faber JE. α_{1D}-Adrenergic receptors and mitogen-activated protein kinase mediate increased protein synthesis by arterial smooth muscle. Mol. Pharmacol 1997;51:764–775. [PubMed: 9145914]

Yamamoto Y, Koike K. Characterization of alpha1-adrenoceptor-mediated contraction in the mouse thoracic aorta. Eur. J. Pharmacol 2001;424:131–140. [PubMed: 11476759]

Zicha J, Kune J. Ontogenetic aspects of hypertension development: analysis in the rat. Physiol. Rev 1999;79:1227–1282. [PubMed: 10508234]

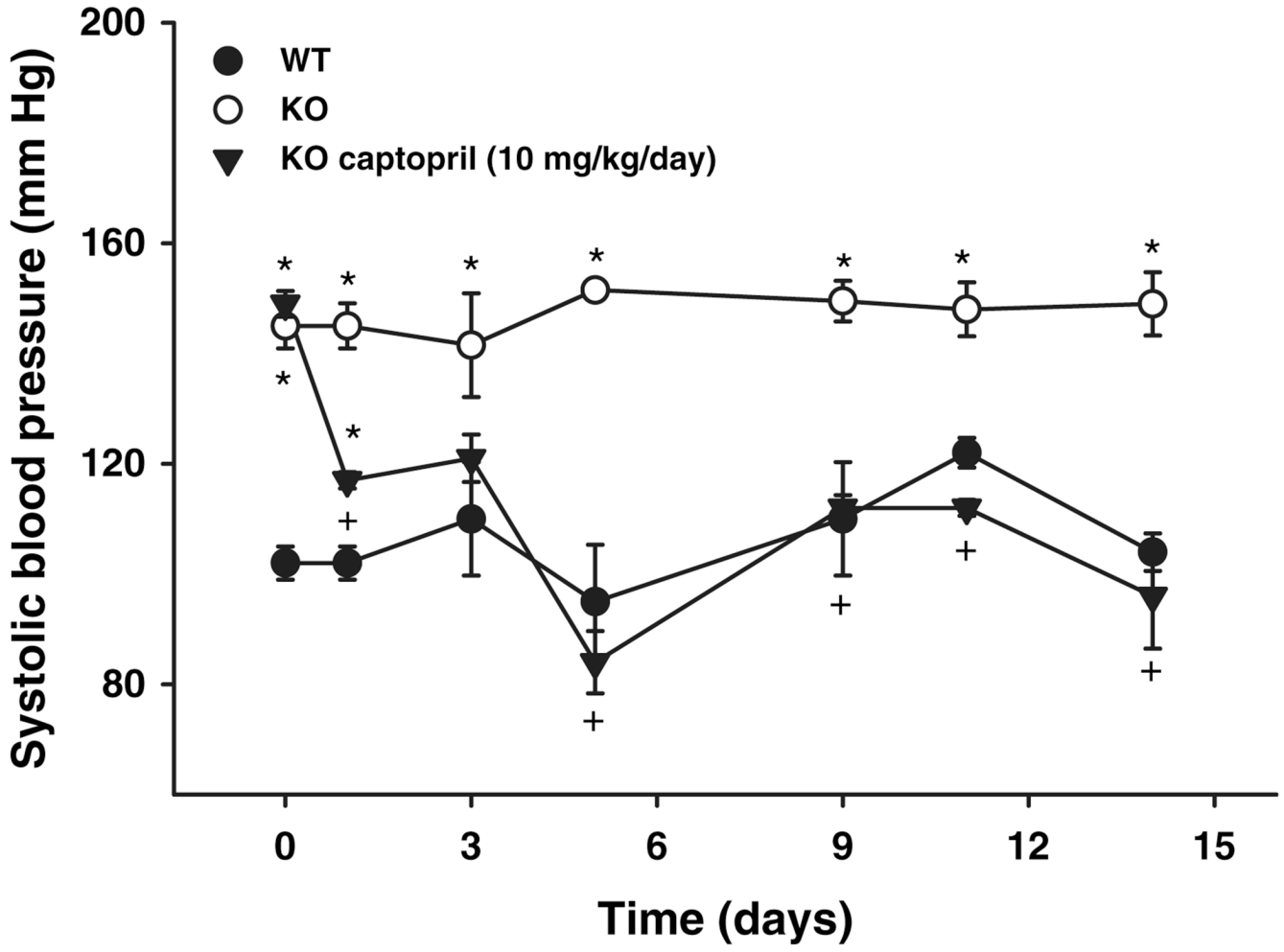

Figure 1.

Systolic blood pressure in AhR$^{-/-}$ mice. Mice were trained for blood pressure measurements, then subjected to assay conditions: WT (●), AhR$^{-/-}$ (○). In some experiments, AhR$^{-/-}$ mice were treated with captopril (▼). Results represent the mean ± SEM of 3–6 animals. *$P < 0.05$ AhR$^{-/-}$ *vs.* WT. $^{+}P < 0.05$ AhR$^{-/-}$ *vs.* AhR$^{-/-}$ + captopril.

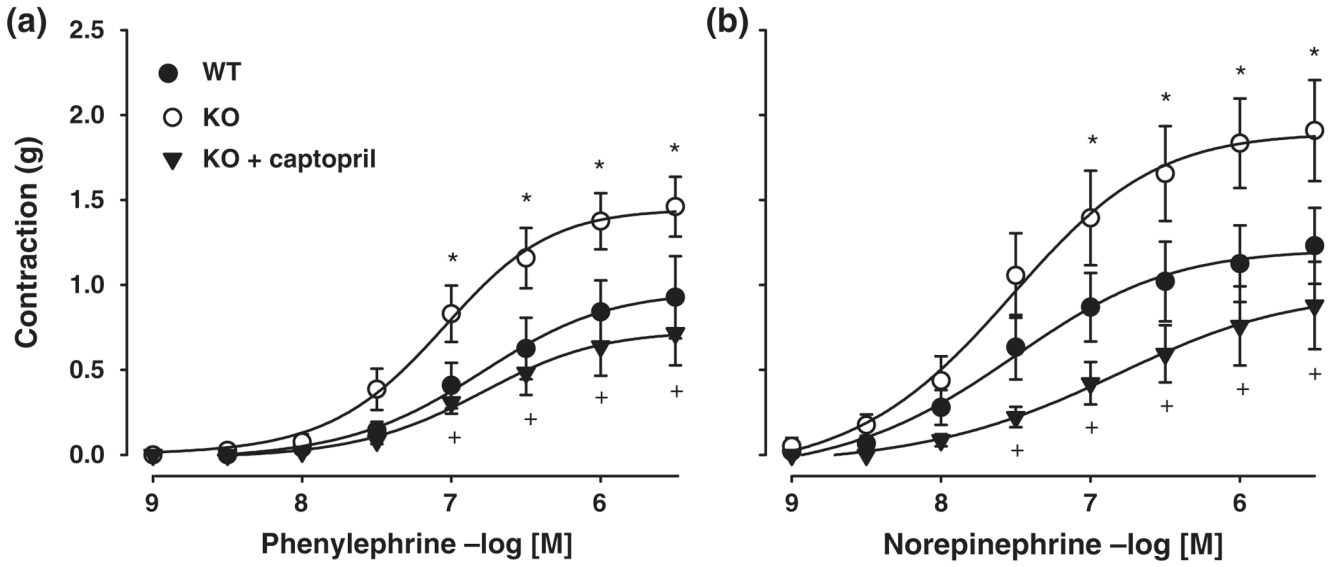

Figure 2.

Contractions of aorta taken from AhR$^{-/-}$ (KO, ○) and WT (•) mice exposed to increasing concentrations of phenylephrine (a) or noradrenaline (b). In some experiments, aorta derived from captopril-treated AhR$^{-/-}$ mice were used (▼). Results are reported as mean ± SEM of 3–6 animals. *P < 0.05 AhR$^{-/-}$ *vs*. WT; +P < 0.05 AhR$^{-/-}$ *vs*. AhR$^{-/-}$ + captopril.

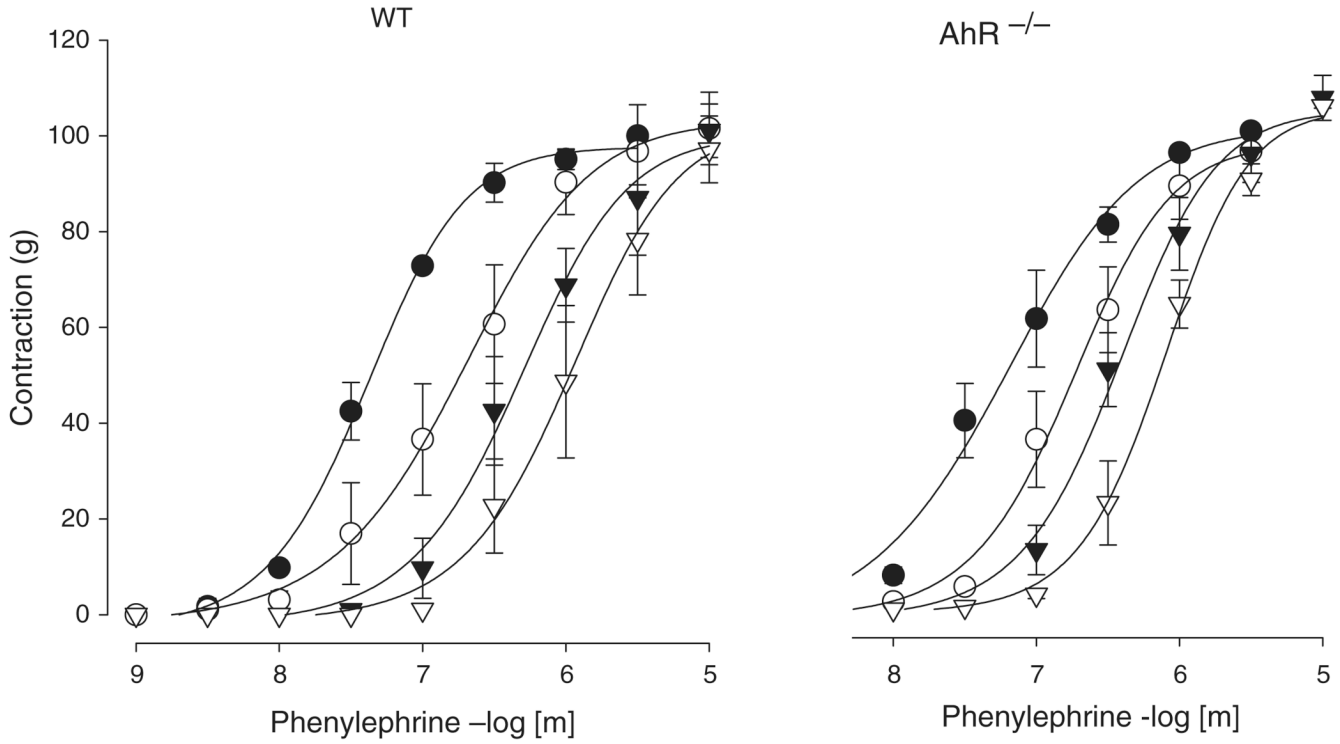

Figure 3.

Effect of α_{1D}-adrenoceptor antagonism in isolated mouse aorta. Isolated aortic rings of WT and AhR$^{-/-}$ mice were incubated with phenyleprhine in the absence and in the presence of increasing concentrations of the selective α_{1D}-adrenoceptor antagonist BMY 7378 (●, control; ○, BMY 3×10^{-9} M; ▼, BMY 1×10^{-8} M; ▽, 3×10^{-8} M). Data are reported as a percentage of the maximum response (mean ± SEM, $n = 3$).

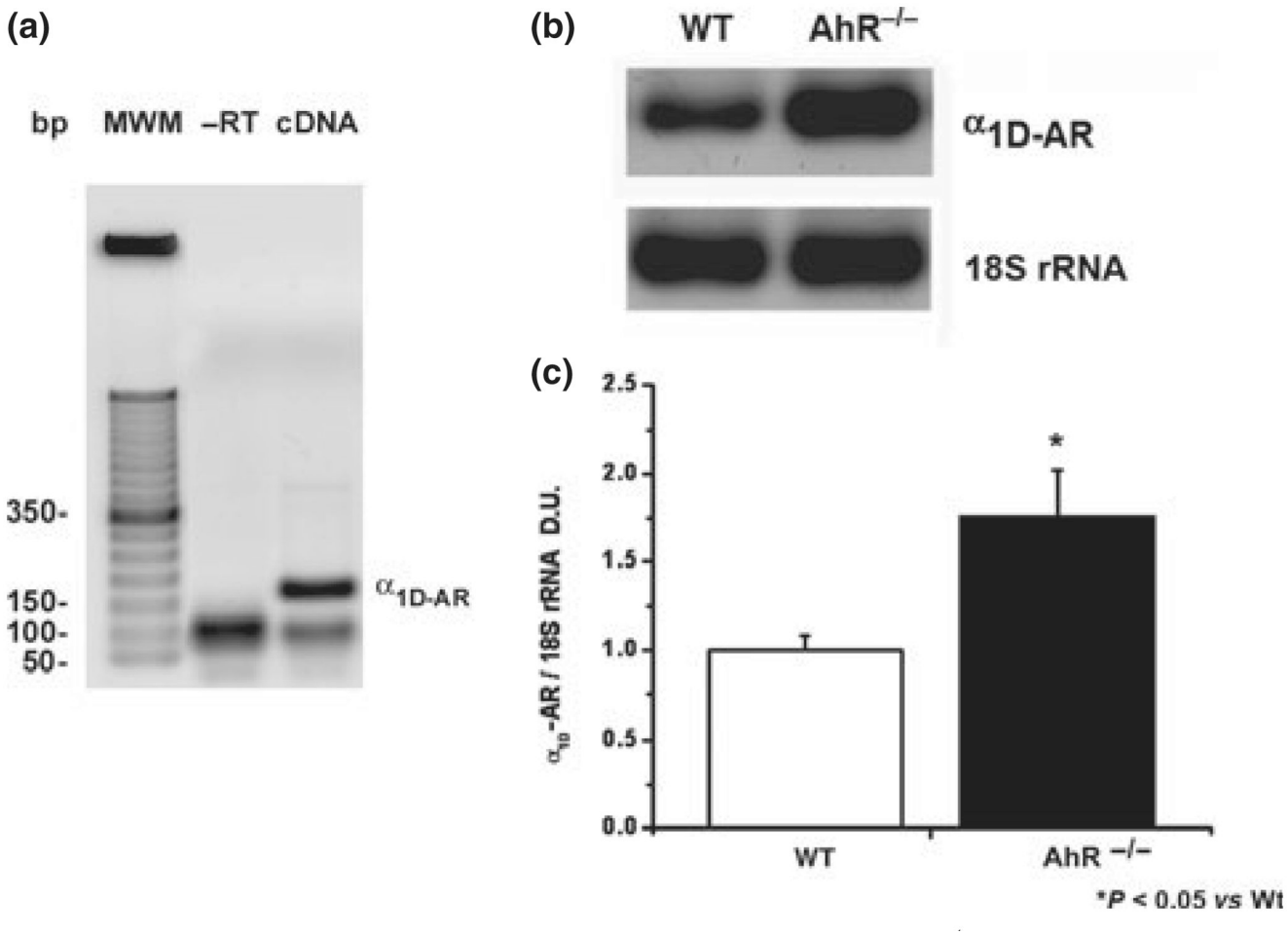

Figure 4.

Reverse transcription and PCR analysis of α_{1D}-adrenoceptor mRNA in $AhR^{-/-}$ mice. (a) PCR amplification of the receptor was visualized in 2% agarose gels as a band of 158 bp. (b) Representative amplification of α_{1D}-adrenoceptor and 18S rRNA in wild type and mutant mice. (c) Relative abundance was normalized *vs.* 18S ribosomal RNA amplification. Results are the mean ± SEM of three animals. $*P < 0.05$ $AhR^{-/-}$ *vs.* WT.

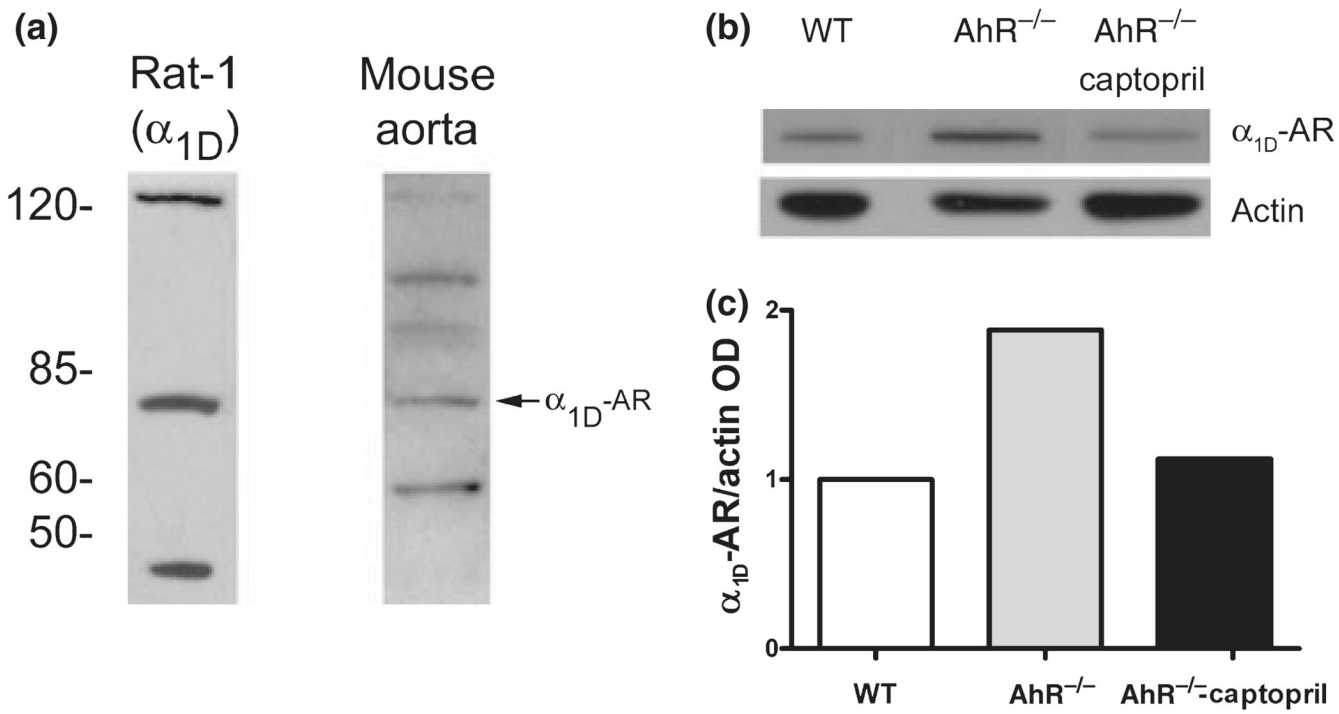

Figure 5.

Western blot analysis of α_{1D}-adrenoceptor protein in $AhR^{-/-}$ mice. (a) α_{1D}-Adrenoceptor is detected as a band of *c*. 85 kDa in Rat-1 cells stably expressing the receptor and in mouse aorta. (b) Representative autoradiography of α_{1D}-adrenoceptor and actin in WT, $AhR^{-/-}$ and captopril-treated $AhR^{-/-}$ mice. (c) Relative abundance analysis of α_{1D}-adrenoceptor protein *vs.* actin in a pool of three mice aorta per group.