

Knockout of the α 1A/C-adrenergic receptor subtype: The α 1A/C is expressed in resistance arteries and is required to maintain arterial blood pressure

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α 1-adrenergic receptors (ARs) play a major role in blood pressure regulation. The three α 1-AR subtypes (A/C, B, and D) stimulate contraction of isolated arteries, but it is uncertain how different subtypes contribute to blood pressure regulation in the intact animal. We studied the role of the α 1A/C subtype by using gene knockout. α 1A/C knockout (KO) mice were viable and overtly normal. The LacZ reporter gene replaced α 1A/C coding sequence in the KO, and β -galactosidase staining was present in resistance arteries and arterioles, but not in the thoracic aorta or its main branches. By tail cuff manometer and arterial catheter in conscious mice, α 1A/C KO mice were hypotensive at rest, with an 8–12% reduction of blood pressure dependent on α 1A/C gene copy number. A61603, an α 1A/C-selective agonist, caused a pressor response that was lost in the KO and reduced but significant in heterozygous mice with a single copy of the α 1A/C. A subtype-nonselective agonist [phenylephrine (PE)] caused a pressor response in KO mice, but the final arterial pressure was only 85% of wild type. The baroreflex was reset in the KO, and heart rate variability was decreased. After baroreflex blockade with atropine, PE increased blood pressure but did not change heart rate. Cardiac and vascular responses to the β -AR agonist isoproterenol were unchanged, and the arterial lumen area was not altered. We conclude that the α 1A/C-AR subtype is a vasopressor expressed in resistance arteries and is required for normal arterial blood pressure regulation. α 1A/C-selective antagonists might be desirable antihypertensive agents.

The sympathetic nervous system plays a major role in blood pressure regulation by modulating cardiac and vascular contractility. Sympathetic effects are mediated by catecholamines acting on G protein-coupled adrenergic receptors (ARs) in three families, β , α 2, and α 1. Vascular contraction is controlled primarily by α 1-ARs, and their importance in blood pressure regulation is emphasized by the efficacy of α 1-AR antagonists in human hypertension, a major medical problem (1, 2).

The α 1-AR family includes three cloned subtypes, A/C[‡], B, and D, which are transcribed at variable levels in different tissues (3). All three α 1-AR subtypes can mediate constriction in many isolated arteries (4, 5). This complexity and the lack of subtype antagonists with sufficient selectivity have so far made it impossible to define the role of any individual α 1-AR subtype in blood pressure regulation in the intact animal.

Distinct roles of α 1-AR subtypes in blood pressure regulation might be important clinically. In the recent, large Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), a nonselective α 1-AR antagonist was an effective antihypertensive agent, but it was associated with an important adverse side effect, an increased incidence of heart failure, raising concern about the use of these drugs in treating hypertension or prostate disease (1, 2, 6, 7). Further, nonselective α 1-AR antagonists can cause orthostatic hypotension and dizziness (1). A subtype-selective agent might treat hypertension without these adverse cardiac and vascular side effects.

The gene knockout approach has been useful in resolving the roles of individual AR subtypes in blood pressure regulation,

demonstrating that the β 2-AR subtype mediates hypotension, whereas the α 2B-AR subtype causes hypertension (8). However, knockout of the α 1B subtype has no effect on resting blood pressure (9), and there is controversy as to whether this subtype increases blood pressure *in vivo* (10).

In this study, we used the knockout approach to test the role in blood pressure regulation of one α 1-AR subtype, the α 1A/C. We replaced the first exon of the α 1A/C with the LacZ gene, encoding β -galactosidase. We could thus test for receptor expression in arteries controlling blood pressure. We measured blood pressure and heart rate in conscious animals by tail cuff manometer, carotid arterial catheter, and ECG telemeter, at rest and after infusion of sympathetic and parasympathetic drugs.

Methods

Targeted Deletion of the α 1A/C. The mouse α 1A/C gene was cloned from a 129/SvJ mouse genomic library (11). The targeting vector (Fig. 1A) contained a 5' arm of \approx 1.1 kb and a 3' arm of \approx 6.5 kb. Homologous recombination replaced the first exon and 415 bp of the intron, including the splice donor, with the *Escherichia coli* β -galactosidase gene LacZ and the neomycin-resistance gene driven by the phosphoglycerate kinase promoter. The LacZ cassette contained a stop codon and an SV40 polyadenylation signal. RW-4 129/SvJ mouse embryonic stem cells (Genome Systems, St. Louis) were electroporated and selected for neomycin resistance, and 5 of 196 clones contained the appropriate short-arm insertion by PCR and sequencing (not shown). Two clones had correct 3' insertion and no random insertions by Southern analysis with external and LacZ probes (not shown). Targeted cells were injected into C57BL/6 blastocysts to obtain three chimeras >90% mosaic. Two chimeras from a single clone transmitted the targeted allele through the germ line when bred into FVB/N and C57BL/6 backgrounds. Mice in this study were from filial (F) generations 2–5 in the FVB/N background. Wild-type (WT) littermates were controls. Routine genotyping was by PCR with a common α 1A/C 5'-flanking primer (AGCTAACCATTTCAGCAAAGA) and specific 3' primers against exon 1 (CAAGATCACCCCAAGTAGAAT) and LacZ (TAACCGTGCATCTGCCAGTTT).

Reverse Transcription (RT)-PCR and RNase Protection. RNA was isolated from tissues rinsed in PBS and homogenized (Polytron) in Trizol (GIBCO/BRL). For RT-PCR, cDNA was transcribed with

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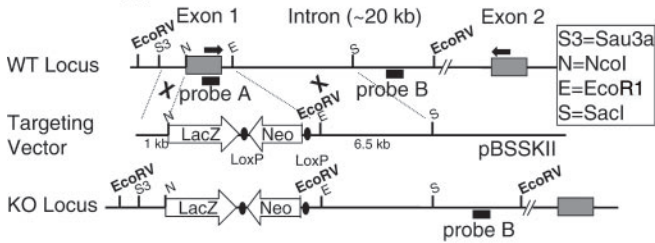
Abbreviations: AR, adrenergic receptor; F, filial; Het, heterozygous knockout; HR, heart rate; KO, homozygous knockout; MAP, mean arterial pressure; PE, phenylephrine; WT, wild type.

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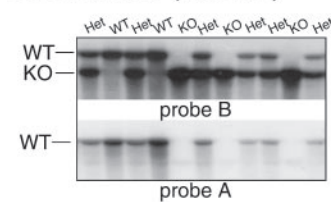
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[‡]Throughout this paper we use the name α 1A/C-AR to indicate that the names "A" and "C" have both been given to the same cloned subtype (24). The cloned α 1D-AR was named originally the α 1A (24), and accession nos. in GenBank for the α 1D sequence (M60654 and M60655) still refer to this gene as the α 1A.

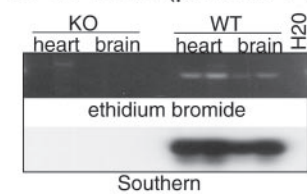
A. Strategy



B. Southern (EcoRV)



C. RT-PCR (primers \uparrow)



D. β -Galactosidase

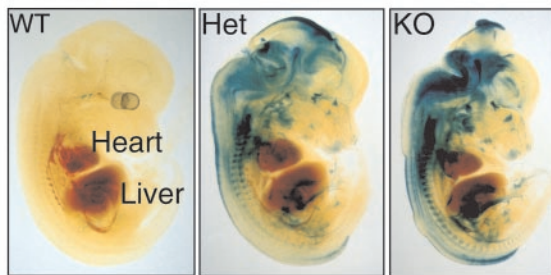


Fig. 1. Targeted deletion of exon 1 of the α 1A/C-adrenergic receptor gene. (A) Targeting strategy. A LacZ-Neo cassette replaced exon 1 of the α 1A/C and introduced an EcoRV site. (B) Southern with EcoRV in an F2 litter. Probe B reveals a 16-kb band with the WT allele and a 11.2-kb band with the KO. Probe A to exon 1 is negative in the KO. (C) RT-PCR. Primers from exons 1 and 2 (arrows) were used with 0.5 μ g of total RNA from heart and brain. Ethidium bromide staining shows a 524-bp product in WT but not in KO (Upper), as confirmed by Southern with a probe to exon 1 (Lower). (D) β -Galactosidase in E12.5 embryos. Blue staining of *E. coli* β -galactosidase is proportional to LacZ copy number in WT (no copies), Het (one copy), and KO (two copies).

an α 1A/C-specific 3' primer within the sixth transmembrane domain in exon 2 (ACTTCCATTACGGAGTCCATC), then amplified by PCR with a 5' primer from the fifth transmembrane domain in exon 1 (CGACAAGTCAGACTCAGAGCAAG) (see Fig. 1A). RNase protection assays for α 1-AR subtype mRNAs used 25 μ g total RNA with rat probes (3, 12).

Radioligand Binding. A 100,000 \times g pellet was used in binding (12). Reactions contained 50–100 μ g protein and [*methoxy*- 3 H]prazosin (70–87 Ci/mmol, NEN; 1 Ci = 37 GBq), 0.4–2 nM for saturation binding and 0.5 nM in competition binding with 5-methylurapidil (0.1 nM to 50 μ M, Sigma RBI #U101). Ten micromolar phentolamine (Sigma RBI #P131) defined nonspecific binding. Data were analyzed with PRISM (GraphPad, San Diego).

β -Galactosidase. To localize *E. coli* β -galactosidase *in situ*, the left carotid artery was catheterized under isoflurane anesthesia, and the mouse was euthanized by thoracotomy and incision of the left atrium. PBS (pH 7.3) was perfused at 2.5 ml/min to clear blood. Then 22.5 ml of 4% paraformaldehyde in PBS pH 7.3 was perfused, incubated 60 min at room temperature, and washed out with PBS. Forty milliliters of staining solution was next perfused [1 mg/ml X-gal/5 mM $K_3Fe(CN)_6$ /5 mM $K_4Fe(CN)_6 \cdot 3H_2O$ /2 mM $MgCl_2$ /

0.02% Nonidet P-40/0.01% deoxycholate in PBS with 40 mM Tris base (pH 7.3)], incubated for 36 h at 30°C, washed out with PBS, and postfixed in 10% buffered formalin. Arteries were dissected and examined with a Zeiss SV11 stereomicroscope and a Spot digital camera. Skin was embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin/eosin. β -Galactosidase activity in tissues was measured by the Galacto-Star Chemiluminescent System (Tropix/Applied Biosystems); and β -galactosidase protein, by Western blot with a monoclonal antibody (Promega).

Blood Pressure and Heart Rate (HR). Tail cuff systolic blood pressure and HR were measured using a noninvasive computerized tail cuff system (BP-2000, VisiTech Systems, Apex, NC; ref. 13). Mice were trained for 3 days, then systolic blood pressure was taken as the mean of 6–12 daily readings, with at least 15 of 20 successful each day. For intraarterial pressure (14), the left carotid artery and right internal jugular vein were catheterized under isoflurane anesthesia with sterile PE-10 tubing filled with heparin (100 units/ml) in saline. Catheters were sealed, tunneled to the back, and coiled in a s.c. pouch. After at least 24 h recovery from surgery, the carotid catheter was connected to a low compliance dome pressure transducer (Cobe Laboratories, Lakewood, CO), and the mice were allowed to move freely in a 36 inches² enclosure. The internal jugular vein catheter was connected to a switching valve with low dead volume for drug infusion. Pressure waveforms were acquired at 500 Hz and transformed simultaneously to mean arterial pressure (MAP) and HR, and reduced to 10-s means (MPS100, Biopac, Santa Barbara, CA). Baseline MAP and HR were established over 40–60 min, then drugs were infused (volumes, 0.2–2.0 μ l/g at 1–5 μ l/s) in the order A61603 (Tocris Pharmaceuticals #1052), phenylephrine (PE; Sigma #P6126), isoproterenol (Sigma #I6405), and atropine (Sigma #A0257). The peak drug response was at \approx 1 min (\approx 2 min for atropine), and the duration was \approx 5 min (longer for atropine). Before each drug was given, the preinfusion baseline was reestablished, except in experiments with baroreflex blockade, where PE and A61603 were given 4 min after atropine. The mean of the 2-min period immediately before infusion was taken as baseline, and the 10-s mean peak response was the treatment value.

Blood Chemistries and Cardiovascular Structure. Blood was drawn from the ventricle under isoflurane anesthesia, and serum electrolytes, blood cell counts, and indices of renal function were assayed in the clinical laboratory of the San Francisco Veterans Affairs Medical Center. Organ wet weights were measured at necropsy. Arteries were fixed *in situ* by perfusion with 10% buffered formalin at 80 mm Hg and sectioned at a set distance from the aorta (in mm: right carotid 2, celiac 1.5, mesenteric 1, right renal 1). Sections were examined with a Nikon SMZ800 stereomicroscope, and lumen area was quantified with NIH IMAGE V.1.61/PPC; the mean of three measurements was used for each artery.

Echocardiography. Echocardiography under anesthesia with isoflurane was performed as described (15).

HR Variability. The supporting information (which is published on the PNAS web site, www.pnas.org) describes implantation of ECG telemeters, drug injections in conscious mice 1 week after surgery, and data analysis.

Statistics. Mean values \pm SE are shown and were compared by an unpaired *t* test or ANOVA followed by Fischer probable least-squares difference (PLSD).

Results

Generation and Confirmation of the α 1A/C-AR KO. Targeting replaced exon 1 of the α 1A/C with Lac Z (Fig. 1A) and introduced an EcoRV site that was used for genotyping (Fig. 1B). Intercrosses of heterozygous F1 mice transferred the targeted allele to both

Table 1. α 1-AR radioligand binding in α 1A/C KO mice

	Brain		Heart		Kidney	
	WT	KO	WT	KO	WT	KO
Bmax, fmol/mg	137 \pm 14	62 \pm 2*	14 \pm 1	10 \pm 2*	56 \pm 2	24 \pm 3*
KO/WT, %		45		70		42
Ki high (nM)	0.6 \pm 0.2	None	1.1 \pm 0.7	None	2.0 \pm 0.7	None
High/total, %	48 \pm 3	0	21 \pm 2	0	65 \pm 3	0
Ki low, nM	86 \pm 18	69 \pm 12	193 \pm 50	280 \pm 113	292 \pm 67	231 \pm 25
Hill coefficient	0.39 \pm 0.03	0.91 \pm 0.03*	0.61 \pm 0.05	1.12 \pm 0.08*	0.45 \pm 0.02	0.85 \pm 0.04*

Membranes from tissues of mice aged 12–16 weeks were used in binding reactions with [3 H]prazosin. Sites with high affinity for 5-methylurapidil (the α 1A/C) were determined by competition. Values are from six mice of each genotype, three male and three female. There were no differences in binding between sexes of either genotype (data not shown). *, $P < 0.05$ vs. WT.

sexes with expected Mendelian frequency in two backgrounds, FVB/N (Fig. 1B Upper) and C57BL/6 (not shown). Exon 1 DNA, which encodes the receptor through transmembrane domain 5, was absent in the KO (Fig. 1B Lower), and α 1A/C mRNA was not detected by RT-PCR (Fig. 1C) or by RNase protection (not shown).

α 1-AR binding in KO mice was reduced by just over 50% in brain and kidney and by \approx 30% in heart (Table 1). In WT mice, 5-methylurapidil, which has higher affinity for the α 1A/C than the α 1B or α 1D, produced shallow competition curves that best fit a two-site model, with Hill coefficients 0.4–0.6 (Table 1). In KO mice the curves best fit a one-site model, with Hill coefficients shifted significantly toward unity (Table 1). The fraction of high-affinity α 1 sites lost in the KO was in good agreement with the reduction of total α 1 sites (Table 1). α 1B mRNA levels were unchanged in the KO heart by RNase protection (data not shown). These results confirmed that the α 1A/C receptor was absent in KO mice, and also suggested that there were no major compensatory changes in the other subtypes.

Because Lac Z was inserted at the α 1A/C translational start site (Fig. 1A, *Nco*I site), β -galactosidase expression was controlled by endogenous α 1A/C regulatory elements. Staining of E12.5 embryos showed an appropriate increase in blue intensity with LacZ copy number (Fig. 1D). β -galactosidase staining was absent in WT mice (Fig. 1D and not shown). In heart and brain, β -galactosidase enzyme activity and protein by Western blot were also proportional to LacZ copy number (not shown). In addition, β -galactosidase was present in the KO (not shown) in the same adult tissues where α 1A/C mRNA was present in WT mice, including brain, heart, and kidney (11). Staining was absent in liver (embryo in Fig. 1D, adult not shown), where the α 1A/C receptor is absent (11). These results indicated that β -galactosidase in KO mice was a valid surrogate to localize the sites of normal α 1A/C transcription.

Expression of the α 1A/C in Resistance Arteries. We used β -galactosidase staining in the KO to localize α 1A/C expression in the vasculature (Fig. 2). Staining was absent in the thoracic aorta and

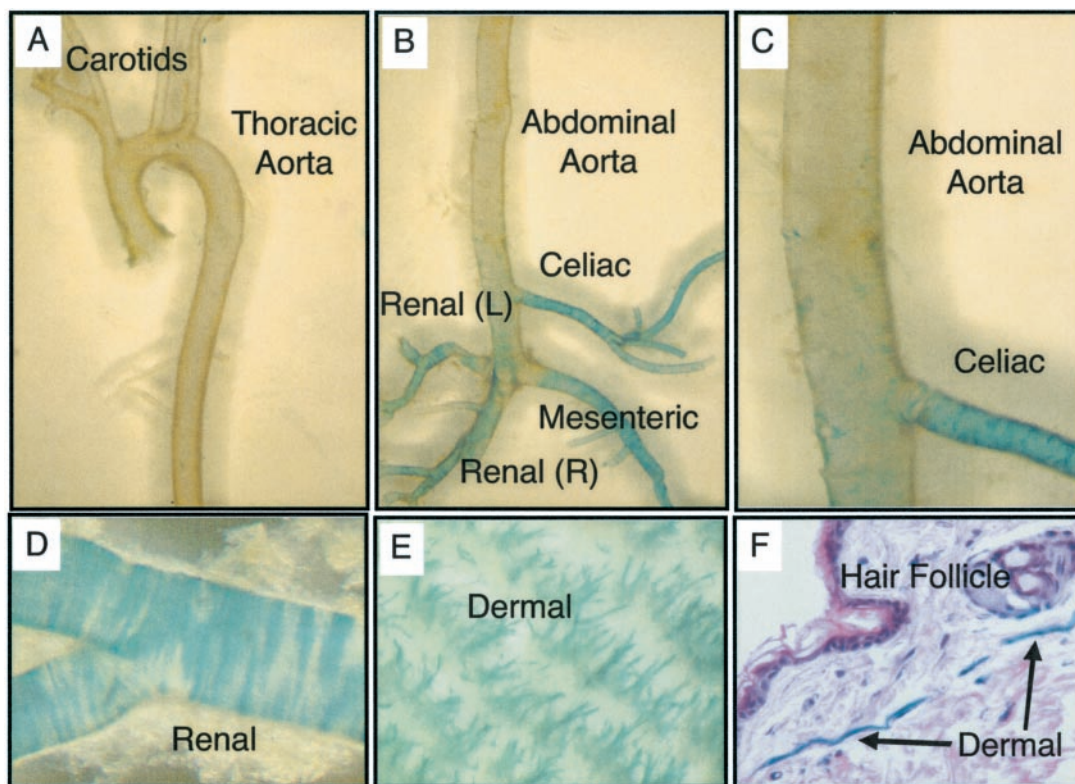


Fig. 2. β -Galactosidase staining localizes arterial expression of the α 1A/C. β -Galactosidase was stained *in situ* (blue), and the major arteries (A–D) or skin from the chest (E) were dissected and photographed. Skin in E is from the epidermal side after removing the fur. (F) A skin section was counterstained with hematoxylin and eosin to show a hair follicle.

Table 2. Blood pressure and HR in conscious $\alpha 1A/C$ KO mice

	Tail cuff manometer				Carotid artery catheter		
	Male		Female		WT	Het	KO
	WT	KO	WT	KO			
Systolic blood pressure, mmHg	114 ± 2	104 ± 5*	111 ± 2	102 ± 3*			
KO/WT, %		91		92			
Mean arterial pressure, mmHg					138 ± 3	127 ± 6*	121 ± 3*
KO/WT, %						92	88
Heart rate, beats per min	602 ± 10	636 ± 28	604 ± 14	630 ± 6	568 ± 33	565 ± 38	624 ± 16
KO/WT, %		106		104		99	110
N mice	12	5	9	10	9	6	8

Blood pressure and heart rate were measured in conscious mice by tail cuff manometer or carotid artery catheter. Mice used for tail cuff were male and female F4 aged 20–28 weeks, and mice for catheterization were female F3 aged 16–20 weeks. *, $P < 0.05$ vs. WT of same sex.

its major branches, including the carotid arteries (Fig. 2A), and in the proximal pulmonary artery and the superior and inferior vena cavae (not shown). β -Galactosidase expression was observed at low levels in the abdominal aorta just above the level of the celiac artery (Fig. 2B), particularly around branch artery origins (Fig. 2C). Staining became prominent lower in the aorta and in the celiac, renal, mesenteric, hepatic, splenic, gastric, testicular, ovarian, iliac, femoral, and tail arteries (Fig. 2B–D and not shown). Remarkably, in medium arteries such as celiac and renal, staining was markedly heterogeneous in a circumferential pattern (Fig. 2C and D). Strong expression of β -galactosidase was also seen in the skin, in ≈ 20 - μ m dermal arterioles near hair follicles (Fig. 2E and F). These results suggested that the $\alpha 1A/C$ was expressed normally in the gut, renal, and skin circulations. These vascular beds have major roles in blood pressure control (16).

Hypotension in the $\alpha 1A/C$ KO. Given the localization of the $\alpha 1A/C$ in arteries controlling overall vascular resistance, we measured blood pressure in KO mice, using two different techniques in conscious mice, tail cuff manometer, and carotid artery catheter.

KO mice were hypotensive, with a significant ≈ 10 – 15 mm Hg reduction in blood pressure (≈ 8 – 12% of WT; Table 2). Carotid artery pressures were higher than tail cuff pressures in both WT and KO mice, and WT values were in the range seen in other studies (13). Heterozygous (Het) mice were intermediately hypotensive, indicating that the normal $\alpha 1A/C$ contribution to blood pressure required receptors expressed from both alleles. Tail cuff pressure was also lower in a smaller cohort of KO mice in the C57BL/6 background, F2 males age 12–16 weeks (WT 106 ± 4 , KO 94 ± 1 or 89% of WT, $n = 6$, $P = 0.02$). Heart rate in the KO was slightly but not significantly higher (Table 2; see below).

Blood pressure is determined by both cardiac output and vascular resistance (17). Thus, we considered the possibility that hypotension in KO mice was due to reduced cardiac output. KO mice of both sexes bred normally, gained body weight identical to WT mice, and had no obvious diseases to at least 12 months of age. Heart weight was identical in KO and WT (heart weight in mg per body weight in gm; female WT 4.2 ± 0.2 , KO 4.4 ± 0.3 , $n = 8$ – 10 , $P =$ not significant; male WT 4.4 ± 0.1 , KO 4.4 ± 0.2 , $n = 3$ – 5 , $P =$ not significant). Heart histology was normal (not shown). Heart function by echocardiography was identical in WT and KO, with ejection fraction 64–66% and fractional

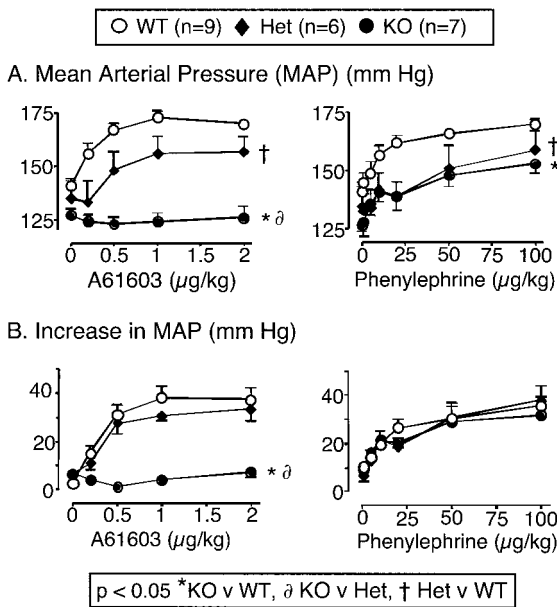


Fig. 3. Carotid artery pressure with A61603 and PE in conscious $\alpha 1A/C$ KO mice. Drugs were infused through a right jugular vein catheter in conscious, unrestrained mice 24 h after catheterization of the left carotid artery. Mice were the same as Table 2 (Carotid artery catheter). In A, the zero dose values were with 50 μ l of saline.

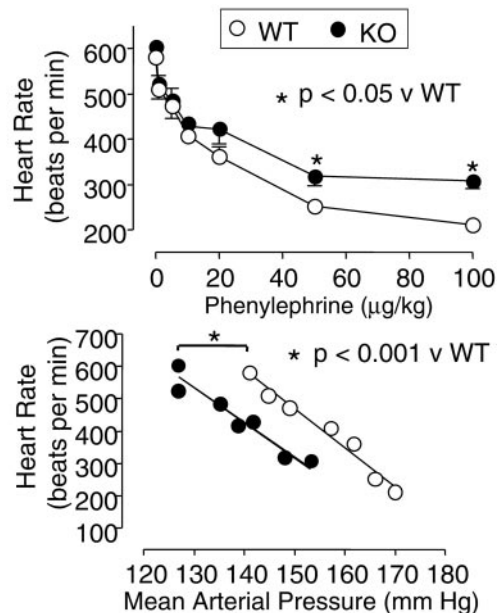


Fig. 4. Altered baroreflex in $\alpha 1A/C$ KO mice. HR data from PE infusions (Fig. 3) are plotted against PE dose (Upper) and MAP (Lower).

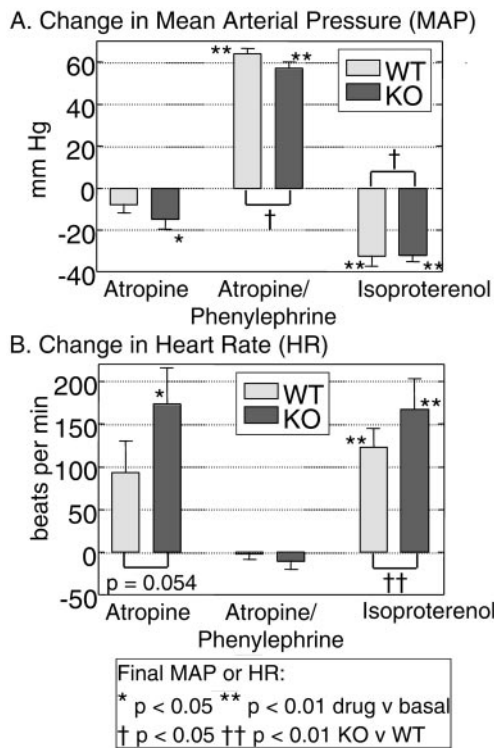


Fig. 5. Blood pressure and HR with atropine and isoproterenol in $\alpha 1A/C$ KO mice. The protocol was as in Fig. 3. Isoproterenol ($3 \mu\text{g}/\text{kg}$) was given after the response to PE had returned to baseline. Then atropine ($1 \text{ mg}/\text{kg}$) was given to block the baroreflex. After 4 min, when parasympathetic blockade was complete and MAP and HR had stabilized, PE was again infused at a maximum dose ($100 \mu\text{g}/\text{kg}$). In these experiments, baseline HR was WT 551 ± 12 ($n = 18$), KO 587 ± 24 ($n = 14$), $P =$ not significant. Baseline MAP was WT 128 ± 3 , KO 115 ± 2 , $P = 0.006$.

shortening 30–31% ($n = 5-7$ of each sex in each genotype, $P =$ not significant; D.G.R., A. Nakamura, E. Foster, and P.C.S., unpublished data). Function of isolated cardiac muscle was normal (25). Thus, there was no evidence for cardiac dysfunction in KO mice. Weights of other major organs were identical to WT, both absolute and normalized to body weight, including lungs, kidneys, and liver (data not shown). Serum levels of sodium, potassium, chloride, bicarbonate, urea nitrogen, and creatinine were the same as WT (data not shown), suggesting that renal function was normal. Blood cell counts were normal (data not shown). Thus, there was no evidence for a nonvascular cause of hypotension.

$\alpha 1\text{-AR}$ Pressor Responses in the $\alpha 1A/C$ KO. To determine whether reduced vascular resistance caused hypotension in KO mice, we studied the pressor response to infusion of $\alpha 1\text{-AR}$ agonists in chronically instrumented, conscious mice (Fig. 3). In WT mice, A61603, an agonist selective for the $\alpha 1A/C$ (18), increased MAP by 35 mm Hg, or 25% over basal, with an EC_{50} $0.3 \mu\text{g}/\text{kg}$ (Fig. 3 Left). In KO mice, A61603 had no effect (Fig. 3 Left), confirming the selectivity of A61603 *in vivo*, and indicating that the $\alpha 1A/C$ receptor was a potent vasopressor in the normal mouse. In Het mice, A61603 stimulated an increase in MAP identical to WT (35 mm Hg, Fig. 3B). The dose–response relationship (Fig. 3B) and time course (not shown) were also identical in Het and WT. However, the final MAP was 15 mm Hg lower in the Het (85% of WT; Fig. 3A). These results confirmed the vasoconstrictor efficacy of the $\alpha 1A/C$ and agreed with the intermediate effect of the Het on resting blood pressure (Table 2).

PE, a subtype nonselective $\alpha 1\text{-AR}$ agonist, in WT mice caused an increase in MAP similar to that seen with A61603, with an EC_{50} $8 \mu\text{g}/\text{kg}$ (Fig. 3 Right). The final MAP was slightly but insignificantly lower with PE than A61603 (Fig. 3), possibly reflecting some β -adrenergic vasodilator effect of the higher PE doses (see below). In KO and Het mice, PE stimulated an increase in MAP similar to WT (Fig. 3B), but the final MAP was 10–15% lower (Fig. 3A). Thus, both copies of the $\alpha 1A/C$ were required to reach maximum levels of MAP with $\alpha 1\text{-AR}$ agonist infusion, but the $\alpha 1B$ and/or $\alpha 1D$ subtypes could increase blood pressure when the $\alpha 1A/C$ was knocked out.

Altered Baroreflex in the KO. Normally, an increase in blood pressure causes a decrease in HR through the parasympathetic baroreflex. In KO mice, higher doses of PE caused less bradycardia than in WT mice (Fig. 4 Upper). A plot of the relationship between MAP and HR showed a significant parallel left shift in the baroreflex in KO mice (Fig. 4 Lower). This resetting of the baroreflex meant that HR was lower for any given pressure, and could account for the insignificant increase in HR in the KO, despite significant hypotension (Table 2).

To test whether the altered baroreflex masked differences in pressor responses between KO and WT mice, the reflex was blocked by the muscarinic receptor antagonist atropine. As expected, atropine caused a small decrease in MAP and a larger increase in HR, but these effects of atropine were significant only in KO mice (Fig. 5). PE increased MAP robustly in KO and WT mice during baroreflex blockade (Fig. 5), and thus baroreflex resetting did not mask a difference in pressor responsiveness. Notably, PE did not change HR when the baroreflex was blocked (Fig. 5).

Effect of β -Adrenergic Stimulation with Isoproterenol. To test for changes in β -AR signaling in KO mice, MAP and HR were measured after infusion of the β -AR agonist isoproterenol. Isoproterenol increased HR and decreased MAP significantly and similarly in KO and WT mice (Fig. 5). Thus, β -AR effects were not changed in the KO. The final HR after isoproterenol was significantly faster in the KO than the WT, and the final MAP was significantly lower, reflecting the different basal values of HR and MAP in the two genotypes (see legend to Fig. 5).

Vascular Structure in KO Mice. The $\alpha 1A/C$ was expressed in the vasculature early in development (Fig. 1D), so we measured arterial size, to test whether abnormal development explained the hypotension in KO mice. In three KO and three WT female mice aged 12 weeks, lumen areas (mm^2) were the same in KO and WT in celiac (0.044 vs. 0.048), mesenteric (0.070 vs. 0.072), and renal (0.092 vs. 0.087) (all $P =$ not significant), arteries with high levels of the $\alpha 1A/C$ (Fig. 2). Areas were also identical in the carotid (0.095 vs. 0.106), an artery with no detectable $\alpha 1A/C$ (Fig. 2).

HR Variability. The significant effect of atropine in the KO but not in the WT was consistent with a relative increase in basal sympathetic activity in the KO. To test for a change in autonomic balance in the KO, we measured heart rate variability by ECG telemetry in conscious mice. As shown in the supporting information, the effects of autonomic drugs indicated that increased sympathetic activity caused decreased heart rate variability. Basal heart rate variability was decreased significantly in the KO vs. WT (14%, $P < 0.05$), suggesting heightened sympathetic balance in the KO (supporting information).

Discussion

The major finding in this study is that the $\alpha 1A/C\text{-AR}$ subtype is required to maintain normal arterial blood pressure. Knockout of the $\alpha 1A/C$ receptor caused reduced blood pressure at rest and following infusion of pressor catecholamines. In addition, several

findings suggested that hypotension in KO mice was due to loss of vasoconstriction mediated by the $\alpha 1A/C$. These results establish the $\alpha 1A/C$ -AR as a potent mediator of vasopressor responses and indicate that selective $\alpha 1A/C$ antagonists might be efficacious in treating hypertension.

A role for the $\alpha 1A/C$ in vasopressor responses was documented by localization of the receptor in resistance arteries and by infusion of an $\alpha 1A/C$ -selective agonist A61603. $\alpha 1A/C$ expression in gut and renal arteries was suspected from prior studies in other species (4). However, the LacZ reporter gene provided new insights. Unexpectedly, the $\alpha 1A/C$ was absent in the thoracic aorta and its major branches. In the gut and renal arteries where the $\alpha 1A/C$ was expressed, the pattern of expression was remarkably heterogeneous. These findings indicate caution in the choice of arterial rings for vascular studies *in vitro*. The mechanism for the heterogeneous expression pattern is unknown, but there might be a relation to the pattern of sympathetic fibers innervating the arteries (19). Also unexpected was $\alpha 1A/C$ expression in skin arterioles. Study of skin vessels by classical AR localization techniques is difficult because of their small size, but the skin accounts for $\approx 9\%$ of resting cardiac output, and thus contributes significantly to vascular resistance (16). Further, localization of the $\alpha 1A/C$ in skin arteries indicates a potential role for this subtype in heat regulation, a common problem in diseases with abnormal sympathetic activity (16).

The skin, gut, and kidney together receive over 50% of resting cardiac output (16), and thus vasoconstriction in these beds is expected to have a major effect on blood pressure. Indeed, we showed that the $\alpha 1A/C$ -selective agonist A61603 caused a substantial hypertensive effect in WT and Het mice, and no effect in KO mice. Thus, we conclude that $\alpha 1A/C$ -AR is a potent vasopressor.

Interestingly, other $\alpha 1$ -AR subtypes were also suggested to be vasopressors, in that the nonselective $\alpha 1$ -AR agonist PE caused an increase in blood pressure in KO mice. However, the final blood pressure with PE in KO mice was only 85% of WT. Thus, the $\alpha 1B$ and/or $\alpha 1D$ were unable to compensate fully for the loss of the $\alpha 1A/C$, either at rest or with $\alpha 1$ agonist infusion. It is unclear whether $\alpha 1B$ or $\alpha 1D$ receptors mediate the residual $\alpha 1$ -AR-mediated vasopressor response in the absence of $\alpha 1A/C$ receptors. The $\alpha 1B$ KO mouse has normal basal blood pressure but, similar to the $\alpha 1A/C$ KO, demonstrates a reduced pressor response to PE (9). However, measurement in the $\alpha 1B$ KO was made shortly after anesthesia (9), which can alter AR responses (14, 20), and further study is warranted.

Several findings suggested that hypotension in $\alpha 1A/C$ KO mice was caused by reduced vascular tone. Blood pressure is a function of vascular resistance and cardiac output, and cardiac output is determined by heart and kidney function (21). The $\alpha 1A/C$ gene was expressed in heart and kidney, albeit at very low levels in the heart, but size and function of these two organs were normal. In addition, arterial size was normal, and the pressor response to PE in the KO

suggested that intrinsic vascular structure and function were normal. Taken together, the data support the conclusion that hypotension in KO mice was caused by loss of $\alpha 1A/C$ -mediated vasoconstriction and consequent reduction of vasomotor tone.

Normal cardiovascular structure in $\alpha 1A/C$ KO mice was noteworthy, given the results from rat models implicating the $\alpha 1A/C$ receptor in cardiac hypertrophy (12). The $\alpha 1A/C$ KO did not alter the normal increase in cardiac size with development, in agreement with an $\alpha 1A/C$ transgenic model with unaltered heart size (22). Adult heart size is also normal in $\alpha 1B$ KO mice (9). Therefore, single knockout of $\alpha 1$ -AR subtypes does not alter normal growth of the heart during postnatal development. However, double knockout of the $\alpha 1A/C$ and $\alpha 1B$ receptors does cause a smaller adult heart (T. D. O'Connell, S. Ishizaka, P. M. Swigart, A. Nakamura, M. C. Rodrigo, S. Cotecchia, D.G.R., W. Grossman, E. Foster, and P.C.S., unpublished data).

Although heart and artery size were unchanged in the $\alpha 1A/C$ KO, the change of the baroreflex was evidence for chronic hypotension. Baroreflex resetting is a known adaptation to chronic changes in blood pressure (21). A shift of the pressure-rate relation to the left, as found in this study, is characteristic of chronic hypotension (21). Resetting of the baroreflex could explain the blunted reflex tachycardia in response to hypotension in the $\alpha 1A/C$ KO. Hypotension would also be expected to stimulate a reflex increase in sympathetic activity. Two findings suggested increased sympathetic activity in the KO, the significant response to parasympathetic blockade in the KO but not the WT, and the decreased heart rate variability in the KO.

Our results have potential clinical relevance. The degree of blood pressure reduction in $\alpha 1A/C$ KO mice was similar to the effect of nonselective $\alpha 1$ -AR antagonists in clinical hypertension (1, 2). It would be interesting to see whether selective $\alpha 1A/C$ antagonists could reduce blood pressure without unwanted side effects such as orthostatic hypotension. Also, low levels of the $\alpha 1A/C$ receptor in heart, as seen here and in other mouse studies (9), might be an advantage. This might lessen the chance that receptor blockade would cause heart failure, as was seen with nonselective $\alpha 1$ -AR antagonism in the ALLHAT trial (2, 6, 7). The distribution of the $\alpha 1A/C$ in mouse arteries found here is similar to that seen in man (4), suggesting that the mouse might be an appropriate model for blood pressure regulation. On the other hand, mouse and human might differ in $\alpha 1A/C$ expression in heart (23), and it will be important to develop methods to test the effects of chronic blockade limited to $\alpha 1A/C$ receptors.

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