

# Hypotension, Autonomic Failure, and Cardiac Hypertrophy in Transgenic Mice Overexpressing the $\alpha_{1B}$ -Adrenergic Receptor\*

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$\alpha_1$ -Adrenergic receptors ( $\alpha_{1A}$ ,  $\alpha_{1B}$ , and  $\alpha_{1D}$ ) are regulators of systemic arterial blood pressure and blood flow. Whereas vasoconstrictory action of the  $\alpha_{1A}$  and  $\alpha_{1D}$  subtypes is thought to be mainly responsible for this activity, the role of the  $\alpha_{1B}$ -adrenergic receptor ( $\alpha_{1B}AR$ ) in this process is controversial. We have generated transgenic mice that overexpress either wild type or constitutively active  $\alpha_{1B}AR$ s. Transgenic expression was under the control of the isogenic promoter, thus assuring appropriate developmental and tissue-specific expression. Cardiovascular phenotypes displayed by transgenic mice included myocardial hypertrophy and hypotension. Indicative of cardiac hypertrophy, transgenic mice displayed an increased heart to body weight ratio, which was confirmed by the echocardiographic finding of an increased thickness of the interventricular septum and posterior wall. Functional deficits included an increased isovolumetric relaxation time, a decreased heart rate, and cardiac output. Transgenic mice were hypotensive and exhibited a decreased pressor response. Vasoconstrictory regulation by  $\alpha_{1B}AR$  was absent as shown by the lack of phenylephrine-induced contractile differences between *ex vivo* mesenteric artery preparations. Plasma epinephrine, norepinephrine, and cortisol levels were also reduced in transgenic mice, suggesting a loss of sympathetic nerve activity. Reduced catecholamine levels together with basal hypotension, bradycardia, reproductive problems, and weight loss suggest autonomic failure, a phenotype that is consistent with the multiple system atrophy-like neurodegeneration that has been reported previously in these mice. These results also suggest that this receptor subtype is not involved in the classic vasoconstrictory action of  $\alpha_1AR$ s that is important in systemic regulation of blood pressure.

The adrenergic receptor family, which includes 3  $\alpha_1$ , 3  $\alpha_2$ , and 3  $\beta$ -receptor subtypes, is a group of heptahelical G protein-coupled receptors that mediate the effects of the sympathetic nervous system. Extensive effort has been spent in classifying the three known  $\alpha_1$ -adrenergic receptor ( $\alpha_1AR$ )<sup>1</sup> subtypes ( $\alpha_{1A}$ ,  $\alpha_{1B}$ , and  $\alpha_{1D}$ ) via molecular cloning techniques (1–4) and pharmacological analyses (5). The most well characterized cardiovascular regulatory actions associated with  $\alpha_1AR$  activation include the contraction, growth and proliferation of vascular smooth muscle cells (6–9), increased cardiac contractility (10), and regulation of the hypertrophic program in the myocardium (11, 12). In other  $\alpha_1AR$ -expressing tissues such as liver and kidney, the function of these receptors is to regulate metabolic processes (13) and sodium and water reabsorption (14), respectively. These responses are transduced primarily via receptor coupling to the  $G_q$ /phospholipase C pathway (5), which leads to the subsequent activation of downstream signaling molecules including protein kinase C and inositol 1,4,5-trisphosphate.

The progress toward elucidating the distinct regulatory role of each  $\alpha_1$  subtype in the various physiologic responses mentioned above has been constrained by a limited number of subtype-selective agonists and antagonists. This is especially true in the  $\alpha_{1B}$  system where there are no selective agonists or antagonists available. We have alleviated this constraint by examining the unique attributes of the  $\alpha_{1B}AR$  in a transgenic mouse model that exhibits constitutive  $\alpha_{1B}AR$  activity targeted only to tissues that normally express the receptor. The appropriate distribution of receptor overactivity was achieved by using the mouse isogenic  $\alpha_{1B}AR$  promoter (15) to drive the overexpression of a transgene containing cDNAs of either the wild type (W) hamster  $\alpha_{1B}AR$  (3) or the constitutively active mutant forms of the receptor. Two such mutants were employed, a C128F single mutant (S) and a C128F/A204V/A293E triple mutant (T), both of which spontaneously couple to  $G_q$  (16, 17). The systemic expression of constitutively active  $\alpha_{1B}AR$ s in these transgenic mice has already led to the identification of a pathology similar to multiple system atrophy suggesting that overstimulation of these receptors leads to neurodegeneration (18). In the present study, we extend this examination of phenotype to the cardiovascular system. Discrete overexpression of

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<sup>1</sup> The abbreviations used are:  $\alpha_1AR$ ,  $\alpha_1$ -adrenergic receptor;  $\alpha_{1B}AR$ ,  $\alpha_{1B}$ -adrenergic receptor; W, wild type  $\alpha_{1B}AR$ ; S, C128F single mutant  $\alpha_{1B}AR$ ; T, C128F/A204V/A293E triple mutant  $\alpha_{1B}AR$ ; NT, non-transgenic; NK, non-knockout; KO, knockout; ANF, atrial natriuretic factor; MAP, mean arterial pressure; gBw, gram body weight.

constitutive  $\alpha_{1B}$ AR activity in the cardiovascular system makes these mice well suited to address questions regarding  $\alpha_{1B}$ AR regulation of cardiovascular homeostasis. Our findings not only confirm the involvement of the  $\alpha_{1B}$ AR in cardiac hypertrophy but suggest that this subtype is not involved with blood pressure-related vasoconstriction. Rather, the hypotension seems to be a manifestation of autonomic failure and not the result of a direct action of the  $\alpha_{1B}$  subtype in the peripheral vasculature. Understanding the  $\alpha_{1B}$ AR control over these processes and the manifestation of disease will further define the therapeutic potential that would come from the development of  $\alpha_{1B}$ AR-selective antagonists and will have an impact on the future development of novel gene therapies.

#### EXPERIMENTAL PROCEDURES

**Mice**—The generation and genotyping of transgenic mice possessing systemic  $\alpha_{1B}$ AR overactivity has been described elsewhere (18). Tissue-specific distribution of systemic  $\alpha_{1B}$ AR overactivity was achieved by using the mouse  $\alpha_{1B}$ AR gene promoter (15) to drive the overexpression of a transgene containing a cDNA coding for the wild type (W)  $\alpha_{1B}$ AR (3) or the constitutively active single mutant (S) C128F  $\alpha_{1B}$ AR (16) or triple mutant (T) C128F/A204V/A293E  $\alpha_{1B}$ AR (17). The Cleveland Clinic Foundation Transgenic Core Facility injected ~200 copies of each transgene into the pronuclei of one cell B<sub>6</sub>/CBA mouse embryos, which were surgically implanted into pseudo-pregnant female mice. 3 W, 5 single mutant, and 3 triple mutant founder mice were identified, and subsequent generations were genotyped by Southern analysis of genomic DNA extracted from tail biopsies. All phenotypic studies detailed below are carried out using equal proportions of male and female mice.

**Echocardiography**—Echocardiographic measurements were performed on mice according to a previously published transthoracic echocardiographic method (19). The mice were anesthetized via intraperitoneal injection of 0.05 mg/g ketamine HCl and 0.1 mg/g thiobutabarbital. The chest area was shaved and ultrasonic gel was applied. Measurements were made as previously described using the Acuson Sequoia 512 system (Mountain View, CA) that employed a dynamically focused symmetrical annular array transducer (13 MHz) for two-dimensional and M-mode imaging. The parasternal long and short axes and apical four chamber views were visualized. Five consecutive cycles of each parameter were measured. Cardiac output was calculated from echocardiographic data using the following equation,  $[\pi \times (\text{PA})^2 \times \text{VTI} \times \text{HR}] / 4$ , where PA was the diameter of the pulmonary artery, VTI was the doppler velocity time integral in the pulmonary artery, and HR was the echocardiographically determined heart rate.

**Mean Carotid Artery Pressure in the Conscious Mouse**—The measurement of the mean carotid artery blood pressure in conscious mice was performed as described previously (20). The mice were anesthetized via intraperitoneal injection of 0.1 mg/g ketamine and 2  $\mu$ g/g acepromazine maleate. A carotid catheter was inserted and connected to a low compliance COBE CDXIII pressure transducer (Cobe Cardiovascular, Arvada, CO). Blood pressure readings were collected on a Model 7D Polygraph (Grass Instrument Division, West Warwick, RI). The recording began immediately after surgery and continued for a 7-h period.

**Mean Femoral Artery Pressure in the Anesthetized Mouse**—The mean femoral artery pressure was determined in mice using a modified version of a previously described method (21). The mice were anesthetized with an initial intraperitoneal injection of ketamine (0.05 mg/g) followed 5 min later by an injection of thiobutabarbital (0.1 mg/g). Supplemental doses of thiobutabarbital were delivered only when necessary to maintain stable anesthesia. A femoral catheter was inserted and connected to a low compliance COBE CDXIII pressure transducer (Cobe Cardiovascular) interfaced with an AH 60-9315 universal oscillograph (Harvard Apparatus, Holliston, MA). The right femoral vein was cannulated similarly for intravenous administration of increasing amounts of phenylephrine at a delivery rate of 0.1  $\mu$ l/g/min using a microinfusion pump.

**Ex Vivo Arterial Contractile Studies**—Four strains of mice were used for this experiment. The  $\alpha_{1B}$  knockout mouse (KO, C57-black) and its non-knockout control (NK mice) were bred in Glasgow from breeding pairs supplied by Professor S. Cotechia (University of Lausanne, Lausanne, Switzerland) (for review see Ref. 8). Tissues were also taken from the W2  $\alpha_{1B}$ AR overexpressor in this study and its appropriate non-transgenic control (NT mice). Mice weighing 25–35 g (KO and their age-matched controls) or 35–55 g (overexpressed and their age-matched

controls) were killed by an overdose of CO<sub>2</sub>. The mesentery was removed, and the branches of first order mesenteric artery were dissected and cleared of connective tissue before mounting on a wire myograph for isometric force recording. The arteries were bathed in Krebs solution, and the temperature was maintained at 37 °C at a pH of 7.4 with a gas mixture of 95% O<sub>2</sub>, 5% CO<sub>2</sub> throughout the experiment. Preliminary studies employed the normalization technique of Mulvany and Halpern (22) in 1977 to obtain vessel internal diameter and normalized resting tension. Thereafter, the vessel segments were set at 0.17 mg of resting tension before construction of concentration response curves. After a “priming” protocol involving challenges of norepinephrine (10  $\mu$ M) and/or KCl (50 mM), tissues were washed and allowed 30 min before beginning construction of a cumulative concentration response curve to phenylephrine (1–10  $\mu$ M). The potency of the agonist was determined by comparing EC<sub>50</sub> values (concentration required to produce 50% of maximum response) obtained in each tissue.

**Catecholamine and Cortisol Determination**—Mice were anesthetized via intraperitoneal injection of thiobutabarbital (0.125 mg/g). An abdominal incision was made, and blood samples were obtained via venipuncture of the vena cava either 5 min after application of the anesthetic or after 1 h of stable anesthesia. Total plasma epinephrine and norepinephrine levels were determined in 100  $\mu$ l of plasma samples using the commercially available plasma catecholamines by high pressure liquid chromatography kit (Bio-Rad). Plasma cortisol levels were determined in parallel in 100  $\mu$ l of plasma samples using the commercially available fluorescence polarization immunoassay kit (Abbott).

**Statistical Analyses**—All reported errors and error bars represent S.E., and significance was determined using either an unpaired two-tailed Student's *t* test (*p* < 0.05) or a one-way analysis of variance (see Figure and Table Legends).

#### RESULTS AND DISCUSSION

**General Characterization of Mice Possessing  $\alpha_{1B}$ AR Overactivity**—We have previously described the genotypic and initial phenotypic analysis of systemic  $\alpha_{1B}$ AR mice (18), confirming transgene integration. The tissue-specific overexpression of wild type and mutant  $\alpha_{1B}$ ARs was confirmed via saturation binding analysis of various tissues from F1 and F2 generation of heterozygous mice. Of the seven transmitting founder lines, five exhibited significant transgene overexpression including two W lines (W1 and W2), one single mutant line (S1), and two triple mutant lines (T1 and T2). The distribution and magnitude of receptor overexpression were not significantly different among the various lines as expected for the housekeeping nature of the promoter. The level of  $\alpha_{1B}$ AR overexpression was ~2-fold in the heart with greater overexpression seen in the liver, lung, brain, and spleen (18). Confirming constitutive signaling of these overexpressed receptors in the transgenic lines, inositol 1,4,5-trisphosphate levels were significantly higher in kidneys from W2+/-, S1+/-, and T2+/- mice than in age-matched NT mice (Fig. 1). Similar constitutive stimulation of inositol 1,4,5-trisphosphate metabolism has been previously shown in the liver (18). The rank order increase in inositol 1,4,5-trisphosphate pool size seen among the various lines (T2 > S1 > W2) corresponds with the strength of constitutive signaling that was found for these receptors *in vitro* (16, 17).

It should be noted that when bred to homozygosity, mice overexpressing constitutively active mutant forms of the  $\alpha_{1B}$ AR (S1 and T2) displayed reproductive problems. This was not seen in the W2 line, suggesting that reproductive failure was unlikely the result of breeding artifacts. Therefore, all phenotypic analyses were performed on heterozygotes. All transgenic lines also displayed a 20–30% reduction in body weight, but this was only apparent in older mice that were more than 12 months of age (18).

**Cardiac Hypertrophy in Mice Possessing  $\alpha_{1B}$ AR Overactivity**— $\alpha_{1A}$ ARs have been shown to evoke a hypertrophic response in cultured cardiac myocytes (23, 24) with the regulation of this process predominated by the  $\alpha_{1A}$  subtype (25, 26). Because

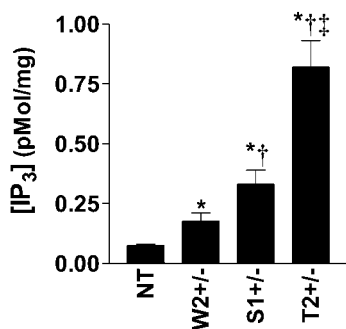


FIG. 1. IP<sub>3</sub> levels were determined in kidneys from 6-month-old NT, W2+/-, S1+/-, and T2+/- mice as described under "Experimental Procedures" ( $n = 3$ ). The asterisk indicates the significance from the NT group. The dagger indicates significant increases compared with the W2+/- group. The double dagger indicates significant increases compared with the S1+/- group. Significance was determined using a two-tailed Student's  $t$  test ( $p < 0.05$ ).

myocardial-targeted overexpression of constitutively active  $\alpha_{1B}$ ARs has also been shown to cause cardiac hypertrophy in mice (11), we performed morphologic and echocardiographic analyses in the context of our systemic transgenic model. Indicative of a hypertrophic phenotype, W2, S1, T1, and T2 mice showed an increased heart to body weight (*heart/Bw*) ratio compared with age-matched (4–6 months) NT control mice (Fig. 2). Body weight was not significantly different among the lines at 4–6 months of age. It should be noted that other organs including the liver, kidneys, lungs, and brain did not exhibit a change in mass relative to body weight (data not shown). Increases in heart mass ranged between 12 and 41% with S1 mice showing the largest increase. These findings were confirmed echocardiographically in W2, S1, and T2 mice, which showed an increased thickness of the posterior wall and interventricular septum compared with age-matched NT control mice (Table I).

Molecular confirmation of cardiac hypertrophy was attempted by measuring *ANF* message levels via Northern blot analysis of poly(A) mRNA purified from 8-month-old NT and T2 mouse hearts. *ANF*, a gene often associated with cardiac hypertrophy (27), was not up-regulated in T2 mice relative to the NT controls (data not shown), suggesting that the morphologic and echocardiographic findings are indicative of an early stage hypertrophy. Besides our model, the hypertrophic cardiomyopathy mouse (28) also shows hypertrophy in the absence of *ANF* up-regulation, suggesting that the progression of cardiac hypertrophy is not always strictly associated with the up-regulation of *ANF* (29) and/or other fetal genes. Another more likely reason for the lack of *ANF* up-regulation is the low level of  $\alpha_{1B}$ AR overexpression present in our model. For example, the *Gaq* overexpression mouse model of cardiac hypertrophy (30) displayed no change in *ANF* expression with a 2-fold increase in the *Gaq* protein, a circumstance similar to the 2-fold overexpression of cardiac  $\alpha_{1B}$ ARs in our mice. However, a 4-fold increase in *Gaq* was sufficient to invoke *ANF* transcription. These findings collectively indicate that a threshold of expression may be necessary to evoke changes in fetal gene transcription.

Despite an increased ventricular diameter in both diastole and systole, the cardiac output in the transgenic lines was lower than that seen in NT mice (Table I). This probably is attributed to the decreased heart rate and increased isovolumetric relaxation time displayed by transgenic animals (Table I). The decreased heart rate, which was confirmed via a tail cuff measurement in conscious mice (Table I), may be the result of a direct effect on Purkinje fiber automaticity, which is thought to be controlled by the  $\alpha_{1B}$ AR (31) and is consistent with the overexpression of the receptor. A similar decrease in heart rate

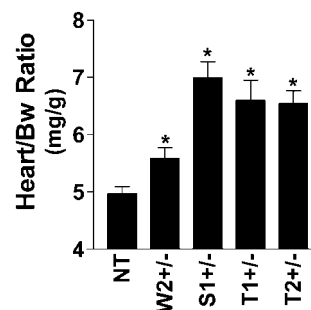


FIG. 2. Heart to body weight (*Heart/Bw*) ratios were calculated in NT, W2+/-, S1+/-, T1+/-, and T2+/- mice at 4–6 months of age ( $n > 12$  for each line). The asterisk indicates the significance from the NT group based on a two-tailed Student's  $t$  test ( $p < 0.05$ ).

was also found in the heart-targeted *Gaq*-overexpressing mouse (30). Overall, because  $\alpha_{1B}$ ARs are coupled to *Gaq*, the decrease in heart rate may be directly related to signaling events downstream of  $\alpha_{1B}$ AR activation, or it may be part of the autonomic dysfunction, which we describe later.

Interestingly, a robust myocardial overexpression (>40-fold) of the wild type  $\alpha_{1B}$ AR has been shown to cause increased diacylglycerol content and *ANF* mRNA without inducing the morphological hallmarks of hypertrophy (12). One conclusion that can be drawn from this earlier study is that only constitutively active  $\alpha_{1B}$ ARs can induce hypertrophy. This raises the possibility that constitutively active receptors may signal through different pathways than wild type receptors. However, in arguing against this possibility, modest developmental and tissue-specific overexpression (2-fold in the heart) of wild type  $\alpha_{1B}$ ARs in our mice caused a cardiac hypertrophy that was less robust but similar to that seen in the heart-targeted constitutively active  $\alpha_{1B}$ AR mouse. Unlike the heart-targeted model, our model may be exhibiting a phenotype that more genuinely represents the end point impact of  $\alpha_{1B}$ AR action in the heart because our use of the isogenic  $\alpha_{1B}$ AR promoter facilitates transgene overexpression in all  $\alpha_{1B}$ AR-expressing cardiac cell types, not just cardiac myocytes. Overall, because several experimentally distinct approaches to genetically induce  $\alpha_{1B}$ AR overactivity have independently led to the manifestation of a somewhat similar cardiac phenotype, the emergence of that phenotype must be  $\alpha_{1B}$ AR-dependent and not simply the spurious outcome of transgenic manipulation. Based on this assumption, we assert that in addition to the  $\alpha_{1A}$ AR, the  $\alpha_{1B}$ AR plays an important regulatory role in the progression of the hypertrophic program in cardiac tissue.

**Hypotension in Mice Possessing  $\alpha_{1B}$ AR Overactivity**—The  $\alpha_{1}$ ARs are widely expressed in the peripheral arteries (4, 5) and possess the capacity to regulate vasoconstriction (32–36), thus implicating them in the control of blood pressure. Regarding the  $\alpha_{1B}$ AR, however, the bulk of the literature suggests that this subtype does not play a significant role in the direct regulation of the peripheral vascular tone (5, 9). Rather, the predominant role of  $\alpha_{1B}$ ARs expressed in the vasculature has been proposed to include the regulation of growth and metabolic activity (6, 37–39). Contrary to these studies, however, the  $\alpha_{1B}$ AR knockout mouse showed a decreased pressor response to phenylephrine infusion (8), which indicates the participation of the receptor in the regulation of peripheral vasoconstriction. Based on these findings from the knockout model, if  $\alpha_{1B}$ ARs participate in vasoconstriction, our constitutively active  $\alpha_{1B}$ AR mice should display hypertension because of the constitutive activation of this process. However, our mice displayed the opposite phenotype, a significantly reduced systemic arterial blood pressure.

4–6-month-old S1 and T2 mice were hypotensive relative to



TABLE I  
Echocardiographic analyses

12-month-old mice were anesthetized with 0.05 mg/gBw ketaset and 0.1 mg/gBw inactin, and the chest area was shaved and swabbed with ultrasound gel. Several cardiac parameters were echocardiographically determined including interventricular septal thickness (IVS), posterior wall thickness (PW), left ventricular internal dimension in diastole (LVIDd) and in systole (LVIDs), isovolumetric relaxation time (IVRT), and heart rate (HR (echo)). For comparison, HR was also determined in conscious mice via a tail cuff. (HR (cuff)), IVS, PW, LVIDd, and LVIDs were normalized to body weight and percent fractional shortening (%FS) was calculated as  $100 \times [(LVIDd - LVIDs)/LVIDd]$ . Cardiac output (CO) was calculated as described under "Experimental Procedures." The asterisks denote significance from NT ( $n \geq 5$ ) based on a two-tailed Student's  $t$  test ( $p < 0.05$ ).

	Wall thickness		Left ventricle dimensions			Heart function			
	IVS	PW	LVIDd	LVIDs	%FS	IVRT	HR (echo)	HR (cuff)	CO
	mm/gBw	mm/gBw	mm/gBw	mm/gBw		ms	beats/min	beats/min	ml/min
NT	$0.031 \pm 0.002$	$0.027 \pm 0.001$	$0.074 \pm 0.002$	$0.028 \pm 0.002$	$62.2 \pm 1.2$	$15.70 \pm 1.2$	$573 \pm 53$	$681 \pm 67$	$24.0 \pm 3.3$
W2+/-	$0.036 \pm 0.002$	$0.034 \pm 0.001^*$	$0.113 \pm 0.011^*$	$0.057 \pm 0.010^*$	$49.6 \pm 6.2$	$25.50 \pm 1.7^*$	$344 \pm 42^*$	$607 \pm 46^*$	$10.4 \pm 1.2^*$
S1+/-	$0.040 \pm 0.002^*$	$0.034 \pm 0.001^*$	$0.102 \pm 0.003^*$	$0.044 \pm 0.006^*$	$57.9 \pm 5.0$	$29.17 \pm 3.1^*$	$337 \pm 16^*$	$590 \pm 63^*$	$18.3 \pm 1.1$
T2+/-	$0.041 \pm 0.002^*$	$0.039 \pm 0.003^*$	$0.100 \pm 0.008^*$	$0.049 \pm 0.008^*$	$51.2 \pm 4.6$	$27.06 \pm 3.2^*$	$382 \pm 41^*$	$588 \pm 55^*$	$15.2 \pm 1.8^*$

age-matched NT control mice. Whereas T2 mice showed modest hypotension while still under the influence of the anesthetic agents (Fig. 3, A and B), fully conscious and unrestrained S1 and T2 mice showed a more significant decrease in basal pressure compared with the NT control (Fig. 3, A and C). The mean arterial pressure in conscious W2 mice was lower than that in NT animals (Fig. 3C); however, this was not statistically significant. Confirming these measurements made in conscious animals, the basal mean femoral artery pressure was also significantly lower in 4-month-old anesthetized S1 mice than in age-matched NT control mice (Fig. 4A). Overall, our observation of basal hypotension in constitutively active  $\alpha_{1B}$ AR mice contradicts the idea that activation of the  $\alpha_{1B}$ AR can induce vasoconstriction and is the first report to indicate that an  $\alpha_1$ AR can affect resting arterial blood pressure. It should be noted that although all transgenic lines demonstrated a hypertrophic phenotype in the heart, only the two constitutively active lines (S1 and T2) demonstrated hypotension. This was probably attributed to the intermediate level of constitutive signaling (see Fig. 1) and variability in the data collected from W2 mice. For example, some parameters of hypertrophy were not significant for the W2 line, and blood pressure was reduced but was highly variable and not significant.

To extend these findings, we compared the potency of the  $\alpha_1$ AR-selective agonist phenylephrine to evoke a pressor response in NT and transgenic mice. Phenylephrine produced a dose-dependent increase in systemic arterial blood pressure in the NT and all transgenic groups. The pressor response in the transgenic group was no greater than that seen in NT animals. Indeed the pressor dose response curve in transgenic animals was shifted to the right of that seen in NT mice (Fig. 4B), arguing that the  $\alpha_{1B}$ AR does not transduce the phenylephrine pressor response. This rightward shift seen in the transgenic lines was probably because of the decrease in basal blood pressure. It should be noted that the dose-response curves could not be completed to saturation because of the lethal effect of high doses of phenylephrine. Because the expression of  $\alpha_{1B}$ ARs has been identified in peripheral arteries via the use of an  $\alpha_{1B}$ -specific antibody (36), these results suggest that vascular  $\alpha_{1B}$ ARs are not directly involved with the regulation of vasoconstriction.

To confirm that the  $\alpha_{1B}$ AR is not directly involved in blood pressure regulation either via vasoconstriction or somehow via a negative influence on the pressor response (i.e. vasodilation, Fig. 4B), contractile-response curves were generated using *ex vivo* segments of the mesenteric artery prepared from several lines of mice. The vasoconstrictory action of phenylephrine was tested in artery segments from  $\alpha_{1B}$ AR knockout mice (8) from our W2 line of mice and from the appropriate non-knockout and non-transgenic control groups. The phenylephrine dose response curves generated for each of these groups were not

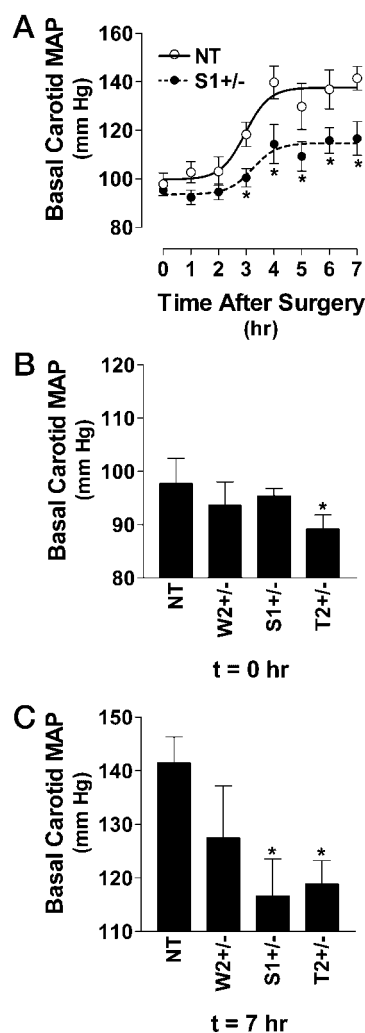


FIG. 3. Mean carotid pressure (Basal Carotid MAP) was determined under basal conditions in conscious NT, W2, S1, and T2 mice via an in-dwelling catheter as described under "Experimental Procedures." A, time course of carotid MAP in NT (open circles) and S1 (closed circles) recovering from anesthesia ( $n > 8$  for each point). B, a summary of carotid MAP in NT, W2, S1, and T2 mice immediately after surgery while still under anesthesia ( $n > 8$  for each line). C, a summary of carotid MAP in fully conscious NT, W2, S1, and T2 mice 7 h after surgery ( $n > 8$  for each line). The asterisks in each part of the figure indicate the significance from the NT group based on a two-tailed Student's  $t$  test ( $p < 0.05$ ).

significantly different from each other (Fig. 5), demonstrating that the  $\alpha_{1B}$ AR does not participate in blood pressure-related vasoconstriction and confirming that the hypotension seen in our transgenic animals is not the result of an arterial event.

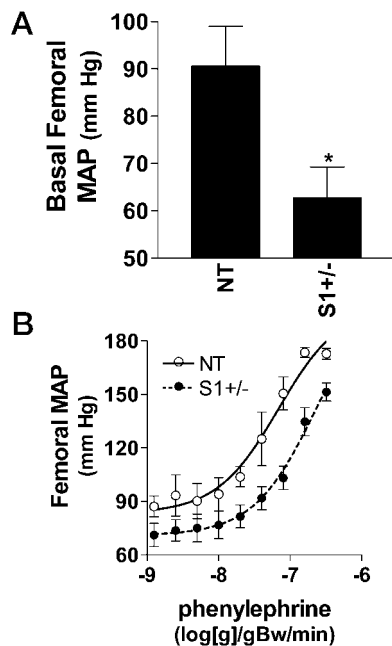


FIG. 4. Mean femoral artery pressure (Basal Femoral MAP) was determined in NT and S1 mice via an in-dwelling catheter under basal conditions and following intravenous presentation of phenylephrine as described under "Experimental Procedures." A, basal femoral MAP in NT and S1 mice under anesthesia ( $n > 6$  for each line). The asterisk indicates the significance from the NT group based on a two-tailed Student's  $t$  test ( $p < 0.05$ ). B, phenylephrine dose effect on femoral MAP in NT (open circles) and S1 (closed circles) mice under anesthesia ( $n > 6$  for each line). Dose response data was analyzed using the non-linear regression functions of the non-iterative curve fitting program GraphPad Prism.

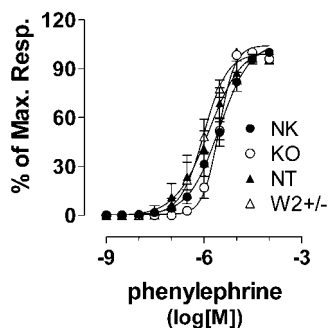


FIG. 5. Concentration-response curves for the  $\alpha_{1A}$ AR agonist phenylephrine in isolated segments of mouse mesenteric artery (first order branches, external diameter 200–220  $\mu$ m) taken from non-knockout (NK, closed circles),  $\alpha_{1B}$ AR knockout (KO, open circles), non-transgenic (NT, closed triangles), and W2 transgenic mice (W2, open triangles). Data points represent the mean for each group ( $n = 11$  for NK and  $n = 5$  for KO, NT, and W2 each).  $\log(EC_{50})$  ( $\log[M]$ ) values were  $-5.53$  for NK mice,  $-5.54$  for KO mice,  $-5.84$  for NT mice, and  $-6.0$  for W2 mice. Dose response data were analyzed using the non-linear regression functions of the non-iterative curve fitting program GraphPad Prism. Groups were determined to not be significantly different from each other based on a one-way analysis of variance.

Because of the apparent lack of direct  $\alpha_{1B}$ AR control over vasoconstriction, the question remains how does systemic  $\alpha_{1B}$ AR overactivity lead to a reduction in blood pressure? It is well established that peripheral vascular tone is partially regulated by sympathetic nervous system activity (40). Lower sympathetic activity, as measured by a reduction of plasma catecholamines, could lead to a lower blood pressure because of a reduced activation of all vascular  $\alpha_1$ -adrenergic targets. This hypothesis was tested by assessing sympathetic function via

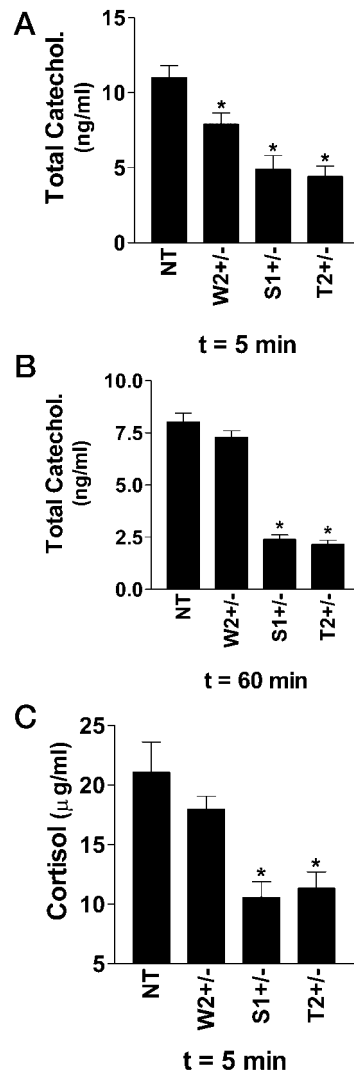


FIG. 6. Total plasma epinephrine and norepinephrine levels in NT, W2, S1, and T2 mice were determined as described under "Experimental Procedures" either 5 min after application of anesthesia (A) or after 1 h of stable anesthesia (B) ( $n = 5$  for each line). (C) total plasma cortisol levels in NT, W2, S1, and T2 mice were also determined 5 min after application of anesthesia as described under "Experimental Procedures" ( $n = 3$  for each line). The asterisks in each part of the figure indicate the significance from the NT group based on a two-tailed Student's  $t$  test ( $p < 0.05$ ).

the measurement of total blood levels of norepinephrine, epinephrine, and cortisol in the transgenic lines. Indicating reduced sympathetic output in transgenic animals, 6-month-old S1 and T2 mice showed a roughly 50% reduction of total blood catecholamine compared with age-matched NT control mice (Fig. 6A). A similar reduction in total catecholamines was seen after a 1-h period of stable anesthesia (Fig. 6B), suggesting that the reduction was not a result of indirect effects of anesthesia or of altered reactivity/stress induced by handling. It should be noted that catecholamine levels seen in our mice (ng/ml) are in the same range as those reported in other transgenic mouse models (41). As expected, because cortisol is released from the adrenal medulla in response to sympathetic stimulation, we also found a corresponding reduction in plasma cortisol (50%) in S1 and T2 mice relative to the NT control (Fig. 6C). Because the reduced plasma catecholamine and cortisol levels were correlated to blood pressure effects, it is possible that these two events may be linked. These data suggest that the hypotension seen in our transgenic mice may be, at least in part, because of a reduction in the sympathetic nerve activity. It is also possible

that the decrease in heart rate and cardiac output may also contribute to the hypotension seen in our transgenic mice. This possibility does not seem probable given the fact that W2 mice, which displayed a reduced heart rate and cardiac output, were not hypotensive.

Our transgenic  $\alpha_{1B}$ AR mice display a Parkinsonian-like syndrome termed multiple system atrophy with associated neurodegeneration in the substantia nigra, olive pontine, thalamus, and locus coeruleus (18). Symptomatically, the presence of multiple system atrophy often involves autonomic failure because of these extensive neurodegenerative lesions in the brain. Therefore, a probable reason for the hypotension seen in our mice is a lowered sympathetic output caused by autonomic dysfunction. Although some patients with autonomic failure have hypertension (42), autonomic neuropathy is a common cause of orthostatic hypotension (43) and is also responsible for the hypotension commonly seen in Parkinson's disease and multiple system atrophy patients (44). Accordingly, these patients also exhibit low plasma levels of norepinephrine (44). Besides hypotension, our transgenic mice displayed reproductive problems, weight loss (at 12 months of age), bradycardia, depressed heart function, and low cortisol and catecholamine levels that are all associated with autonomic dysfunction. Autonomic failure produces distinct abnormalities depending upon the location of the lesions (44). Therefore, our model is probably the outcome of autonomic dysfunction that is caused by  $\alpha_{1B}$ AR-induced neurodegeneration.

Overall, our analysis of  $\alpha_{1B}$ AR control of blood pressure from a systemic perspective has led us to conclude that  $\alpha_{1B}$ AR overactivity does not cause an elevation in pressure but rather induces a net hypotension. The mechanism driving this hypotension is probably rooted in an autonomic failure because many of the symptoms displayed by our mice are consistent with this diagnosis. The data presented in this study are also counterindicative of a vasoconstrictive role for  $\alpha_{1B}$ ARs. Our findings support an emerging hypothesis, which predicts that  $\alpha_{1B}$ ARs do not play a major role in contractile regulation in vascular smooth muscle (9) but rather are predominately coupled to various metabolic and cellular processes at vascular sites where the receptor is expressed (6, 37–39). This realization has important implications in the pharmacotherapeutic approach to the manipulation of blood pressure.

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