duction in receptor density (Fig. 2), or from a combination of these effects. Our results and those of Link *et al.* (*11*) indicate that subtype-selective ligands might provide a therapeutic advantage in the treatment of hypertension.

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10. Agonist competition for [^{3}H]RX 821002 was performed (*13*). Median effective concentration (EC_{50}) values in the absence of Na^+ were as follows for epinephrine: wild-type, 1.6 μM; D79N, 0.5 μM; and for dexmedetomidine: wild-type, 37.5 nM; D79N, 3.6 nM. The potency of agonists was reduced by the inclusion of Na^+ in the binding incubations for the wild-type α_{2a}AR but not for the D79N α_{2a}AR (*9*).
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13. Mouse brains (mice were about 2 months old) were homogenized in hypotonic lysis buffer (*14*) and membranes were resuspended so that final assay conditions were 25 mM glycylglycine, 40 mM Hepes (pH 8), 5 mM EGTA, 5 mM EDTA, 5 mM $MgCl_2$, 100 μM 5′-guanylylimidodiphosphate, and 1 μM prazosin. Membranes (1 to 1.5 mg of protein per incubation, as estimated with the Bradford assay) were incubated with various concentrations of [^{3}H]RX 821002 and 100 mM NaCl or 100 mM *N*-methyl-D-glucamine (pH 8) at 25°C for 60 min. The maximum binding capacity (B_{max}) and dissociation constant (K_d) values reported in the figure were in the presence of Na^+. In the absence of Na^+, values were as follows for B_{max} (fmol of [^{3}H]RX 821002 bound per milligram of protein): wild-type, 210.8 ± 13.6; D79N, 43.2 ± 2.3; and for K_d (nM): wild-type, 2.9 ± 0.3; D79N, 2.8 ± 0.3. Competition studies were done with 6 nM [^{3}H]RX 821002. Nonspecific binding was defined as binding not displaced by 10 μM phentolamine (*14*). Nonlinear regression fit of the data was done with GraphPad Prism software.
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19. A region of the α_{2a}AR coding sequence [base pairs (bp) 113 to 385] from wild-type and D79N mouse tail DNA was amplified by polymerase chain reaction (PCR) and subcloned into pBluescriptII SK(+). Multiple transformants were sequenced with Sequenase 2.0 (USB).
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23. For carotid catheterization, male mice (2 to 3 months old) were anesthetized with halothane (1.75% v/v in O_2), and a PE-10 polyvinyl catheter tubing was inserted into the left common carotid artery (*11*). Mice recovered for 18 to 24 hours before the catheter was connected to a Gould amplifier, by a Gould-Statham pressure transducer, for hemodynamic measurements. Systolic, diastolic, and mean arterial blood pressures, and heart rate were recorded on a microcomputer equipped with DataFlow software (Crystal Biotech). α_2AR agonists were injected directly into the arterial catheter in a 10-μl bolus. For the femoral catheter experiments, a Microrenathane (0.064 cm outer diameter, 0.030 cm inner diameter cannula; Braintree Scientific) was inserted into the femoral artery of anesthetized male mice (2 to 3 months old). Mice recovered for 4 to 6 hours before arterial blood pressure was recorded on a Grass Model 7 polygraph by means of a Cobe blood pressure transducer. Heart rate was calculated from blood pressure tracings. UK 14,304 was injected into the arterial catheter; each sequential dose of UK 14,304 was injected after the blood pressure of wild-type mice had returned to baseline or after a comparable time (about 5 min) for D79N mice. Vehicle injection had no effect on the recorded cardiovascular responses.

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Cardiovascular Regulation in Mice Lacking α_2-Adrenergic Receptor Subtypes b and c

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α_2-Adrenergic receptors (α_2ARs) are essential components of the neural circuitry regulating cardiovascular function. The role of specific α_2AR subtypes (α_{2a}, α_{2b}, and α_{2c}) was characterized with hemodynamic measurements obtained from strains of genetically engineered mice deficient in either α_{2b} or α_{2c} receptors. Stimulation of α_{2b} receptors in vascular smooth muscle produced hypertension and counteracted the clinically beneficial hypotensive effect of stimulating α_{2a} receptors in the central nervous system. There were no hemodynamic effects produced by disruption of the α_{2c} subtype. These results provide evidence for the clinical efficacy of more subtype-selective α_2AR drugs.

α_2ARs have a prominent role in the cardiovascular system and influence vascular tone at multiple points in a complex reflex arc. α_2ARs located in the brain stem are targets for antihypertensive therapy because stimulation of these receptors produces a long-lasting drop in systemic blood pressure. Paradoxically, stimulation of α_2ARs on arterial smooth muscle cells increases blood pressure by increasing vascular resistance. Three subtypes of α_2AR (α_{2a}, α_{2b}, and α_{2c}) have been isolated and share a high degree of structural similarity (50 to 60% identity). All these receptors couple to the inhibitory heterotrimeric GTP-binding protein (G_i) and inhibit adenyl cyclase. The three α_2ARs differ, however, in their patterns of tissue expression (*1*). Little is known about the role of the three α_2AR subtypes in cardiovascular physiology. Studies have been hampered both by the lack of subtype-

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selective α_2AR drugs and by cross-reactivity of α_2AR ligands with other receptors such as α_1ARs and the recently defined imidazoline-preferring sites (I_1 and I_2) (*2*). As an alternative approach, we have generated lines of knockout mice deficient in either the α_{2b} or α_{2c} subtype and measured the hemodynamic responses of these mutants to stimulation by α_2-selective agonists. Our results, together with those of MacMillan *et al.* (*3*), demonstrate that stimulation of α_{2b} receptors counteracts the therapeutic antihypertensive effect of drugs acting at α_{2a} receptors in the central nervous system.

Three α_2AR genes have been isolated in the mouse (*Adra2a*, *Adra2b*, and *Adra2c*) that encode homologs of the human α_{2a}, α_{2b}, and α_{2c} receptor subtypes (*4*, *5*). We produced *Adra2c*$^-$/*Adra2c*$^-$ mice by gene targeting in murine embryonic stem cells (*6*). Despite their deficiency in the α_{2c} receptor subtype, adult *Adra2c*$^-$/*Adra2c*$^-$ mice are viable, fertile, and appear grossly normal. We used a similar strategy to generate mice lacking a functional copy of the *Adra2b* gene, which encodes the murine α_{2b} pharmacologic subtype. Viable *Adra2b*$^-$/*Adra2b*$^-$ mice were recovered from heterozygote intercrosses and were fertile and appeared grossly normal. However, significantly fewer *Adra2b*$^-$/*Adra2b*$^-$ animals were produced from these crosses than predicted by Mendelian ratios (*7*). Protein immunoblot and ligand-binding studies confirmed the absence of α_{2b} expression in *Adra2b*$^-$/*Adra2b*$^-$ mice (Fig. 1). These results demonstrate that the α_{2b} receptor is not absolutely required for embryonic development or for adult survival.

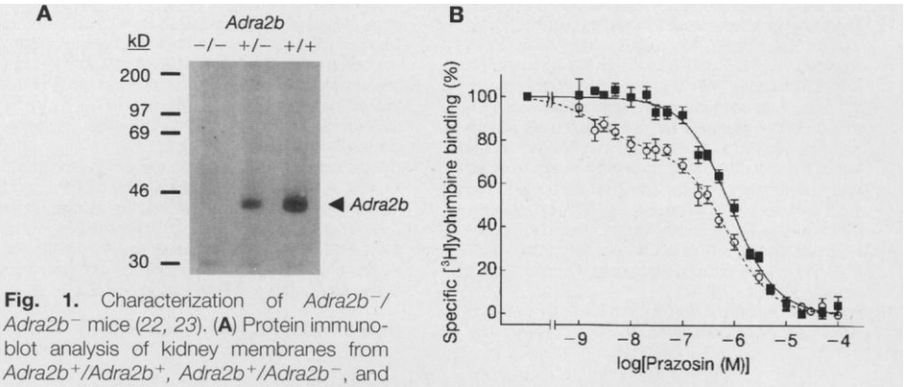

Fig. 1. Characterization of *Adra2b*$^-$/*Adra2b*$^-$ mice (*22*, *23*). (**A**) Protein immunoblot analysis of kidney membranes from *Adra2b*$^+$/*Adra2b*$^+$, *Adra2b*$^+$/*Adra2b*$^-$, and *Adra2b*$^-$/*Adra2b*$^-$ mice (*24*). The genotype of each animal and the position of the nonglycosylated *Adra2b* protein are shown. (**B**) Competition of [^{3}H]yohimbine binding to kidney membranes from *Adra2b*$^+$/*Adra2b*$^+$ (○) and *Adra2b*$^-$/*Adra2b*$^-$ (■) mice by prazosin (*25*). Each point represents the mean (± SE) derived from three mice. Specific α_2 receptor binding in kidney membranes of *Adra2b*$^-$/*Adra2b*$^-$ mice was decreased to 62% of that in membranes from control mice. Competition binding experiments revealed two prazosin-sensitive binding sites in *Adra2b*$^+$/*Adra2b*$^+$ mice, a high-affinity site (0.58 nM; 24% of binding) and a low-affinity site (>750 nM; 76% of binding). The high-affinity prazosin-sensitive α_2AR site (α_{2b} subtype) was absent in membranes from *Adra2b*$^-$/*Adra2b*$^-$ mice.

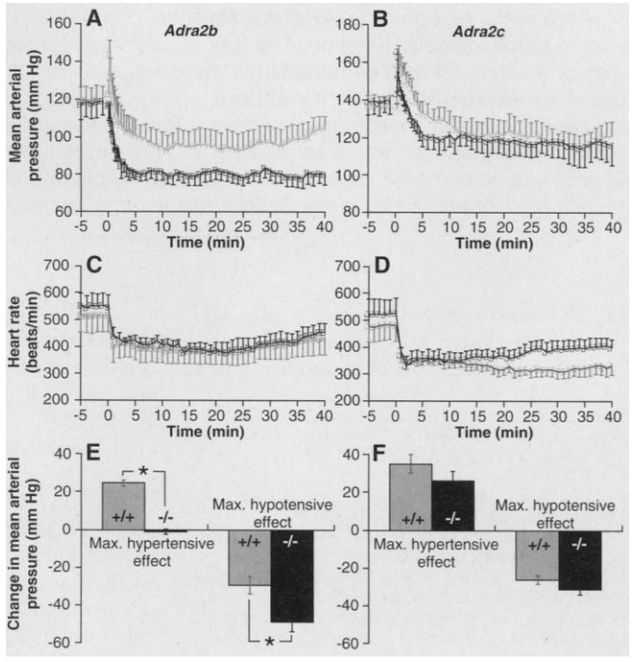

Fig. 2. Representative hemodynamic measurements for control (gray symbols), *Adra2b*$^-$/*Adra2b*$^-$ (filled squares), and *Adra2c*$^-$/*Adra2c*$^-$ (filled circles) mice (*13*). Representative tracings of mean arterial blood pressure (**A** and **B**) and heart rate (**C** and **D**) (± SE) after administration of dexmedetomidine. At time 0, a bolus of dexmedetomidine (5 μg/kg) was administered through the arterial catheter. No response was observed after injection of vehicle alone. Blood pressure and heart rate responses were attenuated by first treating with the α_2-selective antagonist atipamezole (*9*, *15*, *26*). (**E** and **F**) Maximal arterial pressure changes observed during the hypertensive and hypotensive phases of the response to dexmedetomidine. Data were derived independently from 7 *Adra2b*$^+$/*Adra2b*$^+$ and 7 *Adra2b*$^-$/*Adra2b*$^-$ mice [shown in (E)] and 7 *Adra2c*$^+$/*Adra2c*$^+$ and 10 *Adra2c*$^-$/*Adra2c*$^-$ animals [shown in (F)]. Error bars are SEs; values significantly different from appropriate controls are denoted by an asterisk ($P < 0.05$, unpaired Student's t test).

In humans and other species, the blood pressure response to the intravenous administration of an α_2 agonist is biphasic (*8*–*10*). During the initial phase, mean arterial blood pressure rises transiently as arterial α_2ARs constrict vascular smooth muscle (*10*, *11*). After the initial hypertensive response, mean blood pressure drops below baseline because α_2ARs in the ventrolateral medulla oblongata attenuate sympathetic and accentuate parasympathetic outflow (*12*).

To explore the role of specific α_2AR subtypes in regulating these hemodynamic properties, we measured real-time mean blood pressure and heart rate in unrestrained, conscious animals by using an intra-aortic catheter connected to a pressure transducer (*13*). Disruptions of the *Adra2c* or *Adra2b* gene did not result in a significant change in mean arterial pressure or heart rate at baseline. To examine the hemodynamic response to α_2 agonists, we administered dexmedetomidine (5 μg per kilogram of body weight) through the catheter as a bolus. Dexmedetomidine is a highly selective agonist at the α_2 subtype (*14*, *15*). The arterial blood pressure response to dexmedetomidine in wild-type mice (*16*) was biphasic. We observed an immediate hypertensive response, followed by a long-lasting (>60 min) drop in mean arterial pressure to below its original value (Fig. 2, A and B). Dexmedetomidine also caused an immediate bradycardia (Fig. 2, C and D).

In contrast, no hypertensive response to dexmedetomidine was observed in *Adra2b*$^-$/*Adra2b*$^-$ mice. Rather, the hypotensive response occurred immediately and was significantly greater than that observed for control animals (Fig. 2, A and E). The bradycardic response to dexmedetomidine, however, did not differ

significantly between *Adra2b*$^{-}$/*Adra2b*$^{-}$ and control mice (Fig. 2C). *Adra2b*$^{-}$/*Adra2b*$^{-}$ mice had a normal hypertensive response to challenge with a nonselective α_1 agonist (0.1 μg of phenylephrine per kilogram of body weight) (*17*), confirming that resistance vessels in *Adra2b*$^{-}$/*Adra2b*$^{-}$ mice are capable of a normal vasoconstrictive response to α_1AR stimulation. We observed no significant difference between *Adra2c*$^{-}$/*Adra2c*$^{-}$ and control mice in the magnitude of the hypertensive, hypotensive, or bradycardic responses to agonist (Fig. 2, B, D, and F).

Our results show that the central hypotensive response to α_2 agonists is not mediated by the α_{2b} or α_{2c} subtypes. This response is lost after a subtle mutation of the α_{2a} subtype in vivo (*3*). These observations strongly implicate the α_{2a} subtype in the control of central sympathetic outflow. The major component of the α_2-agonist-induced increase in systemic blood pressure appears to be mediated by α_{2b} receptors. The magnitude of the long-lasting hypotensive response to an α_2 agonist in *Adra2b*$^{-}$/*Adra2b*$^{-}$ mice was also significantly greater than that in control animals. This finding suggests that the magnitude of hypotension seen with circulating "central" α_2 agonists such as clonidine actually represents the summation of central and peripheral effects. It may be possible, therefore, to enhance the therapeutic potency of α_2 agonists as antihypertensive drugs by developing compounds with a lower relative affinity for the α_{2b} subtype.

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1. The α_{2c} subtype is expressed primarily in the central nervous system, although small amounts are present in kidney (*18*). The α_{2b} subtype is expressed primarily in the periphery, with the highest amounts in kidney. The α_{2a} subtype is expressed widely throughout both the nervous system and peripheral tissues. The identity of the α_2AR subtypes present on resistance arterioles is unknown.
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13. Mice were anesthetized with inhaled methoxyflurane (1 to 2%). The left carotid artery was isolated and secured through a midline neck incision. An Intramedic PE-10 polyethylene catheter (Clay-Adams, Parsippany, NJ) was stretched to approximately one-half of its original diameter, inserted into the artery, and secured in place with 4-0 silk. The catheter was tunneled to the back of the neck and flushed with heparinized saline, and the end was sealed with glue and buried under the skin for later retrieval. The mice were allowed to recover in a standard rodent cage for at least 12 hours with food and water available ad libitum. For hemodynamic measurements, the arterial catheter was flushed with saline and connected to a Spectramed DTX Plus pressure transducer with a side port for infusing medications. The analog input was amplified with a Gould (Cleveland, OH) model 11-1202-25 preamplifier and model 13-4615-52 amplifier and digitized with a Data Translation (Marlboro, MA) DT2801 analog-to-digital converter. The waveform was analyzed to derive mean blood pressure and heart rate through use of Dataflow data acquisition software (Crystal Biotech, Hopkinton, MA) on a Gateway 2000 486DX2 66 MHz microcomputer (Sioux City, SD). For agonist studies, boluses of dexmedetomidine (5 μg/kg) or phenylephrine (10 μg/kg) were administered through the arterial catheter. For blocking studies, intra-arterial atipamezole (200 μg/kg) was administered 5 min before dexmedetomidine.
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17. In *Adra2b*$^{-}$/*Adra2b*$^{-}$ mice, mean blood pressure increased by 26 ± 3% (n = 5), whereas in *Adra2b*$^{+}$/*Adra2b*$^{+}$ mice mean blood pressure increased by 25 ± 8% (n = 2) after intravascular administration of phenylephrine.
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23. Gene targeting in mouse embryonic stem cells and production of germline chimeric animals was done essentially as described (*6*). The R1 line of murine embryonic stem cells (*19*) was provided by A. Nagy (Mount Sinai Hospital, Toronto, Canada). Southern hybridization was done as described (*4*) with the following ^{32}P-labeled DNA probes: for *Adra2b*, a 0.4-kb Sac I to Sac II fragment derived from sequences 5′ to the *Adra2b* coding sequence, and *Neo*, a 0.82-kb Pst I to Xba I fragment of the *neo* coding sequence.
24. For protein immunoblot analysis, membranes were prepared from whole kidneys of *Adra2b*$^{+}$/*Adra2b*$^{+}$, *Adra2b*$^{+}$/*Adra2b*$^{-}$, or *Adra2b*$^{-}$/*Adra2b*$^{-}$ mice with a modification of the protocol of Uhlen and Wikberg (*20*). Protein electrophoresis through 10%-polyacrylamide gels and transfer to nitrocellulose membranes were done as described (100 μg of membrane protein per lane) (*21*). After transfer to nitrocellulose and blocking in BLOTTO (5% nonfat dried milk protein, 0.1% Tween 20 in phosphate-buffered saline), membranes were incubated sequentially with affinity-purified primary antibody to *Adra2b* (D. Daunt, Stanford University, Stanford, CA; 1:500 dilution) and then with peroxidase-labeled secondary antibody to rabbit immunoglobulin G (Amersham; 1:1000 dilution). After washing in 0.1% Tween 20 in phosphate-buffered saline, proteins were detected by enhanced chemiluminescense (ECL, Amersham).
25. [^{3}H]Yohimbine saturation-binding analysis was done as described (*4*). Binding of [^{3}H]yohimbine (12 nM, 70 Ci/mmol; New England Nuclear) was competed with unlabeled prazosin over a concentration range of 10^{-10} to 10^{-4} M. Each tube contained kidney membrane protein (300 μg) derived from an *Adra2b*$^{+}$/*Adra2b*$^{+}$ or *Adra2b*$^{-}$/*Adra2b*$^{-}$ mouse. Nonspecific binding was determined by incubation with 100 μM WB4101. The data were analyzed with a nonlinear least-squares curve-fitting technique (GraphPad software; GraphPad Software, San Diego, CA).
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