Abnormal Regulation of the Sympathetic Nervous System in α2A-Adrenergic Receptor Knockout Mice

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Received December 4, 1999; accepted March 19, 1999 This paper is available online at http://www.molpharm.org

ABSTRACT
α2-Adrenergic receptors (ARs) play a key role in regulating neurotransmitter release in the central and peripheral sympathetic nervous systems. To date, three subtypes of α2-ARs have been cloned (α2A, α2B, and α2C). Here we describe the physiological consequences of disrupting the gene for the α2A-AR. Mice lacking functional α2A subtypes were compared with wild-type (WT) mice, with animals lacking the α2B or α2C subtypes, and with mice carrying a point mutation in the α2A-AR gene (α2A-D79N). Deletion of the α2A subtype led to an increase in sympathetic activity with resting tachycardia (knockout, 581 ± 21 min⁻¹; WT, 395 ± 21 min⁻¹), depletion of cardiac tissue norepinephrine concentration (knockout, 676 ± 31 pg/mg protein; WT, 1178 ± 98 pg/mg protein), and down-regulation of cardiac β-ARs (Bₘₐₓ: knockout, 23 ± 1 fmol/mg protein; WT, 31 ± 2 fmol/mg protein). The hypotensive effect of α2 agonists was completely absent in α2A-deficient mice. Presynaptic α2-AR function was tested in two isolated vas deferens preparations. The nonsubtype-selective α2 agonist dexmedetomidine completely blocked the contractile response to electrical stimulation in vas deferens from α2B-AR knockout, α2C-AR knockout, α2A-D79N mutant, and WT mice. The maximal inhibition of vas deferens contraction by the α2 agonist in α2A-AR knockout mice was only 42 ± 9%. [3H]Norepinephrine release studies performed in vas deferens confirmed these findings. The results indicate that the α2A-AR is a major presynaptic receptor subtype regulating norepinephrine release from sympathetic nerves; however, the residual α2-mediated effect in the α2A-AR knockout mice suggests that a second α2 subtype (α2B or α2C) also functions as a presynaptic autoreceptor to inhibit transmitter release.

α2-Adrenergic receptors (ARs) play a prominent role in the regulation of the sympathetic nervous system (Ruffolo et al., 1991). Activation of α2-ARs in the brainstem leads to a reduction in sympathetic tone, with a resultant decrease in heart rate and blood pressure. This effect is augmented by stimulation of α2-ARs on sympathetic nerve terminals. These presynaptic α2-ARs serve as autoreceptors regulating catecholamine release. There are three α2-AR subtypes (α2A, α2B, and α2C), and studies using gene-targeting strategies indicate independent functions for each (Link et al., 1996; MacMillan et al., 1996; Sallinen et al., 1997). Resting blood pressure and heart rate were not significantly altered by disruption of either the α2B or α2C gene, indicating that neither receptor is necessary for normal sympathetic regulation (Link et al., 1996). Intra-arterial administration of clonidine-like α2 agonists produced a biphasic blood pressure response in wild-type (WT) mice so an initial brief pressor effect was followed by a sustained fall in arterial blood pressure (Link et al., 1996; MacMillan et al., 1996). This is a characteristic cardiovascular response pattern of clonidine-like drugs in other mammals (Hoeke and Kobinger, 1966). The vasopressor response to α2 agonists was absent in the α2B knockout (KO) mice, indicating that the α2B-AR mediates vasoconstriction in some vascular beds. The response to α2 agonist was not altered in the α2C-deficient mice. Recent studies indicate that the α2C-AR plays a role in several aspects of behavior (Sallinen et al., 1998).

Much has been learned about the function of the α2A-AR from mice with a targeted mutation of the α2A-AR in the second transmembrane at position 79 (α2AD79N) (MacMillan et al., 1996). In cultured cell lines, the α2AD79N mutant receptor failed to activate K⁺ currents but exhibited normal inhibition of voltage-gated calcium channels and CAMP production (Surprenant et al., 1992). The α2AD79N mice were developed to study the physiological importance of K⁺ current regulation by the α2A-AR. Surprisingly, targeted mutation of the α2A-AR gene reduced expression of the α2AD79N mutant receptor by 80% as determined by radioligand binding assays in brain (MacMillan et al., 1996). α2AD79N mu-

ABBREVIATIONS: AR, adrenergic receptor; KO, knockout; ES, embryonic stem; KAC, potassium acetate; CYP, iodocyanopindolol.
tant mice had normal resting heart rate and blood pressure. The initial hypertensive response to an α2 agonist was similar in α2A-D79N and WT mice; however, the hypotensive response to α2 agonists was absent, demonstrating that the α2A-AR mediates this brainstem response.

We now report the physiological effects of disrupting the α2A-AR gene and describe differences between these mice and the α2A-D79N mice. Cardiovascular studies show that unlike the α2A-D79N mice, α2A-KO mice have a resting tachycardia. This difference can be attributed to the loss of pre-synaptic autoregulation in α2A-KO mice, which is preserved in α2A-D79N mutant mice.

Materials and Methods

Gene Targeting. The murine α2A-AR gene (4 kb) and flanking regions (5' arm, 3 kb; 3' arm, 6 kb) was subcloned into pBluescript II SK(–) A 1672-bp neomycin resistance cassette (neo) containing the PGK promoter, neomycin resistance gene, and bovine growth hormone poly(A) sequence was inserted into a unique BglII site within the α2A-AR gene. The neo sequence was inserted in the opposite orientation relative to the α2A-AR gene, resulting in a premature termination codon within the third transmembrane domain. A herpes simplex virus thymidine kinase cassette (hsv-tk) was inserted downstream of the 6-kb 3' flanking region. R1 embryonic stem (ES) cells were electroporated with 40 μg of linearized targeting construct and placed under selection with G418 and ganciclovir. Resistant ES cell colonies were screened, and 37 of 132 were correctly targeted. Targeted ES cells clones were aggregated with eight cell FVB/N mouse embryos and cocultured overnight. The resulting blastocysts were transferred to pseudopregnant mice. Twenty-two male chimeric mouse embryos and cocultured overnight. The resulting blastocysts were screened, and 37 of 132 were correctly targeted.

The generation of α2A-AR KO mice, α2A-KO mice, and α2A-D79N mice has been described previously (Link et al., 1995, 1996; MacMillan et al., 1996).

Saturation and Competition Binding. Brain membranes were prepared by homogenizing whole brain in lysis buffer (10 mM Tris·HCl, 5 mM EDTA, pH 7.4), followed by centrifugation at 10,000g. The pellet was washed in Tris·HCl buffer (75 mM Tris·HCl, 12.5 mM MgCl2, 1 mM EDTA, pH 7.4), followed by centrifugation at 10,000g. The pellet was resuspended in potassium acetate (KAC) binding buffer (50 mM KAC, pH 7.4), and protein concentration was determined. For α2-AR saturation binding experiments, 180 to 250 μg of homogenate protein was used in a 500-μl reaction containing 1 to 10 nM [3H]RX81002, with or without 1 μM atipamezole, and KAC binding buffer. For α2-AR competition binding experiments, 200 to 250 μg of homogenate protein was used in a 500-μl reaction containing 1 nM [3H]RX81002, 1 to 1000 nM yohimbine, with or without 1 μM atipamezole, and KAC binding buffer. All binding assay mixtures were incubated at room temperature for 1 h. For β-AR saturation binding, heart homogenates were prepared by Polytron homogenization of whole heart in lysis buffer, followed by centrifugation at 10,000g. The pellet was washed, and 50 to 100 μg of homogenate protein was used in a 500-μl reaction containing 1 to 300 pM [125I]iodocyanopindolol ([125I]ICYP), with or without 1 μM (dl)-propranolol, in Tris·HCl binding buffer. The binding assay mixtures were incubated for 2 h. Binding reactions were terminated by filtration using a Brandel cell harvester. Membrane-bound [3H]RX81002 was determined by scintillation counting, and [125I]ICYP was determined by gamma emission. The results were analyzed with a nonlinear least-squares curve-fitting technique (Prism; GraphPAD, San Diego, CA).

In Vivo Cardiovascular Physiology. Studies were performed on eight WT and eight α2A-AR KO adult mice (10–20 weeks old) that were generated from α2A-AR heterozygote breeding. The mice were anesthetized with isoflurane (1–3%), and a polyethylene (PE10) catheter that had been stretched (0.5 mm o.d.) was inserted into the left internal carotid artery. The catheter was tunneled s.c. to exit at the base of the neck and placed within a s.c. pouch. After 24 h of recovery, the catheter was removed from the s.c. pouch, flushed with saline, and connected to a Spectramed DTX Plus pressure transducer. Heart rate and mean aortic blood pressure were recorded with a Gould eight-channel recorder and digitized on the Crystal Biotech Dataflow system (Hopkinton, MA). Baseline hemodynamics were continuously recorded for 1 h after the animal was placed in the study cage. To examine the role of vagal tone on baseline heart rate, atropine sulfate (1 mg/kg i.a.) was administered, and hemodynamics were recorded. Similarly, to examine the role of sympathetic tone on baseline heart rate, hemodynamics were recorded after propranolol administration (3 mg/kg i.a.). The next day, hemodynamic responses to dexametomidine (5 μg/kg i.a.) were recorded.

Tissue Norepinephrine Levels. Tissue norepinephrine concentrations were measured from the supernatants of whole heart and kidney homogenates. Tissue samples were homogenized on ice in 0.1 M sodium phosphate (pH 7.4), and a small sample was removed for protein determination. Perchloric acid was added (0.6 M final concentration), and samples were centrifuged at 10,000g for 3 h at 4°C. The resulting supernatants were analyzed by HPLC.

Vas Deferens Contractions. Vasa deferentia were isolated after sacrifice by cervical dislocation. The tissue was suspended in a force transducer and placed in an organ bath filled with physiological buffer solution consisting of 118 mM NaCl, 4.7 mM KCl, 3.0 mM CaCl2, 1.22 mM KH2PO4, 25 mM NaHCO3, and 10 mM glucose, oxygenated with a mixture of 95% O2/5% CO2. The samples were allowed to equilibrate for 30 min, and then 200 mg of resting tension was applied. Electrical field stimulation was applied to produce nerve terminal depolarization and neurotransmitter release (two electrical pulses every 10 s: 30 V, 0.9-ms width, 100-ms interval). The force of muscle contraction was digitized and displayed on a MacLab data analysis package. After contractions had equilibrated, dexmedetomidine was added to the organ chamber in a cumulative fashion every 1.5 min without changing the bath solution. In a separate group of animals, yohimbine was administered to the organ baths, also in a cumulative manner every 1.5 min without changing the medium.

[3H]Norepinephrine Release. The release of [3H]norepinephrine from mouse vas deferens was determined as described previously for other mouse tissues (Limerber et al., 1995; Wahl et al., 1996), with minor modifications. Briefly, small pieces of the vas deferens were incubated with [3H]norepinephrine (0.1 μM) in physiological buffer (Wahl et al. 1996) for 30 min. Tissue pieces were then superfused with [3H]norepinephrine-free medium containing 1 μM desipramine at a rate of 1.2 ml/min. Transmitter release was elicited by rectangular pulses of 1-ms width and 47-V/cm voltage, giving a current strength of 80 mA. There were six stimulation periods in each experiment, applied at intervals of 18 min (S1 to S6). Each period consisted of one train of 20 pulses at 50 Hz. Medetomidine was added at cumulatively increasing concentrations 12 min before S2 to S6. At the end of experiments, tissues were solubilized, and tritium was determined in superfusate samples and tissues. Electrically evoked overflow of total tritiated compounds was calculated as the difference between total tritium outflow and estimated basal outflow and was expressed as a percentage of tissue tritium at the time of stimulation (Wahl et al., 1996). A logistic curve was fitted to the concentration-inhibition data of medetomidine (Trendelenburg et al., 1993). The electrically evoked overflow of total tritium reflects exocytotic release of [3H]norepinephrine (Taube et al., 1977) and is termed thus in this report. Medetomidine (racemic) was used in these experiments because of the limited availability of the dextroisomer dexametomidine. Both compounds are nonselective α2 agonists.

Statistical Analysis. All results are expressed as mean ± S.E.M. Baseline hemodynamics and tissue catecholamine levels were ana-
lyzed by independent t test. Responses to dexmedetomidine were analyzed by two-way ANOVA for repetitive measures. The Mann-Whitney test was used for statistical comparison between experimental groups in the catecholamine release studies.

Results

Generation of α2A-AR-Deficient Mice. To disrupt the α2A-AR (Adra2a) in embryonic stem cells, a targeting vector was constructed that interrupted the coding region of the α2A-AR by insertion of a neomycin resistance cassette (neo; Fig. 1a). The disrupted α2A gene could be distinguished from the WT allele as a 5.1-kb HindIII fragment by a 5′ Southern probe that lies outside the region of homology with the targeting vector (Fig. 1a). From the correctly targeted stem cell clones, three germline transmitting chimeras were generated by morula aggregation. One hundred thirty-one F2 mice were generated from heterozygous Adra2a KO (FVB/N, 129/SV) intercrosses and genotyped to determine whether disruption of the α2A-AR gene had a significant impact on development or viability before weaning. The distribution of WT, heterozygous, and homozygous (KO) mice did not deviate significantly from that predicted by Mendelian genetics, indicating that there was no significant increase in mortality rates in α2A-AR-deficient mice (data not shown). KO mice could not be distinguished from WT or heterozygous littermates by appearance, body weight, fertility, or viability.

**α2A-AR Expression in Brain.** Saturation binding with [3H]RX821002, a nonsubtype-selective α2 antagonist, was performed on membranes prepared from brains of α2A-AR KO mice compared to WT mice (Fig. 2A). α2A-AR KO mice showed a 90% reduction in specific [3H]RX821002 binding (B_max: α2A-AR KO 29 ± 2 fmol/mg protein; WT, 281 ± 27 fmol/mg protein). The residual α2-AR binding in α2A-AR KO mice was anticipated based on previous reports suggesting that ~10% of the α2-AR is present in the brain is of the α2C subtype (Ordway et al., 1993). To confirm that the residual binding in the KO mice was not of the α2A-AR subtype, competition assays were performed with yohimbine (Fig. 2B). Yohimbine, an α2 antagonist, has an unusually low affinity for the rodent α2A-AR subtype (Link et al., 1992). In the WT mice, yohimbine inhibited binding of [3H]RX821002 in a concentration-dependent manner displaying a small population (4 ± 6%) of high-affinity receptors (K_i = 1 nM) and large population of low-affinity receptors (K_i = 37 ± 3 nM). In KO mice, only high-affinity sites were present (K_i = 4.0 ± 0.3 nM), confirming that the residual α2A-AR binding in the KO mice is not of the α2A-AR subtype.

Cardiovascular Physiology. Mean aortic blood pressure and heart rate were recorded 24 h after the insertion of a left carotid artery catheter and while the mice were quietly resting (Fig. 3). Heart rate was significantly higher in α2A-AR KO mice (KO, 581 ± 21 min⁻¹; WT, 395 ± 21 min⁻¹). Mean aortic blood pressure was not significantly different (KO, 131 ± 8 mm Hg; WT, 128 ± 5 mm Hg). Atropine was then administered to determine whether the observed tachycardia in α2A-AR KO mice...
was due to a reduction in vagal tone (Fig. 3). Atropine significantly increased heart rate in both WT and α2A-KO mice. This increase was greater in WT mice, although heart rate remained significantly higher in KO mice. Propranolol was then administered to block sympathetic influence on heart rate. Propranolol produced a greater reduction in heart rate in KO mice so that heart rates were no longer significantly different between genotypes (KO, 479 ± 13 min⁻¹; WT, 437 ± 23 min⁻¹). In a separate group of WT and α2A-KO mice, propranolol was administered without pretreatment with atropine. This was done to determine whether propranolol alone could normalize the heart rate. The reduction in heart rate was greater in KO mice, so the heart rates were not significantly different after β-blockade (before propranolol: KO, 520 ± 46 min⁻¹; WT, 409 ± 29 min⁻¹; after propranolol: KO, 370 ± 32 min⁻¹; WT, 365 ± 44 min⁻¹, n = 3).

The hemodynamic response to infusion of the nonsubtype-selective α2 agonist dexmedetomidine in WT and α2A-KO mice is shown in Fig. 4. Dexmedetomidine produced a biphasic blood pressure response in WT mice. The initial pressor response was greater in α2A-KO mice (maximum increase: KO, 20 ± 4 mm Hg; WT, 10 ± 4 mm Hg). However, dexmedetomidine failed to reduce blood pressure in α2A-KO mice (maximum decrease: KO, 0 ± 2 mm Hg; WT, 25 ± 4 mm Hg). Administration of the α2 agonist caused a marked decrease in heart rate in both WT and α2A-KO mice (Fig. 4b); however, the maximal bradycardic response to dexmedetomidine was reduced in α2A-KO compared with WT mice.

Tissue Norepinephrine Levels. The increased heart rate in α2A-KO mice suggested that norepinephrine release from sympathetic terminals was enhanced in vivo. Because reliable determinations of plasma catecholamines are difficult to obtain in mice, tissue norepinephrine concentrations were determined in WT and α2A-KO heart and kidney (Fig. 5a). The concentrations of norepinephrine in heart were significantly reduced in α2A-KO compared with WT mice, suggesting that the sympathetic catecholamine stores are depleted due to enhanced transmitter release. Similarly, norepinephrine concentration were reduced in the kidney, but this achieved only borderline significance (p < .1).

Cardiac β-AR Down-Regulation. The results presented above suggest that loss of presynaptic autoregulation in sympathetic nerves of α2A-AR KO mice leads to baseline tachycardia and depletion of catecholamine stores. To investigate the effect of the loss of presynaptic α2A-AR function on postsynaptic ARs, we measured the density of cardiac β-ARs using the nonselective β antagonist [125I]IYP. It has previously been shown that chronic agonist infusion leads to down-regulation of cardiac β-AR (Chang et al., 1982; Nanoff et al., 1989). Saturation binding studies revealed a significant reduction in cardiac β-AR density in α2A-KO mice compared with WT mice (Fig. 5b).

Presynaptic α2-Autocepter Function. A vas deferens contraction assay was used to examine α2-AR regulation of sympathetic transmitter release. Electrical stimulation of the vas deferens suspended in an organ bath leads to release of norepinephrine and ATP from intramural sympathetic nerve endings and subsequent contraction due to activation of α1-ARs and P2-purinergic receptors. Although the smooth muscle of the mouse vas deferens contains α2-ARs, they play only a minor role in mediating neurogenic contractions (Bültmann et al., 1991). Stimulation of presynaptic α2-AR on the sympathetic nerve terminals, however, markedly inhibits transmitter release and therefore the neurogenic contraction. Concentration-inhibition curves for the nonselective α2 agonist dexmedetomidine in vas deferens isolated from the α2B-AR KO, α2C-AR KO, α2D79N mutant, and WT mice are shown in Fig. 6. Dexmedetomidine at sufficient concentrations completely blocked the contractile response to electrical stimulation in vas deferens from all of these mice in a similar manner. The data indicate that disruption of the α2B-AR and 

![Fig. 3](image1.png)  
**Fig. 3.** Heart rate regulation in unrestrained, conscious WT and α2A-KO mice. Baseline heart rate was significantly elevated in α2A-KO mice compared with WT mice (*p < .001, KO versus WT). Injection of atropine led to an increase in heart rate in both groups of animals (*p < .001, atropine versus control), but α2A-KO mice were still tachycardic compared with WT mice (*p < .001, KO versus WT). Subsequent injection of the β antagonist propranolol abolished the difference in heart rate between WT and α2A-KO mice.

![Fig. 4](image2.png)  
**Fig. 4.** Hemodynamic effects of the α2 agonist dexmedetomidine on mean arterial blood pressure (a) and heart rate (b) in unrestrained, conscious WT and α2A-KO mice. At time 0, a bolus of dexmedetomidine (5 μg/kg) was administered through the arterial catheter. In α2A-KO mice, the initial hypotensive effect of the α2 agonist was greater than that in WT mice. However, the hypotensive effect of the α2 agonist was completely abolished in α2A-KO mice. The bradycardic response to dexmedetomidine was significantly blunt in α2A-KO mice compared with WT mice. Data are derived from eight animals per group (mean ± S.E.M.).
α2C-AR genes or mutation of the α2A-AR (D79N) does not alter presynaptic function in sympathetic nerves. In contrast, the inhibition by dexmedetomidine in the α2A-KO mice (Fig. 7a), although not abolished, was markedly impaired. The maximal inhibition of vas deferens contraction by the α2 agonist in α2A-KO mice was only 42 ± 9% compared with nearly complete inhibition in the WT mice. The concentration-inhibition curve of dexmedetomidine was also shifted to the right in α2A-KO mice (EC50: α2A-KO, 4.2 ± 1.6 nM; WT, 0.7 ± 0.2 nM).

In vasa deferentia from a separate group of α2A-KO and WT mice, increasing concentrations of yohimbine were added to block presynaptic α2-ARs and thereby disinhibit transmitter release (Fig. 7b). In this way, the extent of autoinhibition could be quantified. The α2 antagonist increased the contractile response in both WT and KO mice (Fig. 7b). This increase was significantly greater in WT mice (maximum increase: WT, 185 ± 40% over baseline; KO, 52 ± 8% over baseline). The vas deferens from KO mice was more sensitive to yohimbine than the vas deferens from WT mice (EC50: α2A-KO, 28 ± 9 nM; WT, 215 ± 45 nM; Fig. 7c).

Contraction responses to phenylephrine in unstimulated vas deferens were similar in WT and α2A-KO mice, indicating no difference in postsynaptic α1-AR function (Fig. 7d). Similarly, dexmedetomidine did not alter the response to phenylephrine, confirming its specific activity at presynaptic receptors.

To more directly investigate the autoreceptor role of the α2A subtype, [3H]norepinephrine release was measured in small pieces of the vas deferens from WT and α2A-AR-deficient animals. Electrical stimulation by single trains of 20 pulses at 50 Hz elicited release of [3H]norepinephrine, which was smaller in the WT tissue (0.17 ± 0.01% of tissue tritium; n = 16) than in the KO tissue (0.30 ± 0.03% of tissue tritium; n = 16). Under the stimulation conditions used, 1 μM rauwolscine increased [3H]norepinephrine release by 38 ± 7% in WT mice (p < .01) and only tended to increase release (15 ± 6%; p > .05) in α2A-KO mice. Thus, little autoinhibition of release is observed because the single-pulse trains are too short for significant autoinhibition to develop (Marshall, 1983; Singer, 1988; Limberger et al., 1995; Wahl et al., 1996). In WT vas deferens, the α2 agonist medetomidine caused concentration-dependent inhibition of the release of [3H]norepinephrine with an IC50 value of 0.44 ± 0.04 nM and by maximally 90.1 ± 0.5% (Fig. 8). In mice lacking the α2A-AR, the effect of medetomidine, although not abolished, was reduced: medetomidine caused inhibition with an IC50 value of 0.76 ± 0.24 nM and a maximum of 74.7 ± 2.2% (Fig. 8). The maximal inhibition was thus decreased by 17%.

Discussion

Disruption of α2A-AR Gene. α2A-AR gene disruption was confirmed by ligand-binding experiments in whole brain. Disruption of the α2A-AR gene resulted in a 90% reduction in total α2A-AR binding in the mouse brain. The 10% residual binding is similar to previous estimates of the extent of α2C-AR expression in brain (Ordway et al., 1993). We found that the residual α2A-AR binding in α2A-KO mice had high affinity for yohimbine. This finding is consistent with the higher affinity of yohimbine for the rodent α2B and α2C subtypes than for the α2A subtype (Link et al., 1992).

![Fig. 5. Tissue norepinephrine levels and cardiac β-AR density are decreased in α2A-KO mice. a, norepinephrine concentrations in heart and kidney were decreased compared with WT mice (p < .01). b, enhanced release of norepinephrine from sympathetic terminals leads to down-regulation of cardiac β-ARs. Saturation isotherms for the β-AR ligand [125I]CYP revealed a reduction in β-AR density in heart membranes from α2A-KO compared with WT mice. Data are derived from five or six animals per group (mean ± S.E.M.).](image)

![Fig. 6. Inhibition of electrically evoked contractions of isolated vas deferens preparations from WT and α2A-AR-deficient mice. Stimulation of presynaptic α2A-ARs by dexmedetomidine decreased the amplitude of the evoked twitch response. No difference was found for the inhibitory effect of dexmedetomidine on twitch contractions in WT and α2A- or α2C-AR-deficient mice (a) or between WT and α2A-D79N mice (b). Data represent mean ± S.E.M. from six to eight vas deferens preparations.](image)
Role of \(\alpha_2A\)-AR in Regulating Heart Rate and Blood Pressure. ARs form the interface between the sympathetic nervous system and the cardiovascular system. The \(\alpha_2\)-ARs also play an important role in regulating the sympathetic nervous system both centrally, by regulating sympathetic tone, and peripherally, by regulating transmitter release from presynaptic nerve terminals. We have used strains of genetically engineered mice to investigate the regulatory functions of specific \(\alpha_2\)-AR subtypes. Previous studies of \(\alpha_2D79N\) mice demonstrated that the \(\alpha_2A\)-AR subtype mediates the hypotensive effects of nonselective \(\alpha_2\) agonists (MacMillan et al., 1996). Several lines of evidence suggest that the hypotensive effect of \(\alpha_2\) agonists could result from actions at sites within the central and/or the peripheral sympathetic nervous system (DeJonge et al., 1981; Urban et al., 1995). However, our results indicate that activation of cardiac and vascular presynaptic \(\alpha_2\)-ARs alone is not sufficient to produce hypotension in response to \(\alpha_2\) agonists. The data presented in Fig. 6 demonstrate that presynaptic regulation of catecholamine release is preserved in \(\alpha_2D79N\) mice; however, these mice failed to become hypotensive in response to administered \(\alpha_2\) agonists (MacMillan et al., 1996).

The only significant difference that we observed between \(\alpha_2AKO\) mice and \(\alpha_2D79N\) mice was in resting heart rate. The resting heart rate of \(\alpha_2D79N\) mice was not significantly different from their WT controls (MacMillan et al., 1996), whereas \(\alpha_2AKO\) mice had resting heart rates more than 180 beats/min greater than the control littermates. The tachycardia in the \(\alpha_2AKO\) mice can be explained by a high basal level of sympathetic tone resulting from the loss of \(\alpha_2A\)-AR-mediated inhibition of the vasomotor center combined with the loss of \(\alpha_2A\)-AR-mediated inhibition of norepinephrine release from peripheral cardiac nerve terminals. The response to atropine was blunted in the \(\alpha_2AKO\) mice, suggesting a state of vagal withdrawal. This finding is consistent with previous reports indicating that central \(\alpha_2\)-ARs stimulate vagal output (Van Zwieten, 1988). However, the difference in heart rate between KO and WT mice persisted after inhibition of parasympathetic muscarinic receptors with atropine (see Fig. 3) and therefore is not principally due to a decrease in parasympathetic activity. The tachycardia observed in \(\alpha_2AKO\) mice was associated with a significant depletion of the tissue.

**Fig. 7.** Presynaptic \(\alpha_2\)-AR function in vas deferens from \(\alpha_2AKO\) mice. a, the inhibitory effect of dexmedetomidine on electrically evoked twitch contractions was reduced by 56% in \(\alpha_2AKO\) mice compared with WT mice. b, the addition of the \(\alpha_2\) antagonist yohimbine caused an increase in the twitch amplitude of vas deferens from WT and \(\alpha_2AKO\) mice. c, the concentration-response curve for yohimbine is shifted to the left in vas deferens from \(\alpha_2AKO\) compared with WT mice. d, the postsynaptic contractile effect of the \(\alpha_1\) agonist phenylephrine is not different in \(\alpha_2AKO\) and WT vas deferens. Contractions were elicited by adding phenylephrine in a cumulative manner to nonelectrically stimulated vas deferens preparations. Contraction responses to phenylephrine were not altered in the presence of the \(\alpha_2\) agonist dexmedetomidine (0.1 \(\mu\)M Dex). Data represent mean ± S.E.M. from six to nine vas deferens preparations.

**Fig. 8.** Effect of medetomidine on \(^{3}H\)norepinephrine release in mouse vas deferens. Pieces of vas deferens from WT or \(\alpha_2A\)-AR-deficient mice were preincubated with \(^{3}H\)norepinephrine, superfused, and, beginning after 54 min of superfusion, subjected to six periods of electrical stimulation 18 min apart (S1 to S6). Each stimulation period consisted of a train of 20 pulses delivered at 50 Hz. Increasing concentrations of medetomidine were added in a cumulative manner 12 min before S2 to S6. a, tritium efflux-versus-time curves from single tissue pieces. Interrupted lines, controls without medetomidine; uninterrupted lines with ○ (WT) or ● (\(\alpha_2AKO\)) circles, medetomidine was added as indicated. b, concentration-response curves of medetomidine. The effect is corrected for time-matched controls. Mean ± S.E.M. from eight or nine tissue pieces.
norepinephrine levels compared with normal mice. Depletion of tissue norepinephrine stores can be explained by enhanced release of norepinephrine from cardiac sympathetic nerves. Because reliable measurements of resting plasma catecholamine concentrations are difficult to obtain in mice, we examined the density of cardiac β-ARs. Chronic agonist exposure leads to pronounced down-regulation of β-ARs in several animal models (Chang et al., 1982; Nanoff et al., 1989). In α2A-KO mice, the level of β-ARs was decreased by 25% compared with WT mice, which is consistent with chronically increased sympathetic activity in these animals.

We were somewhat surprised that baseline blood pressure was unaffected in α2A-KO mice. This may be due to the fact that the sympathetic nervous system mediates vasoconstriction through α1-ARs and the α2B-AR and vasodilatation through both β1 and β2-ARs in mice (Rohrer et al., 1998). Thus, the net effect of increasing sympathetic tone on total vascular resistance may be minimal; however, it is possible that there are differences in the distribution of blood to different vascular beds as a result of different distributions of vasoconstricting and vasodilating ARs. It is also possible that changes in other systems that regulate systemic blood pressure, such as the renin-angiotensin system, are compensating for the chronic elevation of sympathetic tone in α2A-KO mice. Finally, the mice examined in these studies were relatively young (less than 20 weeks old). The effects of chronic elevation of sympathetic tone may become evident as the α2A-KO mice age.

More Than One Presynaptic α2-Autoreceptor. The α2A-AR has been suggested to be the main presynaptic α2-AR in mammalian tissues (Trendelenburg et al., 1993). However, previous studies indicated that at least in certain tissues such as the rat heart, a second α2 subtype might also regulate neurotransmitter release. Limberger et al. (1992; Trendelenburg et al., 1997). Ho et al. (1998) studied the release-enhancing effect of α2 antagonists and concluded that in addition to α2A-ARs, either α2B- or α2C-ARs mediate presynaptic inhibition in rat heart atria.

Our experiments on the vas deferens support the view that the α2A subtype is the principal presynaptic autoreceptor. No attenuation of dexmedetomidine-induced presynaptic inhibition of neurogenic contractions was observed in the α2BKO or in the α2C-KO mice, whereas this inhibition was greatly reduced in the α2A-KO animals. Moreover, yohimbine, which blocks α2-ARs and interrupts α2 autoinhibition, increased neurogenic contractions much less in vasa deferentia from α2A-KO than from WT mice, indicating a decrease of endogenous autoinhibition. Neurogenic responses of the mouse vas deferens to sympathetic nerve stimulation are mediated by the two cotransmitters norepinephrine and ATP, and the inhibition of the responses may be due to a decrease of norepinephrine as well as of ATP release (von Kugelgen and Starke, 1991). However, in direct [3H]norepinephrine release experiments, the inhibitory effect of medetomidine, the racemate of dexmedetomidine, was also reduced in the α2A-KO tissues.

Although the α2A-AR appears to be the principal regulator of catecholamine release, two of our findings indicate that at least one other α2-AR subtype also functions as an autoreceptor. First, presynaptic inhibition by dexmedetomidine or medetomidine was reduced but not abolished in mice that lack the α2A-AR. The maximal inhibition of neurogenic contractions was reduced by 56% in the α2A-KO tissues, whereas the maximal inhibition of [3H]norepinephrine release was reduced by only 17%. The difference may be due to the different patterns of nerve stimulation (pairs of pulses with an interval of 100 ms in contraction experiments versus 20 pulses at 50 Hz in [3H]norepinephrine experiments) or to the fact that release of norepinephrine and release of ATP are subject to differential presynaptic α2 modulation (Driessen et al. 1993). Second, endogenous autoinhibition, as revealed by the effect of yohimbine on contractions, was attenuated but not abolished in the α2A-KO tissues. Although neither the α2BKO nor the α2C-KO showed altered responses to dexmedetomidine, it is anticipated that one of these receptors is responsible for the residual response to α2 agonists and yohimbine in α2A-KO mice. In support of this view, the potency of yohimbine at enhancing neurogenic contractions was increased in the α2A-KO tissue, in accord with its higher affinity for the α2B and α2C subtypes than for the α2A subtype (see above). Moreover, in [3H]norepinephrine release experiments on vasa deferentia from α2A-KO mice, α2 antagonists shifted the concentration-inhibition curve of medetomidine to the right in a manner compatible with the α2B or α2C subtype (A. U. Trendelenburg, unpublished observations).

The question remains why loss of the α2B or α2C-AR did not interfere with presynaptic autoreceptor function. It is possible that the α2A autoreceptor is functionally dominant (more abundant or more efficiently coupled), so loss of the other α2-AR subtype cannot be detected with the assays used. It is also possible that in α2A-KO mice, the α2B-AR or α2C-AR subtype is up-regulated to compensate for the loss of the α2A-AR. Of interest, α2A-D79N mice had normal presynaptic function in the vas deferens contraction assay. Because the α2A-D79N mutant receptor fails to activate K+ channels but does inhibit voltage-dependent Ca2+ channels (see the introduction), this observation favors coupling of the α2A autoreceptor to the latter transduction mechanism (Starke et al., 1989; Hille, 1994).

In conclusion, disruption of the α2A-AR gene in mice has no apparent effect on viability or fertility. Examination of these mice confirms that the α2A-AR is the subtype mediating the beneficial hypotensive effects of α2 agonists on blood pressure. The α2A KO mice have altered sympathetic regulation due to loss of α2A-mediated inhibition of sympathetic tone in the brainstem and loss of α2A-mediated inhibition of catecholamine release from sympathetic nerve terminals. Our results provide evidence that at least one other α2 subtype also plays a contributing role in regulating catecholamine release.

Acknowledgments

We thank Dr. Mervyn Maze (Stanford University) for HPLC analysis of norepinephrine from tissue samples and E. Gaiser for help with the [3H]norepinephrine release experiments.

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