Feedback Inhibition of Catecholamine Release by Two Different $\alpha_2$-Adrenergic Subtypes Prevents Progression of Heart Failure

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Background—Elevated plasma norepinephrine levels are associated with increased mortality in patients and in animal models with chronic heart failure. To test which $\alpha_2$-adrenergic subtype operates as presynaptic inhibitory receptors to control norepinephrine release in heart failure, we investigated the response of gene-targeted mice lacking $\alpha_2$-adrenoceptor subtypes ($\alpha_2$-KO) to chronic left ventricular pressure overload. In addition, we determined the functional consequences of genetic variants of $\alpha_2$-adrenoceptors in human patients with chronic heart failure.

Methods and Results—Cardiac pressure overload was induced by transverse aortic constriction. Three months after aortic banding, survival was dramatically reduced in $\alpha_2A$-KO (52%) and $\alpha_2C$-KO (47%) mice compared with wild-type and $\alpha_2B$-deficient (86%) animals. Excess mortality in $\alpha_2A$- and $\alpha_2C$-KO strains was attributable to heart failure with enhanced left ventricular hypertrophy and fibrosis and elevated circulating catecholamines. The clinical importance of this finding is emphasized by the fact that heart failure patients with a dysfunctional variant of the $\alpha_2C$-adrenoceptor had a worse clinical status and decreased cardiac function as determined by invasive catheterization and by echocardiography.

Conclusions—Our results indicate an essential function of $\alpha_2A$- and $\alpha_2C$-adrenoceptors in the prevention of heart failure progression in mice and human patients. Identification of heart failure patients with genetic $\alpha_2$-adrenoceptor variants as well as new $\alpha_2$-receptor subtype–selective drugs may represent novel therapeutic strategies in chronic heart failure and other diseases with enhanced sympathetic activation. (Circulation. 2002;106:2491-2496.)

Key Words: receptors, adrenergic, alpha ■ genetics ■ heart failure ■ catecholamines

Chronic heart failure is one of the leading causes of mortality in developed countries. Adrenergic activation is a compensatory mechanism to maintain cardiac output in the presence of decreased cardiac contractility but is also associated with disease progression and decreased survival of heart failure patients. $\beta$-blockers exert a beneficial long-term effect on morbidity and mortality of patients with congestive heart failure. In addition to inhibition of myocardial $\beta$-adrenoceptors, activation of $\alpha_2$-adrenoceptors has recently been investigated as a therapeutic strategy in experimental and in clinical studies of heart failure.

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To date, 9 different adrenoceptor subtypes have been identified ($\alpha_{1A,B,D}$, $\alpha_{2A,B,C}$, and $\beta_{1,2,3}$). However, the physiological and therapeutic significance of both presynaptic and postsynaptic adrenergic receptor subtype diversity has not been resolved yet. Transgenic mouse models have recently gained great value to dissect the specific function of individual adrenoceptor subtypes in vivo. Whereas experiments with pharmacological ligands predicted that a single $\alpha_2$-adrenoceptor subtype is the presynaptic inhibitory receptor controlling sympathetic norepinephrine release, studies in gene-targeted mice have identified two $\alpha_2$-adrenoceptor subtypes at this site. In isolated tissues, $\alpha_2A$-adrenoceptors were the major feedback regulators, but $\alpha_2C$-adrenoceptors also contributed to inhibition of norepinephrine secretion from sympathetic nerves. Several functional differences were identified between presynaptic $\alpha_2A$- and $\alpha_2C$-receptor subtypes. In mouse atria, the $\alpha_2A$-subtype inhibited norepinephrine release at high-stimulation frequencies, whereas the $\alpha_2C$-receptor operated at lower levels of sympathetic nerve activity.

Several sequence variants have been identified in the coding regions of human $\alpha_2A$-, $\alpha_2B$-, and $\alpha_2C$-adrenoceptor genes. Most importantly, a 4-amino acid deletion in the third intracellular loop of the $\alpha_2C$-adrenoceptor was associated with decreased G protein coupling of this receptor variant, suggesting that humans carrying this mutation may be more prone to develop heart failure than people with the fully functional $\alpha_2C$-adrenoceptors.
In this study we used a mouse model of heart failure to identify the contribution of individual α2-adrenoceptor subtypes to presynaptic control of sympathetic activation and investigated the functional consequences of genetic α2-receptor variants in human patients with congestive heart failure. Our data demonstrate that two presynaptic regulators, α2A- and α2C-adrenoceptors, can prevent excess sympathetic activity and thus disease progression in experimental and clinical heart failure.

Methods

Generation and Genotyping of α2-Adrenoceptor–Deficient Mice

The generation of the mouse lines lacking α2-adrenoceptor subtypes has been described previously.20–22 Mice were maintained in a specified pathogen-free facility. All animal procedures were approved by the University of Würzburg and the Government of Unterfranken (protocol No. 621-2531.01-28/01).

Cardiac Catheterization and Rapid MR Imaging

For left ventricular catheterization with a 1.4F pressure-volume catheter,23 mice were anesthetized with tribromoethanol (13 μL of 2.5% solution per gram of body weight) and placed on a 37°C table.16,24 For MR imaging of the heart, mice were anesthetized with isoflurane (2.0% isoflurane [vol/vol] in 1 L/min oxygen flow). Images of the heart were taken with a 7.05-T BIOSPEC 70/20 scanner.25

Transverse Aortic Constriction

Mice 4 to 5 weeks old were anesthetized with tribromoethanol, and a nylon suture was placed around a 27G hypodermic needle to constrict the aortic arch.26 The degree of aortic stenosis was assessed by MR imaging (7 weeks after the operation) by the hemodynamic pressure gradient across the stenosis and by morphometric analysis of paraffin sections of the aortic arch. Cardiac histology and morphometry was determined from paraffin sections, as described.27

Norepinephrine Release and Plasma Catecholamine Determination

In vitro release of [3H]-norepinephrine was determined from isolated atria of mice 3 months after aortic constriction, as described.16 Catecholamines were measured in plasma obtained from tribromoethanol-anesthetized mice by high-performance liquid chromatography combined with electrochemical detection.16

Heart Failure Patients

Ninety-one patients with chronic heart failure (NYHA class II through IV) were recruited in the course of routine cardiac catheterization, with a left ventricular end-diastolic volume >110 mL/m2 and an ejection fraction <55% (by ventriculography).28 At the time of blood sample acquisition (1989 to 1991), all patients were stable through IV) were recruited in the course of routine cardiac catheterization, with a left ventricular end-diastolic volume >110 mL/m2 and an ejection fraction <55% (by ventriculography).28 At the time of blood sample acquisition (1989 to 1991), all patients were stable

Detection of α2-Adrenoceptor Polymorphisms

A deletion polymorphism in the third intracellular loop of the human α2C-adrenoceptor (α2C-Dele322-325) and an amino acid exchange in the α2A-adrenoceptor (α2A-Asn251Lys) were detected in genomic DNA isolated from human blood samples, as described previously.17,18

Figure 1. Increased mortality of α2A-adrenoceptor–deficient mice after left ventricular pressure overload. Survival over 3 months after constriction of the aortic arch was significantly reduced in mice lacking α2A- or α2C-adrenoceptors compared with wild-type or α2C-KO mice (Kaplan-Meier plots, *P<0.05, log-rank test, n=15 to 22 mice per genotype).

Statistical Analysis

Data displayed show mean±SEM. For all experiments, one-way or two-way ANOVA tests followed by appropriate post-hoc tests or t tests were used to determine statistical significance (P<0.05) using Prism 3.0 software (GraphPad).

Results

Increased Mortality of α2A-Adrenoceptor–Deficient Mice After Transverse Aortic Constriction

Postoperative survival, development of left ventricular hypertrophy, and heart failure were followed for 3 months after aortic constriction. A total of 86% of the wild-type mice and α2B-KO mice survived until the end of the observation period (Figure 1). Surprisingly, survival of α2A-KO and α2C-KO mice was dramatically reduced to 52% and 47% after aortic banding, respectively. The aortic banding operation resulted in similar degrees of stenosis in wild-type and α2A-adrenoceptor–deficient mice at 7 weeks after the operation (stenosis diameter 0.43±0.03 mm, MR imaging 7 weeks after banding, Figure 2a) as well as 14 weeks after banding (assessed by histomorphometry) (data not shown).

Heart Failure in α2A- and α2C-KO Mice After Aortic Constriction

Multiple cardiovascular indices suggested that the excess lethality after cardiac pressure overload in α2A-KO and α2C-KO mice was attributable to cardiac hypertrophy and heart failure. Without aortic banding, none of the mouse lines lacking single α2-adrenoceptor subtypes showed any defect in cardiac contractility or structure (Table, Figure 3a, top).16,20,22 Only sham-operated α2A-KO mice were tachycardic at baseline due to enhanced sympathetic norepinephrine release (Table).20 Rapid MRI revealed that left ventricles of α2A- and α2C-KO mice were hypertrophied and dilated 7 weeks after aortic constriction compared with wild-type mice (Figure 2b). Left ventricular ejection fraction was significantly decreased to 38% and 35% in α2A- and α2C-KO mice, compared with 60% in wild-type mice and α2C-KO mice, after aortic stenosis (Figure 2c). Decreased cardiac contractility was evident 3 months after aortic constriction as a reduction of the maximal rate of left ventricular pressure increase (dp/dt max) in α2A- and α2C-KO mice (Figure 2d). Similarly, stroke volume and cardiac output were lower in α2A-KO and in α2C-KO animals.
Sympathetic Norepinephrine Release and Circulating Catecholamines in \( \alpha_2 \)-Adrenoceptor–Deficient Mice

To assess the presynaptic feedback regulation of norepinephrine release after aortic constriction, mouse atria were isolated and incubated in vitro in the presence of \(^{[3]}\)H-norepinephrine, and the release of radioactive neurotransmitter was activated by stimulation with short electrical impulses. In atria from wild-type mice, the nonsubtype-selective \( \alpha_2 \)-agonist UK14304 inhibited norepinephrine release by 84% (Figure 4a). However, in atria from \( \alpha_2A \)-KO or \( \alpha_2C \)-KO mice, the inhibitory effect of the \( \alpha_2 \)-agonist was significantly blunted (51% in \( \alpha_2A \)-KO, 68% in \( \alpha_2C \)-KO), demonstrating that both \( \alpha_2A \)- and \( \alpha_2C \)-adrenoceptors are required to control sympathetic catecholamine release after aortic banding. Feedback inhibition in \( \alpha_2B \)-KO atria did not differ from inhibition in wild-type atria (data not shown).

Transverse aortic constriction caused significant sympathetic activation in wild-type and \( \alpha_2 \)-adrenoceptor–deficient mice. Plasma norepinephrine levels were increased in wild-type, \( \alpha_2A \)-KO, \( \alpha_2B \)-KO, and \( \alpha_2C \)-KO mice after aortic banding compared with sham-operated mice (Figure 4b and data not shown). However, in \( \alpha_2C \)-KO mice, circulating norepinephrine levels were significantly higher than in other genotypes, supporting the role of the \( \alpha_2A \)-adrenoceptor as the major presynaptic regulator of sympathetic norepinephrine release. In contrast, plasma epinephrine concentrations were only elevated in \( \alpha_2A \)-KO and \( \alpha_2C \)-KO mice after aortic constriction (Figure 4c). Thus, total circulating levels of the catecholamines epinephrine plus norepinephrine were significantly higher in \( \alpha_2A \)-KO and \( \alpha_2C \)-KO mice than in wild-type or \( \alpha_2B \)-adrenoceptor–deficient mice. Taken together, \( \alpha_2A \)-KO and \( \alpha_2C \)-KO mice were more likely to develop lethal heart failure after cardiac pressure overload than wild-type mice or mice lacking \( \alpha_2B \)-receptors.

Enhanced Heart Failure in Human Patients Carrying a Deletion Variant of the \( \alpha_2C \)-Adrenoceptor

The present findings in mice may be of considerable relevance for human heart disease. Several sequence variants have been identified in the coding regions of human \( \alpha_2 \)-adrenoceptor genes. \(^{17,18}\) To test whether heart failure patients show clinical and hemodynamic traits that are associated with the \( \alpha_2 \)-receptor genotype, we investigated healthy control subjects and patients with chronic heart failure for the presence of \( \alpha_2 \)-receptor variants (Figure 5). Because of low allele frequency, we could not find any subject carrying the single amino acid variation Asn251Lys in the \( \alpha_2A \)-adrenoceptor in our study population (\( \alpha_2A \)-Asn251Lys). \(^{18}\) In contrast, 11% of the heart failure patients had a 4-amino acid deletion in the third intracellular loop of the \( \alpha_2C \)-adrenoceptor (\( \alpha_2C \)-Del322-325), which was associated with decreased G protein coupling of this receptor variant. \(^{17}\) The frequency of the \( \alpha_2C \)-Del322-325 variant was similar in the healthy control population (11.4%). Patients with the \( \alpha_2C \)-Del322-325 polymorphism did not differ in age, underlying cause of heart failure, or drug therapy from those patients with fully functional \( \alpha_2C \)-adrenoceptors. However, heart failure patients
carrying the α2C-receptor deletion variant had a worse clinical status (NYHA class, Figure 5c) and significantly decreased cardiac function, as determined by invasive catheterization and by echocardiography (Figures 5d and 5e).

Discussion

Our study demonstrates that genetic dysfunction of α2-adrenoceptors is associated with disease progression in transgenic mouse models and in human patients with heart failure. Targeted deletion of α2A- or α2C-adrenoceptors in mice impaired feedback inhibition of norepinephrine release from sympathetic nerves, thus leading to enhanced norepinephrine release and elevated circulating catecholamine levels. After aortic constriction, enhanced sympathetic activity is an essential mechanism to increase left ventricular contractility to maintain arterial blood pressure and organ perfusion distal to the aortic stenosis. In wild-type mice or animals lacking functional α2A-adrenoceptors, increased sympathetic tone led to compensated left ventricular hypertrophy, and only few animals (14%) died from cardiac decompensation and failure within 3 months after the aortic constriction (Figure 1). However, when one of the two presynaptic feedback regulators, α2A- or α2C-receptors, was lacking in sympathetic nerves because of genetic deletion in mice, excessive activation of the adrenergic system led to significantly higher levels of circulating catecholamines, thus facilitating the progression from compensated cardiac hypertrophy to heart failure.

Interestingly, deletion of one α2-adrenoceptor subtype could not be compensated for by the other α2-receptor subtype. This finding additionally supports the hypothesis that α2A- and α2C-adrenoceptors may have distinct roles in the presynaptic regulation of neurotransmitter release.16,29 In isolated tissues, α2A-receptors inhibited norepinephrine release at higher stimulation frequencies than α2C-receptors, and presynaptic inhibition mediated by α2A-receptors occurred much faster than inhibition by the α2C-subtype. Under resting conditions, only deletion of the α2A-receptor caused increased norepinephrine release and tachycardia,20,30 but α2C-receptor–deficient animals showed unaltered cardiovascular function.22 Thus, α2A- and α2C–receptors may differentially control the adrenergic system at rest and during times of maximal activation.

These findings may have great relevance for human cardiac disease. We have observed that heart failure patients who carry a signaling-deficient variant of the α2C-adrenergic receptor (α2C-Del322-325) suffer from more severe heart failure and decreased cardiac function than patients with intact α2A-adrenoceptors. When expressed in Chinese hamster ovary cells, α2C-adrenoceptors with a deletion of 4 amino acids (Gly-Ala-Gly-Pro) in the third intracellular receptor domain showed decreased high-affinity agonist binding, indicating impaired formation of the receptor G protein complex.17 Thus, to a certain extent, the biological consequences of the loss of function of the human α2C-adrenoceptor variant α2C-Del322-325 may be similar to the targeted deletion of the α2C-receptor gene in mice. This hypothesis is supported by the fact that α2C-adrenoceptors were previously identified by pharmacological ligands to control the release of norepineph-
Identification of human heart failure patients who carry mutations in the genes encoding for \( \alpha_2 \)-adrenoceptors may thus represent an important strategy for pharmacogenetic risk stratification in chronic heart failure. Future studies in healthy subjects are required to demonstrate that also in humans dysfunction of the \( \alpha_2 \)-receptor is linked to enhanced catecholamine release.

Several experimental and clinical studies have recently tested the concept of sympathetic inhibition by \( \alpha_2 \)-receptor agonists. \(^{32}\) Activation of central \( \alpha_2 \)-adrenoceptors by clonidine or moxonidine suppressed the sympathetic nervous system in congestive heart failure.\(^7\)–\(^9\) However, moxonidine had serious adverse effects and was even associated with increased lethality of patients.\(^8,9\) Our data from gene-targeted mice suggest that subtype-specific activation of \( \alpha_2 \)-receptor subtype may be advantageous over nonselective \( \alpha_2 \)-receptor stimulation to prevent serious side effects of \( \alpha_2 \)-agonists. Some of the biological functions of \( \alpha_2A \)-adrenoceptors, eg, central hypotension\(^{20,33}\) and sedation,\(^{34}\) contribute to the clinically unwanted side effects of nonsubtype-selective \( \alpha_2 \)-agonists. In contrast, \( \alpha_2C \)-receptors do not play a major role in the central regulation of sympathetic tone or sedation,\(^{35}\) but they control sympathetic catecholamine release primarily at the peripheral nerve terminals.

Thus, \( \alpha_2A \) and \( \alpha_2C \)-adrenoceptors are essential to control circulating levels of epinephrine and norepinephrine, and dysfunction of these receptors is associated with heart failure progression in transgenic mouse models as well as in patients with chronic heart failure. In addition, \( \alpha_2C \)-adrenoceptors represent a novel therapeutic target to attenuate or prevent the development of heart failure and other diseases that are attributable to chronic dysfunction in regulating catecholamine release.

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Figure 5. Decreased cardiac function in patients with chronic heart failure carrying a deletion variant of the \( \alpha_{2C} \)-adrenoceptor. (a) In the third intracellular domain of the human \( \alpha_{2C} \)-adrenoceptor, a 4-amino-acid deletion (\( \alpha_{2C} \)-Del322-325) was detected that was deficient in receptor G protein coupling.37 b, DNA sequence chromatogram (antisense strand) of a region corresponding to part of the third intracellular loop of the \( \alpha_{2C} \)-adrenoceptor from a heterozygous individual. Sequencing with a reverse primer revealed a 12-base pair deletion (red box). The allele frequency of this \( \alpha_{2C} \)-receptor variant was similar between healthy humans (5.5%; control, 12 of 210 [5.7%]). Heart failure patients with the 2C-Del polymorphism showed a more severe clinical phenotype in comparison with subjects carrying the wild-type receptor (NYHA class, c) and impaired left ventricular ejection fraction (d) and contractility (e). *P<0.05, \( \alpha_{2C} \)-Del vs WT.

References


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