Direct Inhibition of Cardiac Hyperpolarization-Activated Cyclic Nucleotide–Gated Pacemaker Channels by Clonidine
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Sympathetic control of heart rate plays an important role in the pathophysiology of arrhythmias, hypertension, coronary heart disease, and chronic heart failure. At present, 3 pharmacological strategies are used in clinical medicine to reduce increased sympathetic tone, including α2-adrenoceptors, β-adrenoceptor antagonists, and, most recently, hyperpolarization-activated cyclic nucleotide–gated (HCN) pacemaker channel inhibitors.1,2 The first antisypathetic drug established in clinical therapy was clonidine (for reviews, see Schmitt3 and Hoefke and Kobinger4). Investigation into the mechanism of action of clonidine led to the identification of α2-adrenoceptors as the main target of the action of clonidine.5 Despite the fact that clonidine has vasoconstrictive properties, it was introduced into clinical practice as an antihypertensive and antisypathetic drug. Clonidine may act at 2 anatomic sites to lower blood pressure.6 In several brain stem nuclei, activation of α2-adrenoceptors leads to a reduction in sympathetic tone. In addition, clonidine may activate presynaptic inhibitory α2-adrenoceptors on postganglionic sympathetic fibers to lower sympathetic norepinephrine release.

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Later, pharmacological ligands were applied to identify subtypes of α2-receptors, which were confirmed by molecular cloning of 3 independent α2-adrenoceptor genes from different species (α2A, α2B, and α2C).7 The physiological significance of the 3 α2-adrenoceptor subtypes was then highlighted by targeted deletions in the murine genes.8 With these gene-targeted mouse models, the 2 major actions of clonidine and other α2-agonists, hypotension and sedation, could be assigned to activation of α2A-receptors, whereas α2B-receptors were involved in vasoconstriction, and α2C took part in modulation of catecholamine release.9-11 However, several
reports had suggested that not all of the effects of clonidine were dependent on \( \alpha_2 \)-adrenoceptors, which led to the development of the "imidazoline receptor hypothesis."\(^{11,12}\) Several different imidazoline binding sites were proposed (for review, see Szabo\(^\text{\textregistered}\)); however, the molecular identity of the putative \( \alpha_2 \) imidazoline receptor that was suggested to be responsible for the hypotensive effect of clonidine and other imidazolines has not yet been uncovered.

To search for non-\( \alpha_2 \)-adrenoceptor effects of clonidine, we have generated mice lacking all 3 \( \alpha_2 \)-adrenoceptors (\( \alpha_{2ABC}^{-/-} \)). This led to the identification of a direct bradycardic effect of clonidine by inhibition of the cardiac hyperpolarization-activated ("pacemaker") current (\( I_f \)). \( I_f \) has been shown to play a key role in the generation of pacemaker potentials in sinoatrial node (SAN) cells of the heart.\(^{1,2,13}\) Moreover, \( I_f \) is enhanced by direct interaction with cyclic adenosine monophosphate and, hence, contributes to the autonomous regulation of heart rate by the sympathetic and parasympathetic nervous system. \( I_f \) is encoded by a family of 4 HCN channels (HCN1–4).\(^{14}\) In mouse SAN, HCN4 and HCN2 are the predominantly expressed HCN channel isoforms.\(^{15-17}\) The same isoforms have been also detected in human heart tissue.\(^{18}\) Mouse SAN does not express substantial levels of HCN1, but higher levels of this subunit (\( \approx 20\% \) of total HCN mRNA) were found in rabbit SAN.\(^{15}\) Here, we show that clonidine blocks both HCN2 and HCN4 channels in the low micromolar concentration range and, as a consequence, lowers the frequency of pacemaker potentials.

**Methods**

**Generation of \( \alpha_{2ABC}^{-/-} \) Mice**

The generation of \( \alpha_{2ABC}^{-/-} \) has been described previously.\(^{19}\) From the initial intercrossing of Adra2a\(^{-/-}\)Adra2b\(^{-/-}\)/Adra2c\(^{-/-}\) mice, a small percentage survived a defect in placental development. These mice were used to establish an independent colony of \( \alpha_{2ABC} \)-deficient mice. Mice were maintained in a specified-pathogen-free facility. All animal procedures were approved by the Universities of Freiburg and Würzburg.

**Autoradiography and Radioligand Binding**

Mouse brain membranes\(^{20}\) were incubated in binding buffer containing (in mmol/L): 25 glycylglycine, 40 HEPES (pH 8), 5 EGTA, 5 MgCl\(_2\), 100 NaCl, 8 \( [H]1,4\)-benzodioxan-2-methoxy-2-y12-imidazoline hydrochloride (RX821002). Nonspecific binding was determined in the presence of 1 \( \mu \)mol/L atipamezol. For receptor autoradiography, transverse cryostat sections of the brain (10 \( \mu \)m) were incubated for 60 minutes in 50 mmol/L Tris-HCl (pH 7.5), 1.5 mmol/L EDTA, and 8 mmol/L \( [H]RX821002 \). Slides were exposed to \( [H] \)-Hyperfilm (Amersham Pharmacia, Freiburg, Germany) for 16 to 24 weeks.

**Norepinephrine Release**

\( [H] \)-norepinephrine release was determined in cardiac atria essentially as described previously with minor modifications.\(^{11,20}\) \( [H] \)-norepinephrine release was evoked by short trains of rectangular electrical pulses (4 pulses, 100 Hz). The amount of radioactivity released from the tissues was determined by liquid scintillation counting.\(^{11}\)

**Twenty-Four-Hour Urinary Catecholamine Determination**

Catecholamine excretion was quantified by high-performance liquid chromatography combined with electrochemical detection of urine samples collected over 24-hour periods in metabolic cages as described previously.\(^{21}\)

**Sedation and Analgesia**

Fifteen minutes after clonidine injection (1 mg/kg IP), mice were placed 3 times on a rotating wheel (rotating speed 10 rpm); maximal cutoff time was 60 seconds. For analgesia testing, a tail-flick assay system (Ugo Basile, Comerio, Italy), equipped with an infrared light source and automatic recording of the reaction time, was used.

**Hemodynamic Measurements**

For cardiac, aortic, or femoral artery catheterization with a 1.4F Millar microtip device, mice were anesthetized by isoflurane (2 vol%) in \( O_2 \) applied by face mask and kept on a heating table at 37°C.\(^{22}\) Hemodynamic parameters were digitized via a MacLab system (AD Instruments, Castle Hill, Australia). Transhoracic echocardiography Doppler examinations were performed in lightly sedated (200 \( \mu \)L 2.5% tribromethanol IP) mice with an echocardiographic system (Acuson Sequoia C512, Siemens AG, Erlangen, Germany) equipped with a 15-MHz linear transducer (Acuson, 15L8). For measurements in conscious, unrestrained mice, blood pressure and ECGs were recorded by telemetry (DSI, Transoma Medical, St. Paul, Minn; PA10 for aortic pressure, TC10 for ECG) 10 to 20 days after surgery.

**Histology**

Hearts were fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), embedded in paraffin, and stained with hematoxylin-eosin. Left ventricular myocyte cross-sectional areas were analyzed by computer-assisted morphometry. To detect interstitial fibrosis, hearts were stained with Sirius red as described previously.\(^{22}\)

**Organ Bath Experiments**

Hearts were rapidly excised and placed in carbogenated modified Tyrode’s solution (concentrations in mmol/L: 119 NaCl, 5.4 KCl, 1.4 CaCl\(_2\), 1 MgCl\(_2\), 22.6 NaHCO\(_3\), 0.42 NaH\(_2\)PO\(_4\), 0.025 EDTA, 10 glucose, 0.2 ascorbic acid, pH 7.4). Right atria of 3- to 4-month-old mice were mounted in an organ bath chamber and were allowed to contract spontaneously. \( \alpha_{2ABC} \)-Knockout (KO) mice were injected 16 hour antemortem with pertussis toxin 150 \( \mu \)g/kg IP (Sigma, Munich, Germany).\(^{23}\)

**Cell Culture and Isolation of Murine SAN Cells**

Human embryonic kidney (HEK)-293 cell lines stably expressing either murine HCN2 or human HCN4 were maintained as described previously.\(^{16,24}\) SAN cells were isolated from 6- to 12-week-old adult \( \alpha_{2ABC}^{-/-} \) and \( \alpha_{2ABC}^{-/-} \) mice of either sex by standard procedures.\(^{25,26}\)

**Electrophysiological Recordings**

Native \( I_f \) and heterologously expressed HCN channels were measured at room temperature with the whole-cell voltage-clamp technique as described previously.\(^{28}\) The extracellular solution was composed of (in mmol/L): 135 NaCl, 5 KCl, 1.8 CaCl\(_2\), 0.5 MgCl\(_2\), 5 HEPES, pH 7.4. For recordings of \( I_f \) in SAN cells, 1 mmol/L BaCl\(_2\) and 2 mmol/L MnCl\(_2\) were added to the extracellular solution. The intracellular solution contained (in mmol/L): 130 KCl, 1 NaCl, 0.5 MgCl\(_2\), 1 EGTA, 5 HEPES, pH 7.4. Spontaneous action potentials of isolated SAN cells were recorded at 30°C with the perforated patch technique with 120 \( \mu \)g/mL amphotericin B. Effects of clonidine were determined with a repetitive stimulation protocol. Hyperpolarizing pulses of 1.0-second duration (for HCN2, HCN1, and native \( I_f \); test potential –100 mV for HCN2 and native \( I_f \) and –90 mV for HCN1) or 1.5-second duration (for HCN4, test potential –110 mV) were applied from a holding potential of –40 mV every 2 seconds (HCN2, HCN1, and native \( I_f \)) or every 3 seconds (HCN4), and the resulting inward currents were determined. The longer pulse duration for HCN4 was chosen with respect to the slow activation kinetics of this channel. For determination of dose-response relationships, the maximum inward current corrected for the instantaneous current component of \( I_f \)\(^{27}\) was obtained after repetitive stimulation for 1 minute. \( I_{\text{Ca}} \) values and Hill coefficients (\( n \)) were calculated by fitting the Hill equation. Steady-state activation curves were determined by hyperpolarizing voltages of –140 to –30 mV from a holding potential of –40 mV for 2.4 seconds followed by a step to
−140 mV. Tail currents, measured immediately after the final step to −140 mV, were normalized by the maximal current (I_{max}) and plotted as a function of the preceding membrane potential. The data points were fitted with the Boltzmann function: $(I-I_{min})/(I_{max}-I_{min}) = 1/[1+exp((V_m-V_{0.5})/k)]$ where $I_{min}$ is an offset caused by a nonzero holding current, $V_m$ is the test potential, $V_{0.5}$ is the membrane potential for half-maximal activation, and k is the slope factor.

**Statistical Analysis**

Data were analyzed by ANOVA followed by appropriate post hoc tests, by Student $t$ test for unpaired samples, by paired-samples $t$ test, or by repeated-measures test when appropriate. A probability value of $<0.05$ was considered statistically significant. Results are displayed as mean±SEM.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Generation of Mice Deficient in α2ABC-Adrenoceptors**

Mice lacking all 3 α2-adrenoceptor subtypes (α2ABC−/−) were derived from matings of male and female Adra2a−/−Adra2b−/−Adra2c−/− mice. Initially, a high percentage of α2ABC−/− mice died during embryonic development due to a defect in placental vascular development; however, from surviving α2ABC−/− mice, a breeding colony could be established (Figure 1). Several methods were applied to document the deletion of all 3 α2-adrenoceptor genes. Autoradiography with the α2c-receptor antagonist [3H]RX821002 revealed a high density of α2c-receptor binding sites in wild-type brain. In the presence of the specific α2c-receptor antagonist atipamezole, this signal was absent in α2ABC−/− brains and was also undetectable in brain sections of α2ABC−/− mice (Figure 1b). Similarly, quantitative radioligand binding did not detect any specific α2c-adrenoceptors in brain membranes of α2ABC−/− mice (Figure 1c).

To further verify the complete deletion of the 3 α2-adrenoceptor genes, pharmacological tests for typical α2-receptor functions were performed. Intraperitoneal injection of the α2-agonist clonidine resulted in a shortened latency time on a rotating wheel, which demonstrates its sedating effect in α2ABC−/− mice (Figure 2a). In contrast, clonidine did not induce sedation in α2ABC−/− mice (Figure 2a). Similarly, the analgesic properties of clonidine could be verified by an increased latency time in the tail-flick assay in wild-type mice but not in α2ABC−/− mice (Figure 2b). Another important function of α2-adrenoceptors is their role in presynaptic feedback inhibition of neurotransmitter release. In isolated heart atria from wild-type mice, norepinephrine (Figure 2c) and the α2-agonist medetomidine (not shown) inhibited the electrically evoked release of [3H]norepinephrine in a concentration-dependent manner. In α2ABC−/− atria, norepinephrine did not inhibit sympathetic transmitter release. Genetic disruption of presynaptic feedback inhibition also resulted in enhanced sympathetic neurotransmitter release in vivo, as evidenced by increased norepinephrine excretion in 24-hour urine samples of α2ABC−/− compared with wild-type mice (Figure 2d).

**Cardiovascular Function in α2ABC-Deficient Mice**

To determine the long-term consequences of enhanced sympathetic tone, we first assessed cardiovascular function in conscious, freely-moving α2ABC−/− mice by telemetry (Figures 3a through 3c). Enhanced sympathetic norepinephrine release in α2ABC−/− mice was accompanied by increased systolic and diastolic blood pressures and elevated heart rate (Figures 3a through 3c). At the age of 6 months, however, cardiac function was already compromised. Left ventricular fractional shortening was reduced to 33% in α2ABC−/− animals compared with 50% in wild-type mice (Figure 3d). In addition, severe cardiac fibrosis and hypertrophy were detected in left ventricles of α2ABC−/− hearts (Figures 3e through 3g).

Next, we assessed the effects of the α2-agonist clonidine on blood pressure and heart rate. In wild-type mice, clonidine significantly reduced mean arterial pressure and heart rate during isoflurane anesthesia (Figure 4a). Surprisingly, the bradycardic effect of clonidine was still present in α2ABC−/− mice (Figure 4a), whereas its hypotensive effect was com-
clonidine was completely absent (Figure 5c). In wild-type but not in α2ABC−/− mice, clonidine (1 mg/kg IP) significantly reduced the latency time on a rotarod (Figure 5c); however, in the presence of 2 mmol/L CsCl, clonidine also lowered heart rate in nephrine inhibited electrically evoked release of [3H]norepinephrine in wild-type atria but not in α2ABC−/− atria (n=6 experiments). Twenty-four-hour urinary excretion of norepinephrine was significantly elevated in α2ABC−/− mice compared with wild-type mice (α2ABC−/− n=8, α2ABC+/+ n=10 male mice). *P<0.05.

To identify the site of the bradycardic action of clonidine, isolated spontaneously beating right atria were tested in an organ bath system. Clonidine reduced beating frequency by 20% to 25% in atria from both genotypes (Figure 5a). Clonidine concentrations reducing the right atrial frequency by 50% did not differ between genotypes (α2ABC+/+ 4.9 μmol/L versus α2ABC−/− 4.4 μmol/L). The concentrations required to lower spontaneous beating rate by 20% did not differ between clonidine and the HCN inhibitor ZD7288 (Figure 5a, inset). After pertussis toxin pretreatment of mice to inactivate G proteins of the Gi family, the bradycardic effect of clonidine was completely eliminated (Figure 5b). Pertussis toxin did not affect the clonidine-induced bradycardia, which ruled out the possibility that Gi-coupled receptors mediated the bradycardia. Similarly, incubation with 100 μmol/L Ba2+, a blocker of inwardly rectifying K+ channels, did not alter the clonidine effect (Figure 5c); however, in the presence of 2 mmol/L Cs+, an established blocker of cardiac If, the clonidine-induced bradycardia was completely absent (Figure 5c).

Clonidine Is an Efficient Blocker of Sinoatrial If
The strong Cs+ sensitivity of the bradycardic effect of clonidine suggested that clonidine may act via If channels. To explore this hypothesis, we first characterized spontaneous action potentials of pacemaker cells isolated from the SAN of wild-type and α2ABC−/− mice (Figures 6a and 6b). Clonidine
profundely lowered the frequency of pacemaker potentials in SAN cells from both genotypes. Clonidine increased the duration between 2 peaks (cycle length) from 455±48 to 1105±223 ms (n=6) in wild-type mice and from 605±45 to 895±98 ms (n=4) in α2ABC−/− mice (Figures 6a and 6b). Although the increase in cycle length induced by clonidine was highly significant in both genotypes, the absolute values of cycle length in the absence and presence of clonidine, respectively, were not statistically different between genotypes. The frequency reduction of pacemaker potentials by clonidine was accompanied by a reduction of the slope of the diastolic depolarization (wild-type: from 79.7±6.6 mV/s; n=6, *P<0.05; α2ABC−/−: from 62.1±9.9 to 34.1±7.9 mV/s; n=4, *P<0.05). In contrast, other parameters of the pacemaker potential (maximum diastolic potential, overshoot, and action potential duration) were unaffected by clonidine (Data Supplement, Table I). The reduction of the slope of diastolic depolarization could be caused by an inhibition of the If current. Indeed, clonidine efficiently blocked this current in a dose-dependent manner (Figures 6c and 6d). The IC50 values at −100 mV were 3.1±0.5 μmol/L (n=5 to 11) and 2.8±0.7 μmol/L (n=5 to 8) for wild-type and α2ABC−/− mice, respectively, which is in excellent agreement with the IC50 values determined in beating right atria (Figure 5a). If had the same amplitude in wild-type and α2ABC−/− cells (−5.3±0.8 versus −6.1±1 pA/pF; n=13, *P=0.2). Moreover, kinetics and voltage-dependence of If were indistinguishable between genotypes (data not shown). Cardiac If is mediated by HCN4 and HCN2 channels; therefore, we tested the effect of clonidine on HEK293 cell lines that stably expressed either or both channels (Figures 7a and 7b). Clonidine inhibited both channels in a dose-dependent manner. The IC50 values were slightly higher than those of native If (9.8±1.4 μmol/L [n=7 to 12] for HCN4 and
Interestingly, in the presence of 100 μmol/L H9262 concentration is given in parentheses.

Figure 7. Inhibition of heterologously expressed HCN4 and HCN2 channels by clonidine. a and b, Action potentials of SAN pacemaker cells from α2ABC+/+ (a) and α2ABC−/− (b) mice under control conditions (black) and in the presence of increasing extracellular clonidine. c, f current traces from an α2ABC−/− SAN cell in the presence of clonidine as indicated. Currents were evoked by stepping from a holding potential of −40 to −100 mV. d, Dose-response relationships for inhibition of f by clonidine in SAN cells of α2ABC+/+ (open symbols) and α2ABC−/− mice (closed symbols). Solid lines are the fits to the Hill equation with the following parameters: IC50=3.18 μmol/L, v=0.56; α2ABC−/−: IC50=2.67 μmol/L, v=0.56. The number of experiments for each concentration is given in parentheses.

Discussion

The main finding of the present study is the identification of a direct inhibitory effect of the α2-receptor agonist, clonidine, on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions of HCN1 and clonidine may competitively bind to this cation, the clonidine binding curve for HCN2 was shifted to the right (IC50=16.3±1.4 μmol/L; n=12). This finding suggested that Cs+ and clonidine may competitively bind to the same channel region. Clonidine not only blocked HCN1 currents, although with significantly lower sensitivity (IC50=40.1±4.34 μmol/L; n=7 to 9). By contrast, clonidine had virtually no effect on voltage-gated calcium and sodium channels (Data Supplement, Figure I).

Clonidine reduces the frequency of spontaneous SAN pacemaker potentials by blocking If. α2ABC+/+ mice (closed symbols) and α2ABC−/− mice under control conditions (open symbols) and in the presence (red) of 10 μmol/L extracellular clonidine. c, f current traces from an α2ABC−/− SAN cell in the presence of clonidine as indicated. Currents were evoked by stepping from a holding potential of −40 to −100 mV. d, Dose-response relationships for inhibition of f by clonidine in SAN cells of α2ABC+/+ (open symbols) and α2ABC−/− mice (closed symbols). Solid lines are the fits to the Hill equation with the following parameters: IC50=3.18 μmol/L, v=0.56; α2ABC−/−: IC50=2.67 μmol/L, v=0.56. The number of experiments for each concentration is given in parentheses.

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To verify that no functional α2-adrenoceptors remained in α2ABC−/− mice, we performed a number of experiments. Radioligand binding experiments and autoradiography confirmed the absence of α2-adrenoceptor protein in α2ABC−/− mice. α2-Receptors were originally described as the adrenoceptors acting in a presynaptic feedback loop to inhibit neurotransmitter release from adrenergic nerves (for review, see Starke28). Indeed, presynaptic feedback inhibition was completely deficient in α2ABC−/− mice on the basis of the following results: (1) The endogenous sympathetic neurotransmitter norepinephrine could not inhibit the electrically evoked release of [3H]norepinephrine from isolated α2ABC−/− atria (Figure 2c). (2) Disruption of presynaptic feedback in sympathetic nerves resulted in elevated excretion of urinary norepinephrine. (3) As a consequence of increased sympathetic norepinephrine release, blood pressure and heart rate

8.2±1.4 μmol/L [n=8 to 10] for HCN2; Figures 7c and 7d). Interestingly, in the presence of 100 μmol/L Cs+ ions, which corresponds to the half-maximal inhibitory concentration of this cation, the clonidine binding curve for HCN2 was shifted through 8d). Clonidine also inhibited HCN1 currents, although with significantly lower sensitivity (IC50=40.1±4.34 μmol/L; n=7 to 9). By contrast, clonidine had virtually no effect on voltage-gated calcium and sodium channels (Data Supplement, Figure I).
were increased in α2ABC−/− mice. (4) Chronic elevation of sympathetic tone led to the typical signs of cardiac damage, left ventricular hypertrophy and fibrosis. In addition, typical pharmacological effects of α2-agonists, including hypotension, sedation, and analgesia, were completely absent in α2ABC−/−-deficient mice. Taken together, these experiments demonstrate that α2ABC−/− mice do not express any functional α2-adrenoceptors.

Most surprisingly, clonidine elicited significant bradycardic effects in vivo and in isolated atria of α2ABC−/− mice. Previously, some authors have reported that clonidine inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations. The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency of isolated right atria of mice (n=6); control, V0.5=−18.0 mV, k=18.0 mV. In α2ABC−/− mice (n=6); control, V0.5=−9.1 mV, k=11.3 mV; at 10 μmol/L clonidine, V0.5=−104 mV, k=13.2 mV. The differences between V0.5 values of Ic of SAN cells from α2ABC+/+ and α2ABC−/− are not statistically significant.

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One of the important questions is whether inhibition of HCN channels contributes to the pharmacological actions of clonidine in vivo. The present experiments with ECG telemetry in conscious, freely moving α2ABC−/− mice demonstrated that clonidine doses starting at 10 μg/kg elicited a significant bradycardic effect (Figure 4b). Most importantly, at all doses tested in the present study, the bradycardic effect of clonidine in α2ABC−/− mice was 26% to 43% of its effect in wild-type mice. This indicates that in vivo inhibition of cardiac HCN channels by clonidine occurs in the same dose range as α2-receptor activation (ie, 10 to 300 μg/kg) (Figure 4b). These clonidine doses are at the lower end of the spectrum of doses (30 μg/kg to 50 mg/kg) that have previously been used to investigate α2-adrenoceptor–mediated functions in mice. The present findings in mice are consistent with a recent report demonstrating that clonidine elicited significant bradycardia but no hypotension in mice with a targeted mutation (D79N) in the α2-adrenoceptor gene. Furthermore, the clonidine doses applied in the present study are significantly lower than doses determined for specific Ic channel inhibitors. For example, the ED50 value of ivabradine, the only Ic channel inhibitor that has been introduced into therapy so far, is ≈5 mg/kg in mice. However, it remains to be determined whether the observed bradycardia also contributes to the therapeutic effects of clonidine in humans. Clonidine doses used for antihypertensive therapy in humans are typically in the range of 150 to 900 μg/d, but doses up to 3600 μg/d have also been used in patients with essential hypertension. Interestingly, in tetraplegic patients with complete cervical spinal cord transection and preganglionic sympathetic denervation, clonidine significantly lowered heart rate without affecting blood pressure. Inhibition of cardiac HCN channels by clonidine in humans may be of particular relevance during high-dose application of the drug, including rapid intravenous injection during hypertensive crisis or opioid detoxification.

The present data do not lend support to the “imidazoline hypothesis” of the action of clonidine. We have not been able to obtain any results that are consistent with the HCN channel being an “imidazoline receptor.” According to the imidazoline hypothesis, Ic receptor agonists should lower blood

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**Figure 8.** Clonidine shifts the voltage dependency of HCN channel activation to more hyperpolarizing voltages. Activation curves of HCN4 (a), HCN2 (b), and native Ic from SAN cells of α2ABC+/+ (c) and α2ABC−/− mice (d) in the absence (black symbols) and the presence (red symbols) of clonidine. Solid lines are fits to the Boltzmann equation with the following parameters: HCN4 (n=6); control, V0.5=−97.0 mV, k=10.6 mV; at 30 μmol/L clonidine, V0.5=−108 mV, k=8.48 mV. HCN2 (n=6); control, V0.5=−90.4 mV, k=7.08 mV; at 30 μmol/L clonidine, V0.5=−98.1 mV, k=6.30 mV. Ic of α2ABC−/− mice (n=3); control, V0.5=−84.5 mV, k=11.8 mV; at 10 μmol/L clonidine, V0.5=−108 mV, k=18.0 mV. Ic of α2ABC−/− mice (n=6); control, V0.5=−91.9 mV, k=11.3 mV; at 10 μmol/L clonidine, V0.5=−104 mV, k=13.2 mV. The differences between V0.5 values of Ic of SAN cells from α2ABC+/+ and α2ABC−/− are not statistically significant.
pressure, but they are not reported to be specific bradycardic agents. The present data are in line with previous reports that indicated that certain derivatives of clonidine, including N-allyl-clonidine (alinidine), act as specific bradycardic agents.

In conclusion, clonidine can directly inhibit cardiac HCN pacemaker channels and elicit a strong bradycardic effect. This finding may be of great relevance for other neuronal effects of clonidine and other ligands with imidazoline structure, because HCN channels are ubiquitously expressed in the nervous system. Thus, clonidine-like drugs with imidazoline structure may become novel lead structures in the search for future HCN channel inhibitors.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

Sympathetic control of heart rate plays an important role in the pathophysiology of arrhythmias, hypertension, coronary heart disease and chronic heart failure. At present, 3 pharmacological strategies are used in clinical medicine to reduce increased sympathetic tone, including α2-agonists, β-adrenoceptor antagonists, and, most recently, hyperpolarization-activated cyclic nucleotide–gated (HCN) pacemaker channel inhibitors. Activation of presynaptic α2-adrenoceptors is the most widely accepted mechanism of action of the antisympathetic drug clonidine; however, other target proteins have been postulated to contribute to the in vivo actions of clonidine. To test whether clonidine elicits pharmacological effects independent of α2-adrenoceptors, we have generated mice with a targeted deletion of all 3 α2-adrenoceptor subtypes (α2ABC−/−). α2ABC−/− mice were completely unresponsive to the analgesic and hypnotic effects of clonidine; however, clonidine significantly lowered heart rate in α2ABC−/− mice by up to 150 bpm. Clonidine-induced bradycardia in conscious α2ABC−/− mice was 32.3% (10 µg/kg) and 26.6% (100 µg/kg) of the effect in wild-type mice. Clonidine inhibited the native pacemaker current (If) in isolated murine sinoatrial node pacemaker cells and the If-generating HCN2 and HCN4 channels in transfected HEK293 cells. Clonidine also inhibited HCN1 currents, although with significantly lower sensitivity. As a consequence of blocking If, clonidine reduced the slope of the diastolic depolarization and the frequency of pacemaker potentials in sinoatrial node cells from wild-type and α2ABC-KO mice. Direct inhibition of cardiac HCN pacemaker channels contributes to the bradycardic effects of clonidine gene-targeted mice in vivo, and thus, clonidine-like drugs represent novel structures for future subtype-selective HCN channel inhibitors.