Direct Inhibition of Cardiac Hyperpolarization-Activated Cyclic Nucleotide–Gated Pacemaker Channels by Clonidine

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Background—Inhibition of cardiac sympathetic tone represents an important strategy for treatment of cardiovascular disease, including arrhythmia, coronary heart disease, and chronic heart failure. Activation of presynaptic α2-adrenoceptors is the most widely accepted mechanism of action of the antisypathetic drug clonidine; however, other target proteins have been postulated to contribute to the in vivo actions of clonidine.

Methods and Results—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. A similar bradycardic effect of clonidine was observed in isolated spontaneously beating right atria from α2ABC−/− knockout and wild-type mice. Clonidine inhibited the native pacemaker current (Ipf) in isolated sinoatrial node pacemaker cells and the If-generating hyperpolarization-activated cyclic nucleotide–gated (HCN) 2 and HCN4 channels in transfected HEK293 cells. As a consequence of blocking Ipf, clonidine reduced the slope of the diastolic depolarization and the frequency of pacemaker potentials in sinoatrial node cells from wild-type and α2ABC−/− knockout mice.

Conclusions—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 HCN channel inhibitors. (Circulation. 2007;115:872-880.)

Key Words: receptors, adrenergic, alpha ■ heart rate ■ ion channels ■ pharmacology

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) 2 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

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reports had suggested that not all of the effects of clonidine were dependent on α2-adrenoceptors, which led to the development of the "imidazoline receptor hypothesis." Several different imidazoline binding sites were proposed (for review, see Szabo); however, the molecular identity of the putative I<sub>i</sub> 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-α<sub>2</sub>-adrenoceptor effects of clonidine, we have generated mice lacking all 3 α<sub>2</sub>-adrenoceptors (α<sub>2ABC</sub>). This led to the identification of a direct bradycardiac effect of clonidine by inhibition of the cardiac hyperpolarization-activated ("pacemaker") current (I<sub>pac</sub>). I<sub>pac</sub> has been shown to play a key role in the generation of pacemaker potentials in sinoatrial node (SAN) cells of the heart.<sup>1,2,11</sup> Moreover, I<sub>pac</sub> 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<sub>pac</sub> is encoded by a family of 4 HCN channels (HCN1–4).<sup>14</sup> In mouse SAN, HCN4 and HCN2 are the predominantly expressed HCN channel isoforms.<sup>15,17</sup> The same isoforms have been also detected in human heart tissue.<sup>18</sup> Mouse SAN does not express substantial levels of HCN1, but higher levels of this subunit (≈20% of total HCN mRNA) were found in rabbit SAN.<sup>15</sup> 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 α<sub>2ABC</sub>-/- Mice**
The generation of α<sub>2ABC</sub>-/- has been described previously.<sup>19</sup> From the initial intercrossing of Adra2<sub>a</sub>-/-/Adra2b<sup>2ABC</sup>/Adra2c<sup>2ABC</sup> mice, a small percentage survived a defect in placental development. These mice were dependent on an independent colony of α<sub>2ABC</sub>-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<sup>20</sup> were incubated in binding buffer containing (in mmol/L): 25 glycyglycine, 40 HEPES (pH 8), 5 EGTA, 5 MgCl<sub>2</sub>, 100 NaCl, 8 [<sup>3</sup>H](1,4-benzodioxan-2-methoxy-2-yl)imidazoline hydrochloride (RX821002). Non-specific binding was determined in the presence of 1 μmol/L atipamezole. For receptor autoradiography, transverse cryostat sections of the brain (10 μm) were incubated for 60 minutes in 50 mmol/L Tris-HCl (pH 7.5), 1.5 mmol/L EDTA, and 8 mmol/L [<sup>3</sup>H]RX821002. Slides were exposed to ²H-Hyperfilm (Amersham Pharmacia, Freiburg, Germany) for 16 to 24 weeks.

**Norepinephrine Release**
[<sup>3</sup>H]Norepinephrine release was determined in cardiac atria essentially as described previously with minor modifications.<sup>11,20</sup> [<sup>3</sup>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.<sup>11</sup>

**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.<sup>21</sup>

**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.

**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.<sup>22</sup>

**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<sub>2</sub>, 1 MgCl<sub>2</sub>, 22.6 NaHCO<sub>3</sub>, 0.42 NaH<sub>2</sub>PO<sub>4</sub>, 0.025 EDTA, 10 glucose, 0.2 acetic 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. α<sub>2ABC</sub>-Knockout (KO) mice were injected 16 hour antemortem with pertussis toxin 150 μg/kg IP (Sigma, Munich, Germany).<sup>23</sup>

**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.<sup>16,24</sup> SAN cells were isolated from 6- to 12-week-old adult α<sub>2ABC</sub>-/- and α<sub>2ABC</sub>-/- mice of either sex by standard procedures.<sup>25,26</sup>

**Electrophysiological Recordings**
Native I<sub>pac</sub> and heterologously expressed HCN channels were measured at room temperature with the whole-cell voltage-clamp technique as described previously.<sup>28</sup> The extracellular solution was composed of (in mmol/L): 135 NaCl, 5 KCl, 1.8 CaCl<sub>2</sub>, 0.5 MgCl<sub>2</sub>, 5 HEPES, pH 7.4. For recordings of I<sub>pac</sub> in SAN cells, 1 mmol/L BaCl<sub>2</sub> and 2 mmol/L MnCl<sub>2</sub> were added to the extracellular solution. The intracellular solution contained (in mmol/L): 130 KCl, 10 NaCl, 0.5 MgCl<sub>2</sub>, 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 μg/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<sub>pac</sub>) 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<sub>pac</sub> was obtained after repetitive stimulation for 1 minute. IC<sub>50</sub> values and Hill coefficients (n) were calculated by fitting with 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: 

\[ I = I_{max} \left[ 1 - \exp \left( \frac{V_m - V_0.5}{k} \right) \right] \]

where \( I_{max} \) 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's 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 \( \alpha_{2ABC} \)-Adrenoceptors**

Mice lacking all 3 \( \alpha \)-adrenoceptor subtypes (\( \alpha_{2ABC}^{-/-} \)) were derived from matings of male and female Adra2a−/−Adra2b−/−Adra2c−/− mice. Initially, a high percentage of \( \alpha_{2ABC}^{-/-} \) mice died during embryonic development due to a defect in placental vascular development; however, from surviving \( \alpha_{2ABC}^{-/-} \) mice, a breeding colony could be established (Figure 1). Several methods were applied to document the deletion of all 3 \( \alpha \)-adrenoceptor genes. Autoradiography with the \( \alpha \_2 \)-receptor antagonist [\( ^{3}H \)RX821002 revealed a high density of \( \alpha \_2 \)-receptor binding sites in wild-type brain. In the presence of the specific \( \alpha_{2ABC}^{-/-} \) antagonist atipamezole, this signal was absent in \( \alpha_{2ABC}^{+/+} \) brains and was also undetectable in brain sections of \( \alpha_{2ABC}^{-/-} \) mice (Figure 1b). Similarly, quantitative radioligand binding did not detect any specific \( \alpha \)-adrenoceptors in brain membranes of \( \alpha_{2ABC}^{-/-} \) mice (Figure 1c).

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

**Cardiovascular Function in \( \alpha_{2ABC}^{-/-} \)-Deficient Mice**

To determine the long-term consequences of enhanced sympathetic tone, we first assessed cardiovascular function in conscious, freely-moving \( \alpha_{2ABC}^{-/-} \) mice by telemetry (Figures 3a through 3c). Enhanced sympathetic norepinephrine release in \( \alpha_{2ABC}^{-/-} \) 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 \( \alpha_{2ABC}^{-/-} \) animals compared with 50% in wild-type mice (Figure 3d). In addition, severe cardiac fibrosis and hypertrophy were detected in left ventricles of \( \alpha_{2ABC}^{-/-} \) hearts (Figures 3e through 3g).

Next, we assessed the effects of the \( \alpha \)-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 \( \alpha_{2ABC}^{-/-} \) mice (Figure 4a), whereas its hypotensive effect was com-
I to inactivate G proteins of the Gi/o family, the bradycardic (Figure 5a, inset). After pertussis toxin pretreatment of mice required to lower spontaneous beating rate by 20% did not in wild-type but not in \( \alpha_{2ABC}^{-/-} \) mice. Similarly, incubation with 100 \( \mu \text{mol/L} \) Ba\(^{2+} \), a blocker of inwardly rectifying K\(^+\) channels, did not alter the clonidine effect (Figure 5c); however, in the presence of 2 \( \text{mmol/L} \) Cs\(^+\), an established blocker of cardiac \( I_f \), the clonidine-induced bradycardia was completely absent (Figure 5c).

Clonidine Is an Efficient Blocker of Sinoatrial \( I_f \)

The strong Cs\(^+\) sensitivity of the bradycardic effect of clonidine suggested that clonidine may act via \( I_f \) channels. To explore this hypothesis, we first characterized spontaneous action potentials of pacemaker cells isolated from the SAN of wild-type and \( \alpha_{2ABC}^{-/-} \) mice (Figures 6a and 6b). Clonidine
profoundly lowered the frequency of pacemaker potentials in SAN cells from both genotypes. Clonidine increased the duration between 2 peaks (cycle length) from 455±22 ms (n=6) in wild-type mice and from 605±45 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±8.1 to 45.6±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 I(V) 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
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⁺, which corresponds to the half-maximal inhibitory concentration of 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 Iₛ, it also shifted the voltage-dependence of channel activation by 10 to 20 mV to more hyperpolarizing potentials (Figures 8a 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 1).

**Discussion**

The main findings of the present study are 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 on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN). To uncover this effect of clonidine, we have generated mice with targeted deletions on cardiac pacemaker channels (HCN).

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
were increased in α_{2ABC}^{-/-} 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 α_{2}-agonists, including hypotension, sedation, and analgesia, were completely absent in α_{2ABC}^{-/-} mice. Taken together, these experiments demonstrate that α_{2ABC}^{-/-} mice do not express any functional α_{2}-adrenoceptors.

Most surprisingly, clonidine elicited significant bradycardic effects in vivo and in isolated atria of α_{2ABC}^{-/-} mice. Previously, some authors have reported that clonidine inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30 The bradycardic effect could be traced back to the sinus node, because clonidine also inhibited spontaneous beating frequency in isolated atria or Langendorff heart preparations.29,30

Figure 8. Clonidine shifts the voltage dependence of HCN channel activation to more hyperpolarizing voltages. Activation curves of HCN4 (a), HCN2 (b), and native I_{f} from SAN cells of α_{2ABC}^{+/+} (c) and α_{2ABC}^{-/-} 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=8): control, V_{0.5}=−97.0 mV, k=10.6 mV; at 30 μmol/L clonidine, V_{0.5}=−108 mV, k=8.48 mV. HCN2 (n=9): control, V_{0.5}=−90.4 mV, k=7.08 mV; at 30 μmol/L clonidine, V_{0.5}=−98.1 mV, k=6.30 mV. I_{f} of α_{2ABC}^{+/+} mice (n=3): control, V_{0.5}=−84.5 mV, k=11.8 mV; at 10 μmol/L clonidine, V_{0.5}=−108 mV, k=18.0 mV. I_{f} of α_{2ABC}^{-/-} mice (n=6): control, V_{0.5}=−91.9 mV, k=11.3 mV; at 10 μmol/L clonidine, V_{0.5}=−104 mV, k=13.2 mV. The differences between V_{0.5} values of I_{f} from SAN cells from α_{2ABC}^{+/+} and α_{2ABC}^{-/-} are not statistically significant.

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, I_{f} receptor agonists should lower blood
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 (alindine), 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


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**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 antisypathetic 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 KO). α2ABC KO mice were completely unresponsive to the analgesic and hypnotic effects of clonidine; however, clonidine significantly lowered heart rate in α2ABC KO mice by up to 150 bpm. Clonidine-induced bradycardia in conscious α2ABC KO 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 (I\(f\)) in isolated murine sinoatrial node pacemaker cells and the I\(f\)-generating HCN2 and HCN4 channels in transfected HEK293 cells. Clonidine also inhibited HCN1 currents, although with significantly lower sensitivity. As a consequence of blocking I\(f\), 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.