Neuronal control of heart rate in isolated mouse atria

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Choate, J. K. and R. Feldman. Neuronal control of heart rate in isolated mouse atria. Am J Physiol Heart Circ Physiol 285: H1340–H1346, 2003. First published May 8, 2003; 10.1152/ajpheart.01119.2002.—A novel mouse isolated atrial preparation with intact postganglionic autonomic innervation was used to investigate the neuronal control of heart rate. To establish whether autonomic activation was likely to alter heart rate by modulating the hyperpolarization-activated current (I_f), the L-type Ca^{2+} current (I_{Ca,L}), or the ACh-activated K^+ current (I_{K,ACh}), the effects of nerve stimulation (right stellate ganglion or right vagus, 1–30 Hz) and autonomic agonists (0.1 μM norepinephrine or 0.3 μM carbachol) on heart rate were investigated in the presence of inhibitors of these currents, cesium chloride (Cs^+, 1 mM), nifedipine (200 nM), and barium chloride (Ba^{2+}, 0.1 mM), respectively. The positive chronotropic response to stellate ganglion stimulation was reduced by ∼20% with Ca^2+ and nifedipine (P < 0.05), whereas the heart rate response to norepinephrine was only reduced with Cs^+ (P < 0.05). Ba^{2+} attenuated the decrease in heart rate with vagal stimulation and carbachol by ∼60% (P < 0.05). These results are consistent with the idea that sympathetic nerve stimulation modulates I_f to increase heart rate in the mouse. Activation of I_{Ca,L} also appears to contribute to the sympathetic heart rate response. However, the decrease in heart rate with vagal stimulation or carbachol is likely to result primarily from the activation of I_{K,ACh}. The presence of pharmacological blockers of I_f and I_{Ca,L} attenuates the heart rate response to cardiac sympathetic nerve stimulation on heart rate in spontaneously beating mouse atrial preparations.

Cardiac sympathetic and parasympathetic neurotransmitters are likely to alter heart rate by modulating sinoatrial node ionic currents, such as the hyperpolarization-activated current (I_f), L-type Ca^{2+} current (I_{Ca,L}), or I_{K,ACh}. Nonmurine studies have produced contradictory results as to the specific sinoatrial node currents that are influenced by cardiac autonomic neurotransmitters. This could be due to the use of different models of autonomic activation (i.e., direct nerve stimulation vs. bath-applied autonomic agonists) or to the use of different preparations (e.g., spontaneously beating isolated atrial preparations, anesthetized animals, or isolated sinoatrial node cells). For example, I_f and I_{Ca,L} are attenuated by bath-applied ACh in isolated cardiac myocytes, whereas the heart rate response to vagal stimulation is not altered, or may even be enhanced in vitro and in vivo, after pharmacological inhibition of these currents (3, 9, 23, 26, 27, 29). Furthermore, I_{Ca,L} blockers either have no effect on, or attenuate, the heart rate response to cardiac sympathetic nerve activation in vivo (1, 12, 14, 22). In contrast, pharmacological inhibition of I_{K,ACh} consistently reduces the heart rate response to vagal stimulation in vitro and in vivo, suggesting that the activation of I_{K,ACh} contributes to vagal bradycardia (3, 26). There is little information available concerning the modulation of I_f by sympathetic nerve activity. A single study (12) reported that the heart rate response to sympathetic nerve stimulation is attenuated after I_f inhibition in vivo. This result is supported by data from sinoatrial node cells indicating that cAMP enhances I_f (10).

In the present study, an isolated cardiac preparation was used to investigate the effects of autonomic nerve stimulation on heart rate, because circulating hormones, hemodynamic reflexes, or anesthetics may alter autonomic nerve activity in vivo. To determine whether sinoatrial node currents were likely to be modulated by autonomic nerve stimulation, the effects of pharmacological blockers of I_f (cesium chloride, Cs^+, 1 mM), I_{Ca,L} (nifedipine, 200 nM), and I_{K,ACh} (barium chloride, Ba^{2+}, 0.1 mM) were examined on the heart rate responses to nerve stimulation in isolated mouse atria. We found that activation of cardiac sympathetic nerves in isolated mouse atrial preparations is likely to modulate I_f and I_{Ca,L} to produce an increase in heart rate.

THIS STUDY USED a novel isolated mouse atrial preparation with intact postganglionic autonomic innervation to investigate the neuronal control of heart rate in this species. The mouse was chosen as the experimental animal because genetically manipulated mice with alterations in cardiac autonomic receptors (e.g., mice with overexpression of cardiac β_1-adrenoceptors (19)) or membrane currents (e.g., ACh-sensitive K^+ current (I_{K,ACh}), knockout mice (28)) have been developed. Cardiovascular diseases such as heart failure and hypertension are associated with abnormal sympathetic and parasympathetic activation of cardiac autonomic receptors and membrane currents (11). Genetically altered mice could therefore be used to investigate the role of the cardiac autonomic innervation in the development of these cardiovascular diseases. Because the neuronal control of heart rate has not been examined in mice, the primary aim of this study was to investigate the effects of cardiac sympathetic and parasympathetic nerve stimulation on heart rate in spontaneously beating mouse atrial preparations.

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rate. Furthermore, the activation of $I_{K,ACb}$ appears to contribute to vagal bradycardia. Because both the sympathetic and vagal heart rate responses persisted when $I_f$, $I_{Ca,L}$, or $I_{K,ACb}$ were inhibited, they are likely to result from the interplay between several different sinoatrial node currents rather than the action of a single current.

METHODS

Isolated mouse double-atrial preparation with intact autonomic postganglionic innervation. The Monash University Animal Ethics Committee approved all experimental procedures on animals. Mice ($n = 119$, 22.0 ± 0.7 g, male, C57BL6/J) were killed by cervical dislocation. The thorax and mediastinum were removed from the body and placed in mouse physiological saline solution [containing (in mmol/l) 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 0.5 Na₂EDTA, 1.2 KH₂PO₄, 25 NaHCO₃, 11 glucose, and 1.75 CaCl₂; pH 7.4] gassed with carbogen (95% O₂:5% CO₂) at room temperature (25°C).

The atria, together with either the right stellate ganglion or right vagus, were dissected free and placed into an organ bath (3 ml volume) maintained at 37 ± 0.1°C (see Fig. 1). A silk suture was placed into the left auricle, and this was attached to an isometric force transducer (SensoNor). A suture in the right auricle was placed over a hook attached to the wall of the organ bath. The force response (in mN) was determined on the heart (in beats/min) from the software calculated heart rate (in beats/min) from the atrial node current [as the difference between the baseline heart rate and the maximum response to autonomic activation (or bath-applied autonomic agonist) were obtained at each stimulation frequency (or agonist). The magnitude of these responses was calculated as the difference between the baseline heart rate (averaged over 3 s immediately before autonomic activation) and the maximum response to autonomic activation (averaged over 3 s). The three responses at each stimulation frequency were averaged. Data are presented as means ± SE. Statistical analyses were performed using SPSS (version 11.0) software. ANOVA with repeated measures was used on group data, with the Student-Newman-Keuls test applied for post hoc analysis when significance was obtained. When appropriate, a comparison between two groups was performed by paired Student’s $t$-tests. For all statistical tests, $P < 0.05$ was accepted as being statistically significant.

RESULTS

Neuronal control of heart rate in an isolated mouse atrial preparation. Stimulation of the right stellate ganglion (1–10 Hz) increased heart rate in a frequency-dependent manner. Propranolol (0.1 μM) significantly reduced the magnitude of the positive chronotropic response to sympathetic nerve stimulation ($P < 0.05$ by ANOVA; increase in heart rate with 5-Hz stimulation: control = 106 ± 11 beats/min and propranolol = 3 ± 1 beats/min).

*Fig. 1. Isolated mouse double-atrial preparation with the right stellate ganglion and its connections to the atria (RA, right atrium; LA, left atrium; SVC, superior vena cava; AA, oval, right stellate ganglion). The silk sutures in the right and left auricles were used to connect the preparation to an isometric force transducer. A silk thread was tied just below the right stellate ganglion so that it could be positioned onto the stimulating electrodes. A stainless steel pin head is also shown.*
beats/min, \( n = 6 \) but did not alter the baseline heart rate. In three preparations, guanethidine (0.1 μM) significantly attenuated the increase in heart rate with sympathetic stimulation (\( P < 0.05 \) by ANOVA; increase in heart rate with 5-Hz stimulation: control = 88 ± 40 beats/min and guanethidine = 4 ± 1 beats/min). The magnitude of the positive chronotropic response to sympathetic activation remained stable for the duration of the experimental protocols (increase in heart rate with 3-Hz sympathetic stimulation: 0 min = 72 ± 9 beats/min, after 20 min = 65 ± 9 beats/min, and after 40 min = 66 ± 8 beats/min, \( n = 4 \)).

Right vagal stimulation (1–30 Hz) evoked a frequency-dependent decrease in heart rate. At stimulation frequencies at and above 10 Hz, the preparations often arrested. Atropine (0.1 μM) significantly reduced vagal bradycardia without altering the basal heart rate (\( P < 0.05 \) by ANOVA; decrease in heart rate with 5-Hz stimulation: control = 89 ± 8 beats/min and atropine = 0 ± 0 beats/min, \( n = 3 \)). The magnitude of vagal bradycardia did not alter during the time course of the experimental protocols (decrease in heart rate with 3-Hz vagal stimulation: 0 min = 64 ± 6 beats/min, after 20 min = 67 ± 7 beats/min, and after 40 min = 62 ± 2 beats/min, \( n = 4 \)).

**Effect of Cs**\(^+\) **on autonomic control of heart rate.** The \( I_f \) blocker Cs\(^+\) (1 mM, \( n = 33 \), combined values for all Cs\(^+\) experiments) decreased heart rate from 339 ± 9 to 270 ± 15 beats/min. The heart rate returned to control levels when Cs\(^+\) was washed out with fresh physiological saline solution [washout = 305 ± 9 beats/min, \( P < 0.05 \) (by ANOVA) for Cs\(^+\) vs. control and washout]. In addition to slowing the heart rate, Cs\(^+\) significantly attenuated the positive chronotropic responses to sympathetic nerve stimulation (1–10 Hz, \( n = 10 \); see Fig. 2, A and B) and 0.1 μM norepinephrine (\( n = 10 \), heart rate response to norepinephrine: control = 177 ± 13 beats/min, Cs\(^+\) = 148 ± 12 beats/min, and washout = 186 ± 14 beats/min; \( P < 0.05 \) (by ANOVA) for Cs\(^+\) vs. control and washout; see Fig. 2C). The decreases in heart rate with vagal stimulation (1–30 Hz, \( n = 8 \), see Fig. 2D) and carbachol (0.3 μM, \( n = 5 \)) were not altered by Cs\(^+\) (heart rate response with carbachol: control = −158 ± 22 beats/min and Cs\(^+\) = −161 ± 19 beats/min).

To determine whether the slowed baseline heart rate with Cs\(^+\) contributed to the attenuated sympathetic

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**Fig. 2.** A: typical raw data traces showing the effects of selective hyperpolarization-activated current (\( I_f \)) inhibition with cesium (1 mM) on baseline heart rate [in beats/min (bpm)] and the increase in heart rate with right stellate ganglion stimulation [sympathetic nerve stimulation (SNS), 5 Hz]. B: averaged data (\( n = 10 \) atria) for the effect of cesium on the magnitude of the heart rate response to SNS. *Change in heart rate with cesium significantly different from changes in heart rate for control and washout, \( P < 0.05 \); †change in heart rate with cesium significantly different from control, \( P < 0.05 \). C: raw data traces showing the effect of cesium on the heart rate response to bath-applied norepinephrine (NE; 0.1 μM). The arrows indicate the point at which NE was added to the cardiac chamber. D: averaged data (\( n = 8 \) atria) illustrating that cesium did not alter the decrease in heart rate with right vagal stimulation.

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and norepinephrine heart rate responses, the effect of reducing the baseline heart rate with carbachol was determined on these β-adrenoceptor-mediated responses. Carbachol (0.1 μM) produced a similar decrease in heart rate (n = 15, heart rate decreased by 85 ± 15 beats/min) to 1 mM Cs⁺ (n = 33, heart rate decreased by 63 ± 14 beats/min). As shown in Fig. 3, in contrast to the inhibitory effects of Cs⁺, carbachol did not alter the heart rate responses to sympathetic nerve stimulation (n = 7) and norepinephrine (n = 8).

**Effect of nifedipine on autonomic control of heart rate.** Nifedipine (200 nM) slowed the baseline heart rate from 319 ± 6 to 267 ± 12 beats/min (n = 28, combined values for all nifedipine experiments; P < 0.05 by paired t-test). It also significantly decreased in the magnitude of the heart rate response to sympathetic nerve stimulation (1–10 Hz, n = 6; see Fig. 4, A and B). Nifedipine had no effect on the heart rate responses to vagal stimulation (n = 6; see Fig. 4C), 0.1 μM norepinephrine (heart rate response: control = 170 ± 11 beats/min and nifedipine = 156 ± 11 beats/min, n = 10) or 0.3 μM carbachol (heart rate response: control = −174 ± 19 beats/min and nifedipine = −166 ± 18 beats/min, n = 6).

**Effect of Ba²⁺ on autonomic control of heart rate.** Vagal bradycardia (1–30 Hz, n = 6; see Fig. 5, A–C) and the heart rate response to carbachol (0.3 μM, n = 5) were significantly reduced (by ~60%) when IK,ACh was inhibited with Ba²⁺ (0.1 mM). This effect was reversed when Ba²⁺ was washed out with fresh physiological saline solution (heart rate response to carbachol: control = −171 ± 22 beats/min, Ba²⁺ = −72 ± 22 beats/min, and washout = −140 ± 18 beats/min; P < 0.05 by ANOVA). Ba²⁺ had no effect on the heart rate responses to sympathetic nerve stimulation (n = 7; see Fig. 5D) and bath-applied norepinephrine (heart rate response to norepinephrine: control = 177 ± 28 beats/min and Ba²⁺ = 141 ± 19 beats/min, n = 5).

**DISCUSSION**

This study investigated the effects of the pharmacological inhibition of If (1 mM Cs⁺), ICa.L (200 mM

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**Fig. 3.** Effect of carbachol (CCh; 0.1 μM) on the heart rate responses to SNS (5 Hz, n = 7) and bath-applied NE (0.1 μM, n = 8).

**Fig. 4.** A: heart rate traces showing the effects of L-type Ca²⁺ channel current (ICa,L) antagonist nifedipine (200 nM) on baseline heart rate and the increase in heart rate with right stellate ganglion stimulation (SNS, 3 Hz). B: averaged data (n = 6 atria) showing that nifedipine attenuated the magnitude of the heart rate response to SNS.

*Change in heart rate for control significantly different from change in heart rate with nifedipine, P < 0.05. C: averaged data (n = 7 atria) illustrating that ICa,L inhibition with nifedipine did not alter the magnitude of vagal bradycardia.
nifedipine), and $I_{K,ACh}$ (0.1 mM Ba$^{2+}$) on the neuronal control of heart rate in mouse atria. We found that the positive chronotropic response to right stellate ganglion stimulation was significantly reduced by Cs$^{+}$ and nifedipine, whereas only Ba$^{2+}$ attenuated vagal bradycardia.

Sympathetic and vagal control of heart rate in an isolated mouse atrial preparation. In this study, the normal physiology of the autonomic control of heart rate was mimicked by the use of an isolated double-atrial preparation with intact cardiac sympathetic or parasympathetic postganglionic innervation. This is the first time that the effects of cardiac sympathetic and parasympathetic nerve activation on heart rate have been specifically investigated in an isolated mouse cardiac preparation. In a recent study (6), our laboratory examined the vagal heart rate responses in mouse atria from normal and neuronal nitric oxide synthase knockout mice. Other studies have indirectly examined neuronal heart rate responses in the mouse by using field stimulation of the atria or by activation of cardiac nerves in vivo via the baroreflex (for example, see Refs. 7 and 28). Consistent with other mammalian species, we found that in mouse atria, right stellate ganglion stimulation caused the release of neurotransmitter(s), which interacted with $\beta$-adrenoceptors to produce an increase in heart rate, and right vagal stimulation caused a decrease in heart rate that resulted from the activation of muscarinic receptors.

Effect of Cs$^+$ on autonomic control of heart rate. Mangoni and Nargeot (18) recently recorded $I_{f}$ in isolated mouse sinoatrial node cells. We found that inhibition of $I_{f}$ with Cs$^{+}$ in isolated mouse sinoatrial node cells. We found that inhibition of $I_{f}$ with Cs$^{+}$ in isolated mouse atria reduced their spontaneous beating rate, indicating that this current is likely to be involved in the generation of sinoatrial node pacemaker action potentials in this species. In addition, Cs$^{+}$ attenuated the positive chronotropic responses to right stellate ganglion stimulation and bath-applied norepinephrine, suggesting that $\beta$-adrenoceptor activation enhances the activation of $I_{f}$ to cause an increase in heart rate. It is unlikely that the sympathetic heart rate response was attenuated in the presence of Cs$^{+}$ due to the associated decrease in baseline heart rate, because a similar slowing of heart rate with carbachol failed to attenuate the sympathetic heart rate response.

In the autonomically decentralized heart of the anesthetized dog, zatebradine, a potent inhibitor of sinoatrial node $I_{f}$, also attenuated the increase in atrial beating rate evoked by cardiac sympathetic nerve activation (12). Furthermore, $\beta$-adrenoceptor agonists increase the amplitude of $I_{f}$ in isolated rabbit sinoatrial...
node pacemaker cells by shifting the activation curve ~11 mV in the positive direction, an effect that would enhance $I_R$ increase the slope of the diastolic depolarization, and elevate the heart rate (10). More recently, Mangoni and Nargeot (18) reported that norepinephrine caused an increase in the amplitude of $I_R$ recorded from isolated mouse sinoatrial node cells. Therefore, norepinephrine released from sympathetic nerve terminals innervating the mouse sinoatrial node could increase the activation of $I_R$ and thus increase the heart rate. This agrees with our data from mouse atria, which indicate that when $I_R$ is inhibited the heart rate response to sympathetic activation is significantly attenuated.

A hyperpolarization-activated current, $I_h$, which is similar to sinoatrial node $I_h$ (also called $I_h$) has been recorded in isolated sympathetic postganglionic neurons (16). It is therefore possible that $Cs^+$ inhibited $I_h$ in both sinoatrial node myocytes and sympathetic neurons in our preparation. Inhibition of $I_h$ with $Cs^+$ in sympathetic neurons causes hyperpolarization of the resting potential, implying that $I_R$ normally contributes an inward current to the resting potential of the neurons (16).

Modulation of $I_R$ is unlikely to contribute to the cholinergic control of heart rate in the mouse because $Cs^+$ did not alter the drop in heart rate with vagal stimulation or bath-applied carbachol. The magnitude of the vagal bradycardia was also unchanged with $I_h$ inhibition in isolated guinea pig atria and the anesthetized dog (23, 26). However, 5 μM carbachol inhibited $I_R$ by 37% in isolated mouse sinoatrial node cells (18). It is possible that high concentrations of ACh inhibit $I_R$ in the mouse sinoatrial node. However, in the current study, concentrations of carbachol above 1 μM arrested the preparations. This suggests that these high concentrations of bath-applied carbachol are unlikely to be physiological.

Effect of nifedipine on autonomic control of heart rate. A recent study (25) using isolated rabbit sinoatrial node cells indicates that $I_{Ca,L}$ contributes to pacemaker diastolic depolarization. It has been established that $I_{Ca,L}$ is also present in murine sinoatrial node myocytes (2). Furthermore, knockout mice deficient in voltage-gated L-type Ca$^{2+}$ channels exhibit bradycardia in vivo relative to their wild-type controls (21). Similarly, in the present study, partial inhibition of $I_{Ca,L}$ with a low concentration of nifedipine (200 nM) reduced the beating rate of spontaneously beating mouse atria. Therefore, modulation of $I_{Ca,L}$ by autonomic neurotransmitters could alter heart rate in the mouse.

Inhibition of $I_{Ca,L}$ with nifedipine significantly attenuated the increase in heart rate evoked by activation of the right stellate ganglion. Nifedipine has also been reported to attenuate the increase in heart rate with cardiac sympathetic nerve stimulation in the dog and cat in vivo (1, 14, 22). It is possible that the attenuated sympathetic heart rate response with nifedipine could be at least partly due to the inhibition of neuronal L-type Ca$^{2+}$ channels. The membranes of postganglionic sympathetic neurons possess several types of voltage-dependent Ca$^{2+}$ channels, including Pr, Qr, Nr, and L-type Ca$^{2+}$ channels (5). The contribution of L-type Ca$^{2+}$ channels in sympathetic postganglionic neurons to calcium entry, and therefore transmitter release, is unclear. In isolated somata from the rat superior cervical ganglion, 80% of the current passed through N-type channels and only 5% through L-type channels (24). In the present study, nifedipine attenuated the positive chronotropic response to sympathetic activation but had no significant effect on a similar increase in heart rate caused by bath-applied norepinephrine. These results are consistent with the idea that $I_{Ca,L}$ contributes to calcium entry in mouse cardiac sympathetic neurons and thus to neurotransmitter release and the postsynaptic heart rate response.

Effect of Ba$^{2+}$ on autonomic control of heart rate. Because Ba$^{2+}$ significantly attenuated the decrease in heart rate with vagal stimulation and carbachol in isolated mouse atria, these responses are likely to be primarily due to the activation of $I_{K,ACh}$. Similarly, Wickman et al. (28) found that mice genetically deficient for $I_{K,ACh}$ had a 50% reduction in the vagal modulation of heart rate (assessed by bradycardia in response to baroreflex activation in conscious mice). Previous studies (3, 4, 26) in isolated rabbit and toad atria, as well as in the anesthetized dog, have also indicated a substantial role for $I_{K,ACh}$ in vagal bradycardia. In all of these studies, inhibition of $I_{K,ACh}$ never completely abolished vagal bradycardia. This indicates that other sinoatrial node channels are likely to be modulated by the parasympathetic neurotransmitter ACh, or by a parasympathetic cotransmitter such as nitric oxide (6), to cause alter heart rate. Our data indicate that $I_R$ and $I_{Ca,L}$ are unlikely to be involved in vagal bradycardia in isolated mouse atria. However, parasympathetic modulation of $I_R$ may contribute to the vagal heart rate response because carbachol has been reported to inhibit this current in isolated rabbit sinoatrial node cells (17).

Perspectives. This study used ion channel blockers to examine the sinoatrial node pacemaker currents that are likely to be modulated by autonomic activation. We understand that the autonomic modulation of ionic currents can only be directly examined in isolated sinoatrial node cells. However, because the aim of this study was to investigate the neuronal control of heart rate in the mouse, a multicellular preparation with intact innervation was required. We attempted to determine the effects of autonomic nerve stimulation on intracellular recordings of sinoatrial node action potentials in isolated mouse atria. However, it was impossible to maintain impalements with the high beating rates in this preparation. As mentioned in the Discussion, it is possible that the pharmacological agents used in this study may affect the autonomic neurons as well as cardiac myocytes. Taken together with the results of this study, the development of genetically manipulated mice with alterations in cardiac membrane currents [e.g., $I_{K,ACh}$ knockout mice (28)] will further our understanding of how autonomic neuro-
transmitters modulate ionic currents to alter heart rate.

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DISCLOSURES

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