The following is the abstract of the article discussed in the subsequent letter:

Choate, JK and Feldman, RR. The neuronal control of heart rate in the isolated mouse atria. Am J Physiol Heart Circ Physiol 285: H1340–1346, 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 (\(I_h\)), the L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)), or the ACh-activated K\(^+\) current (\(I_{\text{K,ACH}}\)), the effects of nerve stimulation (right stellate ganglion or right vagus, 1–30 Hz) and autonomic agonists (0.1 mM norepinephrine or 0.3 mM 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 Cs\(^+\) 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 \(I_h\) to increase heart rate in the mouse. Activation of \(I_{\text{Ca,L}}\) 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 \(I_{\text{K,ACH}}\).

Autonomic Modulation of Heart Rate: Pitfalls of Nonselective Channel Blockade

To the Editor: In a recent study, Choate and Feldman (2) reinvestigate the longstanding and contentious question of the ionic basis of autonomic modulation of heart rate using an isolated atrial preparation with intact autonomic innervation. Although experiments are conducted with care, their rationale is based on the assumption that the contribution of a single ion channel to activity can be inferred by changes caused by ion channel blockers to activity per se. This assumption has contributed to the continuing misunderstandings and controversies in this field for decades for the following reasons.

First, action potential generation and spontaneous activity are dynamic processes generated by the interplay between several voltage-dependent currents. If any one of these currents is abolished, the shape of the action potential, hence, of the time-dependent development of all remaining components and their contribution to activity, is bound to change, too. This implies that modifications of activity and autonomic response may be caused not only by abolishment of the target current but also by indirect modifications of other components. One method able to dissect the contribution of an individual channel to activity is the “action potential clamp,” which, however, can only be applied to isolated cells.

Second, the method critically depends on the selectivity of the drugs used to remove individual current components. That is, a drug should entirely eliminate its target current with no direct or secondary effects on any other current. This is clearly not the case for at least Cs\(^+\) and nifedipine. In this paper, Cs\(^+\) (1 mM) was used on the assumption that it specifically blocks the sinoatrial node (SAN) hyperpolarization-activated (\(I_h\)) current. This assumption is questionable for two reasons. First, Cs\(^+\) does not block \(I_h\) fully. At ~50 mV, for example, Cs\(^+\) (5 mM) blocks only some 70% of the current in the SAN (3). Furthermore, block is voltage dependent, which complicates the time course of \(I_h\) current inhibition during action potential. Second, Cs\(^+\) is a known K\(^+\) channel blocker, and action potential clamp analysis reveals block of an outward K\(^+\) component in SAN cells (4). Conclusions that strictly depend on the assumption of full and selective \(I_h\) block by Cs\(^+\) are therefore not justified and may be severely misleading.

A similar line of reasoning applies to the block of Ca\(^{2+}\) influx by nifedipine. A further complication here arises because blocking Ca\(^{2+}\) influx is likely to disrupt intracellular Ca\(^{2+}\) homeostasis and have secondary effects on other currents. For example, nifedipine can be shown in action potential clamp experiments to not only modify Ca\(^{2+}\) currents but also Ca\(^{2+}\)-activated K\(^+\) currents (4). Furthermore, reduction of intracellular Ca\(^{2+}\) transients inhibits the adrenergic signaling cascade (1) making it questionable to extrapolate autonomic function under conditions of Ca\(^{2+}\) disruption to normal physiology.

Whereas development of new blocking drugs with higher specificity and the use of channel gene knock-out techniques will help clarify the role of ionic components in SAN automaticity, the continuing use of partial or imperfect channel blockers to define individual channel contributions to activity should not be encouraged.

REFERENCES


REPLY

To the Editor: We appreciate the comments from Baruscotti and his colleagues on our study. They make two main points to illustrate why the effects of selective ion channel blockers on action potentials do not indicate the contribution of that single ion channel to the action potential. We agree with their comments and will highlight how they are relevant to our study.

First, they mention that selective inhibition of an ionic current may change the activation of the remaining unblocked ionic currents that contribute to an action potential, and that the “action potential clamp” technique could be used to overcome this problem (see Ref. 6). This technique will not further our understanding of how autonomic nerve activity alters pacemaker action potentials and thus heart rate; this requires multicellular cardiac preparations. To establish which pacemaker currents are modulated by autonomic neurotransmitters, researchers are limited to using multicellular cardiac preparations with selective ion channel blockers or with genetic inactivation or overexpression of ion channels. It is possible to apply autonomic agonists to isolated cells to mimic the effects of nerve stimulation, but in isolated atrial preparations autonomic agonists cause different changes in the shape of pacemaker action potentials to those produced by autonomic nerve stimulation. This suggests that they are likely to modulate different ionic currents (2).

Second, Baruscotti et al. indicate that there are some problems with the selectivity of Cs+ and nifedipine. We agree that the block of If by Cs+ is voltage dependent and that it has been reported to block K+ channels (6). Nifedipine (at a concentration higher than that used in our study) may alter a Ca2+-activated K+ current (6); inhibiting Ca2+ influx with nifedipine is also likely to alter intracellular Ca2+ fluxes. We chose these ion channel blockers because they were the most appropriate that were available to us. Furthermore, we felt that because nifedipine is used in the clinical environment, it is important that we understand how it may alter cardiac autonomic function. In retrospect, we would use the If blocker ZD-7288 because it selectively blocks If, with no significant effects on the delayed rectifier current IK (1). Consistent with the effects of Cs+ on the sympathetic heart rate response in our study, ZD-7288 has been reported to attenuate the increase in heart rate with norepinephrine in isolated guinea pig atria (3).

It is true, as Baruscotti et al. mentioned, that cardiac preparations from genetically modified mice with alterations in ion channel function should provide further information about the autonomic control of cardiac function. As with putatively selective ion channel blockers, results from these mice need to be treated with caution; “knockout” of an ion channel may cause the upregulation of other ion channels or of downstream intracellular signaling pathways. Genetic knockout of the If channel would be expected to produce mice with decreased resting heart rates; this has not been found. Four isoforms of a hyperpolarization-activated cation channel (HCN1–4) have been cloned, but the contributions of the isoforms to the physiological functions of If are not clear (4). Mice lacking the type 2 isoform of the hyperpolarization-activated cation channel (HCN2) have similar resting heart rates and heart rate responses to isoproterenol as those of wild-type mice, suggesting that the HCN2 isoform of this channel is not involved in the sympathetic modulation of heart rate (4). This result contradicts previous studies indicating that pharmacological inhibition of If decreases resting heart rate and sympathetic heart rate responses (3). It would be interesting to know whether If blockers decrease heart rate in the HCN2 mice, plus to examine heart rate modulation in HCN1 knockout mice; this isoform has been identified in the rabbit SAN (5). In an ideal study, data from isolated sinoatrial pacemaker cells with action potential clamp would be viewed together with data from autonomically innervated multicellular cardiac preparations and intact animals with ion channel blockers. In addition, cellular and multicellular results from mice with both knockout and overexpression of cardiac ion channels would be compared with those from normal mice and the effects of pharmacological ion channel blockers examined in these mice.

REFERENCES


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