Autonomic Rate Modulation

The SAN of mammalians is densely innervated with autonomic nervous fibers, regulating cardiac chronotropism. Sympathetic β-adrenergic stimulation acts by accelerating, and parasympathetic muscarinic stimulation by slowing cardiac rate. As mentioned above, the original description of I_f in the SAN was accompanied by the finding that the current is also increased by adrenaline, in a way that could explain the accelerating action of sympathetic stimulation.[19]

When SAN cells are superfused with solutions containing low concentrations of adrenergic agonists, acceleration of spontaneous rate is associated with a steeper slope of diastolic depolarization, with little modification of action potential duration and shape. A post hoc analysis of data from Brown et al (1979)[19] illustrates this observation for their original records, as shown in Figure 1A (right), where the trace recorded in adrenaline is shifted so as to have superimposition of control and adrenaline action potentials.

Clearly, the adrenaline-induced rate acceleration is almost entirely attributable to the shortening of the diastolic duration associated with a faster slope of diastolic depolarization, whereas only minimal changes occur in the action potential shape and duration. This agrees with the hypothesis that the process responsible for diastolic depolarization (ie, I_f activation) is an important target of sympathetic rate regulation.

Although the description of the I_f response to adrenaline was a first indication of the involvement of I_f in autonomic modulation of heart rate, much work was still needed to define more fully other aspects of the I_f function. Several additional observations provided detailed description of I_f features and more evidence supporting its relevance to pacemaker generation and rate control. It was shown for

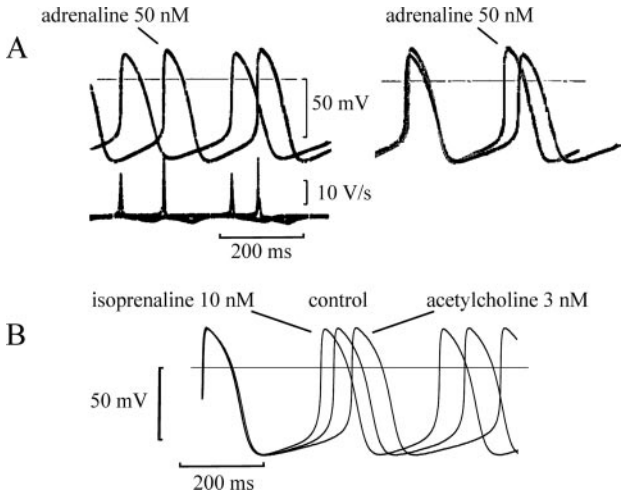

Figure 1. Low concentrations of autonomic transmitters alter rate by changing the slope of diastolic depolarization. A, Spontaneous activity recorded from a SAN preparation in a control Tyrode solution and during perfusion with 50 nmol/L adrenaline, as indicated (left) (adapted from Brown et al[19]); shifting horizontally the traces so as to make the upstroke of the action potentials coincide shows that most of the rate acceleration in adrenaline is attributable to steepening of the diastolic depolarization (right). B, In single-cell recordings, acceleration attributable to isoprenaline 10 nmol/L and slowing attributable to ACh 3 nmol/L also result from changes of diastolic depolarization slope, with little modification of action potential duration.

example that β-adrenergic receptor (AR) stimulation increases I_f by shifting the activation curve of the current to more positive voltages, without modification of the conductance, a result also confirmed by single-channel measurements.[28,29] The depolarizing shift of I_f activation curve is attributable to the β-AR-dependent increase of intracellular cAMP, the second messenger in I_f modulation. As was later shown by macropatch analysis, cAMP positively shifts the f-channel activation curve not by a phosphorylation-dependent process, but directly by binding to channels.[30] This was the first evidence, later confirmed with the cloning of HCN channels, that funny and CNG (cyclic-nucleotide gated) channels have similar properties and in fact belong to the same superfamily.

A more complete understanding of the I_f role in heart rate control was achieved in the mid/late 1980s, with the demonstration that I_f is strongly inhibited by parasympathetic stimulation, according to a mechanism opposite to that associated with β-AR stimulation, ie, a negative shift of the current activation curve attributable to muscarinic-induced inhibition of adenylate-cyclase and cAMP reduction.[31,32]

Vagal stimulation induces a negative chronotropic effect by releasing acetylcholine (ACh) (Figure 1B), and when the muscarinic modulation of I_f was discovered, the established view, based on early experiments,[33] was that the mechanism responsible for ACh-induced slowing is the activation of an ACh-dependent K^+ current.[34] The new evidence challenged this view and raised the question whether, and to what extent, the muscarinic-dependent I_f inhibition was involved in the vagal-induced negative chronotropism; why should 2 different mechanisms operate simultaneously to slow cardiac rate on vagal stimulation? This question was addressed by investigating the ranges of ACh concentration required to activate the 2 mechanisms. The result was surprising, in that the concentrations were quite different: whereas low doses of ACh (up to 0.01 to 0.03 μmol/L) were shown to inhibit I_f, 20-fold higher concentrations were necessary to activate the K^+ current conductance; also, the low doses of ACh active on I_f inhibition (but not on $I_{K,ACh}$ activation) were perfectly capable of slowing the rate of spontaneous activity of pacemaker cells (Figure 1B).[35] The consequence of this finding was the introduction of a novel concept in the physiology of autonomic heart rate regulation: these results showed that the negative chronotropic effect of low-to-moderate vagal stimuli is mediated by I_f inhibition, not by activation of a K^+ current.

Along with the cAMP-dependent modulation, other mechanisms control the function of funny channels, as shown by studies of native channels and/or individual HCN isoforms; these include accessory subunits such as MiRP1,[36,37] membrane phospholipids such as PIP2 (phosphatidylinositol 4,5-bisphosphate),[38] and mechanisms affecting compartmentation of channels into membrane lipid rafts.[39]

Phosphorylation by serine/threonine kinases (p38 mitogen-activated protein kinase)[40] and by tyrosine kinases (Src) also contributes to regulate funny channel activity. The action of Src tyrosine kinase has been characterized with some detail. Phosphorylation by Src tyrosine kinase of residues Y476 (of mHCN2) or the analogous Y554 (of hHCN4), for example, was found to induce acceleration of channel activation and shift to more positive voltages the current activation curve.[41,42] Specific screening of tyrosine residues of hHCN4 involved in Src-dependent phosphorylation further revealed that a major role in mediating the Src action is played by the tyrosine residue Y531.[43] Src-dependent phosphorylation involves a sequence of HCN2 comprising part of the C-linker and the cyclic nucleotide binding domain, which might suggest an interaction between the actions of cAMP and phosphorylation; however, cAMP-dependent modulation is essentially preserved when Src phosphorylation is inhibited, except for a slowing of activation kinetics at saturating cAMP concentrations.[41] Thus, cAMP-dependent and phosphorylation-dependent control of HCN2 appear to operate mostly through separate mechanisms.