PERSPECTIVES

Role of neuronal potassium M-channels in sympathetic regulation of cardiac function

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Noradrenaline (NA), the neurotransmitter released from postsynaptic cholinergic neurons of the sympathetic nervous system, plays an essential role in the control of heart rate, myocardial excitability, rate and force of contraction, and relaxation. As such, NA is a major player in physiological adaptation responses, including stress, emotions and physical activity, and in pathological conditions such as arterial hypertension and cardiac insufficiency. In cardiac tissue, NA stimulation of β1-adrenergic receptors has powerful effects on the contractile properties of the cardiomyocytes. Hence, regulation of NA release is essential under both normal and pathophysiological conditions. In the sympathetic nervous system, release of NA at adrenergic synapses is evoked by action potentials induced by stimulation of nicotinic acetylcholine receptors residing in the ganglia; it is highly dependent on the firing pattern of action potentials. The active and passive discharge properties are modulated via stimulation of a variety of G protein-coupled receptors expressed on the ganglionic neurons, which are targets for acetylcholine, several peptides and other neuro-humoral factors. The modulation is conveyed via regulation of the activity of multiple ion channels expressed by these neurons, including 'M-type' K^+ channels, responsible for the voltage-dependent M-type K^+ current (M-current). M-current was first described in sympathetic neurons (Brown & Adams, 1980) as a subthreshold, slowly activating and non-inactivating K^+ current thought to stabilize membrane potentials and control neuronal excitability by limiting the frequency of action potentials. Hence, alterations of M-channel conductance could serve as an effective mechanism regulating cardiomyocyte excitability and contractility, via tuning of the amount of NA released from sympathetic neurons. However, whereas the role of M-current in control of excitability is widely studied in the nervous system, its function in the cardiovascular system remains obscure.

In a recent issue of *The Journal of Physiology*, this subject was addressed by Zaika *et al.* (2011) looking at the effect of M-current on myocardial excitability in the context of sympathetic regulation. The authors use an *in vitro* preparation of sympathetic neurons of the superior cervical ganglia (SCG) that form functional adrenergic synapses when co-cultured onto a spontaneously contracting myocardial syncytium, examining the contraction rate of the cardiomyocytes in response to nicotinic stimulation of the neurons. This experimental approach presents an ideal model system to study neuro-regulation of cardiomyocytes by sympathetic innervation, as such *in vitro* synapses have been shown to contain much of the same organization as *in vivo* synapses, providing a physiologically relevant environment. Implementing several experimental strategies, the authors cleverly exploited the model to discern M-channel involvement in the regulation of myocardial automaticity. Thus, the modulatory effect of M-current was studied by specific manipulations of M-channel activity: current enhancement by application of the M-channel opener, retigabine, which is specific for neuronal M-channels (without affecting the cardiac isoform constituting I_{KS}), and current suppression by stimulation of bradykinin B_2 and purinergic P2Y $G_{q/11}$-coupled receptors (GPCRs). Although M-current is most famous for its suppression by muscarinic acetylcholine receptors, the authors chose B_2 and P2Y receptors since their action on M-channels involves IP_3-mediated signalling, rather than depletion of phosphatidylinositol 4,5-bisphosphate (PIP_2), hence affecting M-channels, but not the N-type voltage-gated Ca^{2+} channels expressed in SCG neurons (Hernandez *et al.* 2008). Further, to exclude stimulation of these receptors of the cardiomyocytes, the myocardial syncytium was prepared from B_2 and $P2Y_2$ receptor knock-out mice. Using such an experimental set-up, which recapitulates sympathetic innervation of myocardium *in vitro*, the nicotine-induced increase in the cardiomyocyte contraction rate was severely blunted by retigabine, but was enhanced by co-application of bradykinin or the purinergic agonist UTP. Taken together, a significant role for GPCR-mediated M-channel control of heart rate via regulation of NA release from ganglionic sympathetic neurons emerges.

Whereas Zaika *et al.* (2011) provide convincing evidence that M-current activity can control neurotransmitter release, the question of whether and how exocytosis is fine-tuned at the presynaptic nerve terminal in an M-current-dependent way is still controversial. Most neuronal M-channels are composed of heteromeric KCNQ2/KCNQ3 subunits. Both KCNQ2 and KCNQ3 subunits have been localized to the somas, dendrites, axon initial segments (AISs) and nodes of Ranvier of many neurons in the central and peripheral nervous systems (Devaux *et al.* 2004). Their location in the AIS, where fast spikes, as well as spike after-depolarization potentials (ADPs) are initiated, is strategic to shape the ADP waveform and modulate spike frequency adaptation (Yue & Yaari, 2004). Thus, *indirectly*, KCNQ2/3 channel activity, by influencing the firing pattern of action potentials invading the terminal, tunes the electrical properties of the plasma membrane at presynapses, consequently influencing transmitter release. But, what about a *direct* action? Notably, KCNQ2 subunits are uniquely expressed on axons and nerve terminals (Cooper *et al.* 2001), where they can directly affect the electrotonic properties of the presynaptic plasma membrane and directly control neurotransmitter release. In support of this intriguing possibility, modulation of M-channels via a direct and specific interaction between KCNQ2 subunits and the exocytotic neuronal SNARE protein syntaxin 1A was recently documented in a heterologous system and supported by data from hippocampus (Regev *et al.* 2009). This line of inquiry is consistent with a work documenting control over release from brain synaptosomes by M-channels (Martire *et al.* 2004, 2007). Taken together

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with the high co-localization of KCNQ2 and syntaxin 1A at presynaptic terminals (Regev *et al.* 2009), we suggest it likely that a direct interaction of syntaxin 1A with M-channels containing KCNQ2 subunits serves a mechanism for fine-tuning of pre-synaptic release.

Zaika *et al.* (2011) demonstrate regulation of presynaptic neurotransmitter release by M-current modulation; however, they do not address this above-mentioned possibility of *direct* regulation of release at pre-synaptic nerve terminals, over and above the documented M-channel control over action potential firing. They acknowledge this critical issue to remain 'murky'. We posit the full addressing of this issue to be important. For sure, such investigations will be extremely challenging technically, being hampered by the tiny dimensions of the pre-synaptic terminal. In the context of the work commented on here in this issue of the journal, the role of M channels in control over NA release from sympathetic neurons is critical to our understanding of cardiac function and dysfunction.

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