COMMENTARY

Impaired cAMP-cGMP Cross-Talk During Cardiac Sympathetic Dysautonomia

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Dysautonomia is a well-established contributor to the development and progression of hypertension and many other cardiovascular diseases. Sympathetic hyperactivity and vagal impairment are features of human hypertension, as well as in subjects with a familial predisposition for hypertension\textsuperscript{1,2}. This neural phenotype is also observed in the spontaneously hypertensive rat (SHR)\textsuperscript{3}. Moreover, it is widely accepted that autonomic imbalance contributes to the pathogenesis of hypertension itself, where emerging research is beginning to shed light on the key cellular and molecular changes that occur in diseased neurons\textsuperscript{3,4}.

The postganglionic sympathetic stellate neurons (PGSNs) of the SHR that predominantly innervate the heart, display increased intracellular calcium [Ca\textsuperscript{2+}], transients linked to impaired neuronal nitric oxide synthase (nNOS) activity\textsuperscript{3}. Together, with downstream reductions in nitric oxide (NO)-cyclic guanosine monophosphate (cGMP), this results in enhanced end-organ neurotransmission\textsuperscript{3}. Pharmacological and genetic techniques aimed at enhancing NO-cGMP-protein kinase G (PKG) signalling have been successful in rectifying the Ca\textsuperscript{2+} phenotype in SHR PGSNs and decreasing sympathetic hyperactivity\textsuperscript{3}. Interestingly, sympathetic impairment is present in young pro-hypertensive SHRs (pro-SHR), suggesting that these intracellular changes form early hallmarks of hypertension; however, the precise nature of events that trigger sympathetic dysfunction remain unclear.

Recently, we published data suggesting that sympathetic hyperactivity in the stellate neurons from the pro-SHR may be triggered by dysregulated Ca\textsuperscript{2+} channel activity, resulting in greater Ca\textsuperscript{2+} influx\textsuperscript{4}. N-type Ca channels (Ca\textsubscript{2.2}) were identified as the major contributor to Ca\textsuperscript{2+} entry in PGSNs, that in turn facilitate sympathetic neurotransmission. Inhibition of the N-type Ca\textsuperscript{2+} channel also reduces the propensity for fatal ventricular arrhythmias and ameliorates autonomic dysfunction in a heart failure mouse model\textsuperscript{2}. When taken together these findings support a significant physiological role of the N-type Ca\textsuperscript{2+} channel in neural modulation associated with cardiovascular disease.

In our study we reported that PGSN whole-cell Ca\textsuperscript{2+} currents of the pro-SHR are greater when compared to normotensive rats\textsuperscript{4}. Moreover, we demonstrated a novel link between impaired cyclic nucleotide signalling and increased N-type Ca\textsuperscript{2+} channel activity in pro-hypertension\textsuperscript{4}. cAMP and cGMP are ubiquitous second messenger cyclic nucleotide signalling molecules that regulate fundamental intracellular processes through direct activation of their respective kinases: protein kinase A (PKA) and PKG. Importantly, cyclic nucleotide regulation of neuronal [Ca\textsuperscript{2+}], is necessary for normal PGSN function. Indeed, site-specific phospho-regulation of several voltage-gated Ca\textsuperscript{2+} channel subtypes has been well documented, where a fine balance between PKA and PKG activity is maintained in order to regulate Ca\textsuperscript{2+}-dependent neurotransmission. Additional regulatory mechanisms are also maintained to ensure high signalling fidelity, whereby cAMP and cGMP are able to modulate alternative signalling pathways through the activation and / or inhibition of specific phosphodiesterases (PDEs)\textsuperscript{5}.

To test a link between N-type Ca\textsuperscript{2+} channels and cyclic nucleotide activity we carried out patch-clamp recordings and Förster Resonance Energy Transfer (FRET) microscopy. Pharmacological increases in cGMP were effective in reducing N-Type
Ca\(^{2+}\) currents in neurons from the pro-SHR to the same level as that recorded from control PGSNs, demonstrating that cyclic nucleotide regulation of the N-type Ca\(^{2+}\) channels is impaired in prohypertensive states. Real-time FRET recordings of cAMP and PKA illustrated divergent responses to cGMP in PGSNs in health and disease, suggesting significantly altered cyclic nucleotide signalling in diseased neurons. Indeed, in healthy neurons, pharmacological increases in cGMP led to cAMP generation and increased PKA activity. Conversely, in pro-SHR PGSNs, cGMP had no effect on cAMP or PKA signalling. These observations suggest that cross-talk between cyclic nucleotide signalling pathways is impaired in disease, and likely arises as a result of dysregulated PDE activity\(^4\).

PDE regulation of cAMP and cGMP cross-talk is considered critically important in maintaining cell-signalling processes and for restricting cyclic nucleotide signalling within specific subcellular microdomains\(^5\). Three families of PDEs (PDE1-3) have affinities for both cAMP and cGMP\(^5\), and recently both PDE2A and PDE3A have been implicated in the pathogenesis of hypertension\(^6,7\). We confirmed the presence of PDE2A and demonstrated pharmacologically a role for PDE3 in PGSNs. Given that PDE2A is activated by cGMP, and PDE3 is inhibited by cGMP, we suggest that the differential cAMP responses to cGMP observed in healthy versus diseased neurons, may arise as a result of altered PDE2/3 activity (fig. 1).

Other interesting but currently unexplored alternative explanations for our observation of impaired cyclic nucleotide cross-talk, might include an increased number of cGMP-specific hydrolysing PDE isoforms in the SHR such as PDE5, 6 or 9; whereby excessive hydrolysis of cGMP in diseased neurons could prevent the activation of cAMP signalling pathways. Notably, PDE9a expression is upregulated in clinical cardiac hypertrophy and heart failure\(^8\). Another possibility is that cAMP-hydrolysing PDE isoforms (e.g. PDE 4, 7, 8) are upregulated in diseased PGSNs, facilitating excessive cAMP hydrolysis following physiological stimuli. However, there is no compelling evidence to date that these PDE isoforms are present in sympathetic neurons. Establishing which PDE isoform/s contribute to sympathetic dysfunction and how this impairment arises is one of the key goals for understanding the basis of autonomic-driven metabolic phenotypes. Moreover, pharmacological or genetic targeting of specific PDE isoform/s within sympathetic nerves may have significant therapeutic potential for rectifying dysautonomia associated with many cardiovascular diseases.

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