

Is it all in our Heads? The Role of CaMKII in Neurogenic Hypertension

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The letter

We wish to draw the attention of your readership to an intriguing development in neurogenic hypertension that was reported in the *Journal of Neuroscience* late last year.¹ While 1 in every 3 American adults now experience elevated blood pressures, the majority present with primary hypertension, the pathology of which is incompletely understood. It has been postulated that many cases of treatment refractory primary hypertension may be of a neurological origin² – i.e. sympathetically-driven increases in the vasoconstrictor tone of resistance vessels, leading to elevated arterial blood pressure.³

The presympathetic neurons of the hypothalamic paraventricular nucleus (PVN) regulate sympathetic outflow through projections to the rostral ventrolateral medulla. It is therefore biologically plausible that hyperactivity of this pathway may contribute towards hypertension.⁴ Increases in glutamatergic output on *N*-methyl-D-aspartate receptors (NMDARs) in the PVN have previously been shown to increase vasomotor tone in a hypertensive rat model, with increases in both presynaptic and postsynaptic NMDAR activity in PVN neurons.⁴ These NMDARs are activated through phosphorylation by numerous kinases including the calcium/calmodulin-dependent protein kinase II (CaMKII), which itself is activated by increases in cytoplasmic calcium.⁵ Although there is a strong association between CaMKII and NMDA activity, its role in the hypertension-promoting PVN NMDAR activity currently remains unclear. Therefore, Li *et al.* set out to determine the role of CaMKII in regulating synaptic NMDAR activity of PVN presympathetic neurons and sympathetic motor tone in spontaneously hypertensive rats (SHRs).¹

Elevated sympathetic outflow has previously been implicated in the development of essential hypertension in SHRs. Sympathetic outflow is regulated via PVN projections to the rostral ventrolateral medulla and the intermediolateral cell column of the spinal cord, which were the targets of these experiments. The major strength of this study lies in the meticulous confirmation of PVN location, which was predicated on several previous proof-of-principle experiments.^{4,6} In the current study, Li and colleagues test the role of both pre- and postsynaptic CaMKII modulation of NMDARs by selective blockade of the suspected constituents involved in elevated sympathetic outflow in SHRs, as summarised in **Figure 1**.

Coronal brain slices were incubated in autocamtide 2-related inhibitory peptide (AIP), a selective CaMKII inhibitor, and electrophysiological recordings of the hypothalamus were made. CaMKII blockade normalized both the inherent raised baseline amplitude of NMDAR-excitatory postsynaptic current (EPSC) and the NMDAR-EPSC/AMPA-EPSC ratio in SHRs compared to the control group. Subsequent puff-application of NMDA on post-synaptic NMDARs was shown to increase SHR receptor current, while receptor currents were not increased in Wistar-Kyoto control rats suggesting the role of PVN NMDAR activity in

the pathogenesis of spontaneous hypertension. AIP blockade in conjunction with puff NMDA diminished SHR receptor current, indicating the direct role of CaMKII on increased postsynaptic activity in SHRs.

In attempt to assess the specific presynaptic role of CaMKII, miniature-EPSC (mEPSC) activity was measured with NMDAR channels blocked by Dizocilpine (MK-801), a non-competitive NMDAR antagonist. The blockade significantly increased mEPSC frequency in SHRs, which was subsequently normalized by application of 2-amino-5-phosphonopentanoic acid (AP-5), a competitive NMDAR antagonist. Increased mEPSC can thus be directly attributed to NMDAR activity. To illustrate the role of CaMKII in this pathway, slices were incubated once again with AIP and a decreased mEPSC frequency was observed. Subsequent application of AP-5 had no effect suggesting that CaMKII is directly responsible for the tonic basal increase in SHR PVN activity.

We currently know that expression of NMDARs is modulated by casein kinase (CK)2-mediated phosphorylation of receptor subunits, which is regulated by CaMKII. Li *et al.* previously demonstrated the role of 5,6-Dichloro-1- β -D-ribofuranosylbenzimidazole (DRB), a selective CK2 inhibitor, in reducing mEPSC activity in the PVN of SHRs.⁵ However, AIP treatment coupled with DRB was not shown to further decrease either NMDAR current amplitude or mEPSC frequency versus AIP treatment alone. This indicates a common role of CaMKII and CK2 in both pre- and postsynaptic presympathetic neurons of SHRs.

Intriguingly, Li *et al.* revealed raised CaMKII phosphorylation of the GluN2M subunit exclusive to the PVN of SHRs versus controls through Western blot analysis,¹ while celiac ganglionectomy surgery did not reduce CaMKII phosphorylation levels compared to sham surgery. This indicates that high blood pressure does not directly increase CaMKII phosphorylation. Importantly, the authors further probed this result by injecting AIP directly into the PVN. In turn, results observed demonstrated a reduction in lumbar sympathetic nerve activity as well as arterial blood pressure in SHR.¹ A similar effect was found with exclusive AP-5 injection, which suggests that CaMKII is responsible for the increased sympathetic activity in SHRs.

The authors acknowledge that it is currently unclear as to the role of NMDAR activity of hypothalamic PVN presympathetic neurons in secondary hypertensive states, such as salt and obesity-induced hypertension. In this regard, a porcine model of mineralocorticoid-induced, metabolic syndrome-associated hypertension may represent a useful tool in exploring the potential role of this pathway.⁷ Overall the study was well designed as the involved pathways were isolated with utmost precision with a focus on probing potential redundancies in the CaMKII-mediated increase in vasomotor tone in SHRs. The stringent attention to detail removed the effect of peripheral mediators in order to define the role of CaMKII alone on the vasomotor pathway. Sequential blockade of constituents in both the presynaptic and

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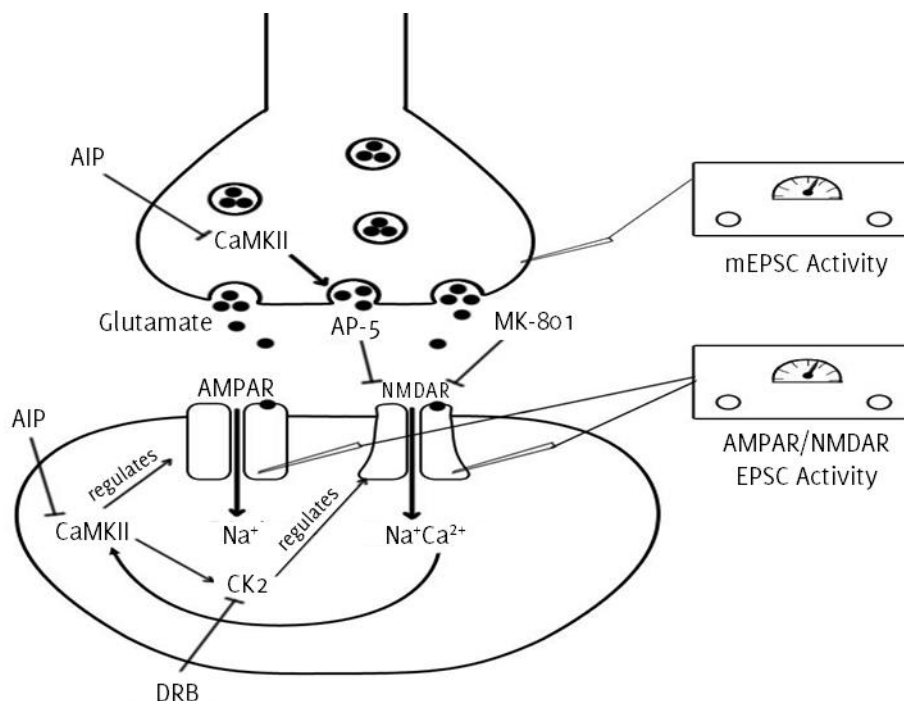
postsynaptic environment, aided in illustrating the direct role of CaMKII in raised sympathetic outflow.

This data represents a significant step in our understandings of neurogenic hypertension molecular underpinnings and provides novel information regarding the central role of CaMKII in synaptic plasticity, thereby revealing potential targets for the future development of pharmacologic treatments. However, further *in vivo* models are warranted to quantify the degree of antihypertensive effects prior to conclusively targeting this pathway for the treatment of hypertension. Previously, there had been indications that treatment-resistant, neurological pathology was mediated by neuro-inflammation – more specifically, the chronic inflammation of the hypothalamic PVN mediated by obesity and the renin angiotensin system.⁸ Indeed, it is difficult to determine whether this represents an entirely alternate hypothesis of neurogenic hypertension or, rather, a contributory/resultant factor of the neuropathology.

While this research is primarily an academic advancement for the field, it is difficult not to extrapolate the translational potential. Despite the armory of pharmaceuticals currently available, essential hypertension is managed satisfactorily in less than half of patients.⁹ Indeed, it has been proposed that much of this discrepancy may be neurologic in origin,¹⁰ indicating that the CaMKII pathway may represent a novel molecular target in the fight against hypertension. We must now assess whether there are any suitably selective drugs currently available, such as the NMDA receptor antagonist Memantine or whether a novel therapy could be designed for this purpose. In terms of the alternate PVN inflammation hypothesis, currently available pharmaceuticals such as angiotensin receptor blockers, immunosuppressants and reactive oxygen species scavengers may offer potency as adjunct therapeutics.

With such insightful data, it may now be time to ask ourselves: is hypertension all in our heads?

Figure 1. Summary of pathways in the PVN of the hypothalamus illustrating the pre- and postsynaptic role of CaMKII and associated locations of EPSC measurement. The drugs used in the study and their associated targets are also shown: AIP, autocalmitide 2-related inhibitory peptide; AP-5, 2-amino-5-phosphonopentanoic acid; DRB, 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazol; MK-801, Dizocilpine; CaMKII, Calcium/Calmodulin dependent protein kinase II; CK2, Casein kinase 2; AMPAR, AMPA receptor; NMDAR, NMDA receptor; EPSC, Excitatory post-synaptic current; mEPSC, miniature excitatory post-synaptic current.



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