Channels 7:2, 57–59; March/April 2013; © 2013 Landes Bioscience

Channels News & Views

The long and short of PKC modulation of the L-type calcium channel

Comment on: Weiss S, et al. Channels 2012; 6:426-37;
PMID:22990911; http://dx.doi.org/10.4161/chan.22016

Jonathan Satin; Department of Physiology; The University of Kentucky College of Medicine; Lexington, KY USA
Email: jsatin1@uky.edu; http://dx.doi.org/10.4161/chan.24147

L-type calcium channel (LTCC) function is critical for electrical and cellular signaling in a diverse range of cell types. In cardiac muscle LTCC function ($I_{Ca,L}$) provides trigger Ca^{2+} for Ca^{2+}-induced Ca release, and $I_{Ca,L}$ kinetics is a critical determinant of action potential duration.[1] LTCC blockade is an effective antihypertensive regimen, likely owing to contributions of $I_{Ca,L}$ in vascular smooth muscle for maintenance of vasotone. Cardiac and vascular smooth muscle finely grade function. In part, precise regulation of $I_{Ca,L}$ contributes to such graded contraction.

The $Ca_V1.2$ pore-forming Ca^{2+}-channel protein is encoded by up to ~50 exons, though there is substantial heterogeneity of $Ca_V1.2$ exon usage. Three key determinants of $I_{Ca,L}$ modulation have been associated with: the cytosolic localized N-terminus, connecting linker between homologous repeats I and II (L_{I-II}), and the C-terminus. For example, L_{I-II} splice variants prominent in vascular smooth muscle confer higher diltiazem sensitivity.[2] Angiotensin II (AngII) is an important regulator of vasotone and contributes to cardiomyocyte signaling. In the heart, AngII elicits a biphasic response.[3] AngII receptor activation via Gq-containing trimeric G-proteins elicits a variety of effects including activation of PKC and $I_{Ca,L}$ modulation. However, the consequence of Gq-signaling of $I_{Ca,L}$ is controversial. Discrepant literature can arise from studies of diverse $Ca_V1.2$ splice variants, and from intricacies of Gq signaling, including, but not limited to differential effects of PKC and Gβγ subunits following receptor activation. In a recent report in *Channels,* the Dascal laboratory sheds new light on the complex regulation of $I_{Ca,L}$ by Gq-signaling pathways.[4] Their new study[4] uses *Xenopus* oocytes as a 'null' background to heterologously express $Ca_V1.2$

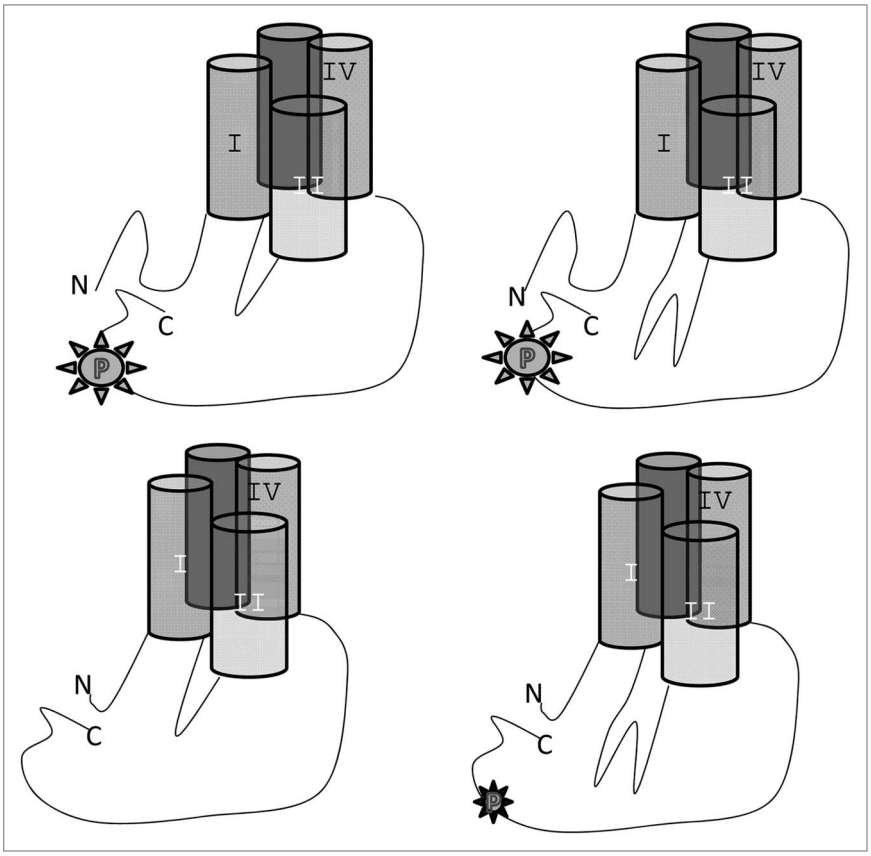

Figure 1. Exon usage influences PKC-mediated C-terminal phosphorylation (note that phosphorylation was not assayed in this study, but was inferred from a Ser to Ala mutation analysis); in turn, C-terminal phosphorylation increases current in long N-terminal splice variants. Four combinations shown: long vs. short N-terminus or LI-II. PKC and Gβγ bind to all N-terminal variants and oppose PKC effects, albeit more efficaciously in short N-terminal variants. Long LI-II partially compensates for short N-terminal.

containing four prevalent combinations of exons expressed in human vasculature or heart. Activated-receptors elicited a biphasic response with an early increase followed by decline of $I_{Ca,L}$ about 10 min after the stimulus. Addition of Gβγ scavenger or Gβγ alone reveals a relatively slowly accumulating inhibition of $I_{Ca,L}$ by Gβγ. The cardiac expressed long N-terminal $Ca_V1.2$ recapitulates native biphasic response to Gq-receptor activation. The early increase requires the relatively long cardiac-expressed N-terminus and is mediated by PKC in opposition to the Gβγ diminution of $I_{Ca,L}$. From here the story gets more complicated. The C-terminus contains a PKC substrate site at Ser1928, and the new study shows a requirement for S1928 to mediate the PKC enhancement of increase of $I_{Ca,L}$ (independently of the Gβγ decrease); however, the N-terminus binds to PKC and Gβγ suggesting N- and C-terminal communication reminiscent, for example of CaM signaling.[5] In a nutshell, this new study separates Gq-PLC vs. Gβγ modulation of $Ca_V1.2$ in an exon-specific fashion (**Fig. 1**).

There are a few caveats also worth noting. First, recapitulation of native $I_{Ca,L}$ modulation is notoriously difficult to achieve. As the authors note, some of these limitations are uniquely overcome in the *X.* oocytes (as opposed to mammalian cells, such as HEK 293 cells). The LTCC is a complex of multiple proteins in mammalian cells. The finding that specific N- and C-termini must communicate to capture native modulation suggests a complicated folding pattern that might encompass interactions with other proteins such as CaM, CaMKII, $Ca_V\beta$ subunits, and RGK proteins. A second issue is that Ba^{2+}, not Ca^{2+} is used as the charge carrier. In *X.* oocytes Ca^{2+} induces a large contaminating Cl- current. Aside from the well-established Ca^{2+}-CaM influence on LTCC kinetics, a recent study showed that the dogmatic auto-inhibition by the distal C-terminus is relieved when Ca^{2+} is the charge carrier,[6] and the accessory protein Rem interacts with $Ca_V1.2$ C-terminus in a Ca^{2+}-CaM dependent fashion.[7] Therefore, caution must be used interpreting the data. This new study is an interesting step forward, but follow-up studies in native systems are of critical importance to verify the complex interplay of channel exon usage and Gβγ and PKC modulation of L-type Ca^{2+} channel function.

References

1. Alseikhan BA, et al. Proc Natl Acad Sci U S A 2002; 99:17185-90; PMID:12486220; http://dx.doi.org/10.1073/pnas.262372999
2. Zhang HY, et al. Br J Pharmacol 2010; 160:1631-40; PMID:20649567; http://dx.doi.org/10.1111/j.1476-5381.2010.00798.x
3. Liang W, et al. Hypertension 2010; 56:422-9; PMID:20696985; http://dx.doi.org/10.1161/HYPERTENSIONAHA.109.149344
4. Weiss S, et al. Channels (Austin) 2012; 6:426-37; PMID:22990911; http://dx.doi.org/10.4161/chan.22016
5. Dick IE, et al. Nature 2008; 451:830-4; PMID:18235447; http://dx.doi.org/10.1038/nature06529
6. Crump SM, et al. Am J Physiol Heart Circ Physiol 2012; PMID:23203963; http://dx.doi.org/ 10.1152/ajpheart.00396.2012
7. Pang C, et al. Channels (Austin) 2010; 4:192-202; PMID:20458179; http://dx.doi.org/10.4161/chan.4.3.11867

Pannexins after stroke: Knocking-out membrane channels to improve neurological function

Comment on: Bargiotas P, et al. Channels 2012; 6:453-6; PMID:23111424; http://dx.doi.org/10.4161/chan.22315;

Anna Rosell; Neurovascular Research Laboratory; Vall d'Hebron Research Institute; Universitat Autònoma de Barcelona; Barcelona, Spain
Email:anna.rosell@vhir.org; http://dx.doi.org/10.4161/chan.24143

New therapeutic approaches are urgently needed to treat stroke patients. According to the World Health Organization this devastating disease affects 15 million people each year, among them about 10 million will die or live as functionally-disabled stroke survivors.

Despite these devastating numbers, only pharmacological and mechanical reperfusion therapies to restore the blood flow have proven to save lives and improve the neurological outcome of ischemic stroke patients.[1] On the other side, hundreds of neuroprotective drugs targeting inflammation, oxidative stress, apoptosis and other cell-death triggers of the ischemic cascade, have failed to prove efficacy in clinical trials after obtaining encouraging supporting data from pre-clinical studies. Hence, enormous disappointment has struck researchers, neurologists and industry partners when translating basic science findings to the clinical practice.

This shocking reality has opened the eyes of many stroke researchers to look for new targets, and most importantly, to find new ways to validate potential therapeutic benefits in a bedside-to-bench strategy. In this regard, the main endpoint of stroke clinical trials designed to prove efficacy is to demonstrate improvement of neurological function in stroke survivors.

In recent publications from Bargiotas and colleagues,[2,3] the authors show this new vision in the stroke research field: the authors focus their interest in pannexins, a family of proteins involved in basic cell-signaling functions, to demonstrate their role not only in brain injury but also in modifying functional outcome in a mouse model of stroke.

It is known that cell-to-cell communication occurs directly through gap-junctions between cells or by indirect paracrine signaling when cells release molecules such as ATP, ions or small metabolites into the extracellular space. With a structure similar to gap-junction forming connexins, recently discovered pannexins are membrane channels described to connect the cytosol with the extracellular space.[4] Pannexin 1 (Px1) and pannexin 2 (Px2) are known to be expressed in the cerebral nervous system, in contrast to the other member of the family, pannexin 3.[4] Interestingly, pannexins have been shown to be expressed in the brain both in neurons and astrocytes,[2,5] while other authors have recently demonstrated their expression in vascular cells of the rat brain (Px1 expression in smooth muscle cells and Px2 in both endothelium and smooth muscle cells).[6] Regarding function, channel activity has been demonstrated to be dependent on pannexins in neurons,[2] whereas channel activity in astrocytes has been reported to be both independent and dependent of pannexins by different authors.[2,7]

Interestingly, Px1- and Px2-deficient mice, but not single knockouts, have recently shown to be protected in front of ischemia in an experimental model reproducing cortical infarcts, by reducing lesion volume and improving neurological outcome at short-term.[2] Why pannexins become involved in brain damage? Several authors speculate that K+ efflux, accumulation of reactive oxygen species and caspase expression after ischemia might activate and open these membrane channels leading to cell death,[8] although the precise mechanisms still need to be fully characterized.

The knowledge on pannexin functions is incipient and continuously evolving. However, Bargiotas and colleagues[2,3] have already explored the final consequences of knocking-out these proteins in the context of cerebral ischemia. Their results position pannexins as therapeutic targets to improve functional outcome in sensorimotor, anxiety and exploration functions after stroke. Certainly these are exciting results, but from a bedside-to-bench point of view it is still required to demonstrate if the reported neurological protection is sustained long-term, in old animals, in females or in other species, and should be validated by independent researchers in other stroke models. Finally, it would be interesting to explore the pharmacological inhibition of pannexins to demonstrate functional benefits in preclinical models before we move to the clinical setting.

References

1. Molina CA. Stroke 2011; 42(Suppl):S16-9; PMID:21164113; http://dx.doi.org/10.1161/STROKEAHA.110.598763
2. Bargiotas P, et al. Proc Natl Acad Sci U S A 2011; 108:20772-7; PMID:22147915; http://dx.doi.org/10.1073/pnas.1018262108
3. Bargiotas P, et al. Channels (Austin) 2012; 6:453-6; PMID:23111424; http://dx.doi.org/10.4161/chan.22315
4. Sosinsky GE, et al. Channels (Austin) 2011; 5:193-7; PMID:21532340; http://dx.doi.org/10.4161/chan.5.3.15765
5. Bruzzone R, et al. Proc Natl Acad Sci U S A 2003; 100:13644-9; PMID:14597722; http://dx.doi.org/10.1073/pnas.2233464100
6. Burns AR, et al. J Vasc Res 2012; 49:101-10; PMID:22301733; http://dx.doi.org/10.1159/000332329
7. Iglesias R, et al. J Neurosci 2009; 29:7092-7; PMID:19474335; http://dx.doi.org/10.1523/JNEUROSCI.6062-08.2009
8. MacVicar BA, et al. Trends Neurosci 2010; 33:93-102; PMID:20022389; http://dx.doi.org/10.1016/j.tins.2009.11.007