REGULATION OF L-TYPE CALCIUM CHANNEL SPARKLET ACTIVITY BY PKC AND C-SRC

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ABSTRACT

Ca^{2+} sparklets are elementary fluorescence events associated with Ca^{2+} entry through L-type Ca^{2+} channels ($Ca_v1.2$) channels and are classified as persistent and low activity Ca^{2+} sparklets. Persistent Ca^{2+} sparklets are characterized by longer and more frequent channel open events and account for approximately 50% of the steady state Ca^{2+} entry through $Ca_v1.2$ channels. Previous studies suggest that the alpha isoform of protein kinase C (PKCα) underlies persistent Ca^{2+} sparklet activity, but the mechanism of PKCα action on $Ca_v1.2$ channels is unclear. c-Src, another highly expressed kinase in vascular smooth muscle, phosphorylates $Ca_v1.2$ to increase whole-cell Ba^{2+} current (I_{Ba}) but it remains unknown if c-Src induces persistent Ca^{2+} sparklet activity through $Ca_v1.2$ channels. Here, I addressed two questions: 1) Does c-Src produce persistent Ca^{2+} sparklets through $Ca_v1.2c$ (the neuronal isoform of $Ca_v1.2$)? 2) Does PKCα activate c-Src to produce persistent Ca^{2+} sparklets? TIRF microscopy was used to record Ca^{2+} sparklets from voltage-clamped HEK 293T cells co-expressing wild type (WT) or mutant $Ca_v1.2c$ + active or inactive PKCα/c-Src. The results indicate that c-Src produces persistent Ca^{2+} sparklet activity, which is significantly reduced in the presence of the c-Src inhibitor, PP2, or with overexpression of kinase-dead c-Src. I tested two potential c-Src phosphorylation sites ($Y^{2122}F$ and $Y^{2139}F$) on $Ca_v1.2c$ for their role in production of persistent Ca^{2+}sparklets. The $Y^{2122}F$ mutation significantly reduced persistent Ca^{2+}sparklet activity while the $Y^{2139}F$ mutation was without any effect, indicating that c-Src phosphorylates $Ca_v1.2c$ at Y^{2122} to induce persistent Ca^{2+} sparklets. $Y^{2122}F$ and $Y^{2139}F$ mutations did not have any significant effect on persistent Ca^{2+} sparklets in cells expressing $Ca_v1.2c$ + PKCα, indicating that PKCα does not act upstream of c-Src to produce persistent Ca^{2+}sparklets. Whether PKCα phosphorylates S^{1901}, the classical PKA phosphorylation site, to produce persistent Ca^{2+}sparklet activity remains to be resolved.