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Short communication

Contribution of Protein Kinase C (PKC) in the modulation of Voltage gated Calcium Channels

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ABSTRACT:

High voltage-gated calcium (CaV) channels are the major source for Ca^{2+} influx that underlies Ca^{2+} - dependent response in excitable cells. Protein kinase C (PKC) is families of protein kinases enzymes that are play important roles in several signal transduction cascades. Ca^{2+}, Ca_V channels and PKC are involved in the processes of pain, insulin secretion, glucose homeostasis, smooth muscle physiology, response to neuro-chemicals, receptor sensitization, in modulating membrane structure events, in mediating immune response, in regulating cell growth, and in learning and memory. The identification of the roles of these proteins will provide us significant knowledge for the understanding of the complexity of the ion channel physiology and regulation of many diseases. In this short communications, we discuss the contribution of PKC in the modulation of calcium channels in *Xenopus* oocytes model.

KEYWORDS: Acetyl-*β*-methylcholine; insulin; pain; phorbol-12-myristate-13-acetate;phosphorylation sites; *Xenopus* oocytes.

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INTRODUCTION

VOLTAGE GATED CALCIUM CHANNELS (CaV)

The intracellular processes such as contraction, secretion, neurotransmission and gene expression are regulate by Ca_V mediate calcium channel influx in response to membrane depolarization[1]. These channels are a family of hetero-multimers classified into six types (Ca_V 1.2, 1.3, 1.4, 2.1, 2.2 and 2.3) based on their pore-forming α1 subunits. The members of $\alpha_1$1.0 subfamily, 1.2, 1.3 and 1.4 encodes the variants of the L-type channels; $\alpha_1$2.1 encodes P/Q-type channels, $\alpha_1$2.2 encodes N-type channels and $\alpha_1$2.3 encodes R-type channels. The low voltage gated calcium channels are known as 3.0 subfamilies[2,3].

The α1 subunit is the largest, and it incorporates the conduction pore, the voltage sensor, and the gating apparatus. The β, α_2/δ, and γ subunit, *viz*., the N and C termini and the intracellular loops between domains I and II, II and III, and III and IV, possess the binding/recognition sites for second messengers such as G protein β subunits or intracellular Ca^{2+} ($[Ca^{2+}]i$) as well as sites that can be phosphorylated by protein kinase C (PKC)[4-6].
The α1 sub-unit generates a functional Ca_V channel when expressed alone and determines the major characteristics of the channels, while the auxiliary sub-units modulate the expression and certain gating properties of the channels[7,8].

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The cytoplasmic regions of α_1 sub-units possess recognition sites for a number of second messengers and enzymes that modulate channel activity and function[9,10].

The Ca_V1 subfamily initiates contraction, secretion, regulation of gene expression, integration of synaptic input in neurons, and synaptic transmission at ribbon synapses in specialized sensory cells. The Ca_V2 subfamily is primarily responsible for initiation of synaptic transmission at fast synapses. The Ca_V3 subfamily is important for repetitive firing of action potentials in rhythmically firing cells such as cardiac myocytes and thalamic neurons[11].

Protein kinase C (PKC)

The family of lipid-activated Ser/Thr protein kinases is known as protein kinase C (PKC), present at different concentration in different cell/tissue types and is divided into three groups of isozymes based on co-factor requirements. The classical cPKCs-α, βI, βII and γ require diacylglycerol, phosphatidylserine (PS) and Ca^{2+} for activation. The novel PKCs-δ,ε,η and θ require diacylglycerol and PS, but are Ca^{2+}-independent, while the atypical PKCs--ζ, ι/λ and μ require neither Ca^{2+} nor diacylglycerol[12-14]. In addition, DAG-sensitive PKC isoforms can be activated pharmacologically using phorbol esters.

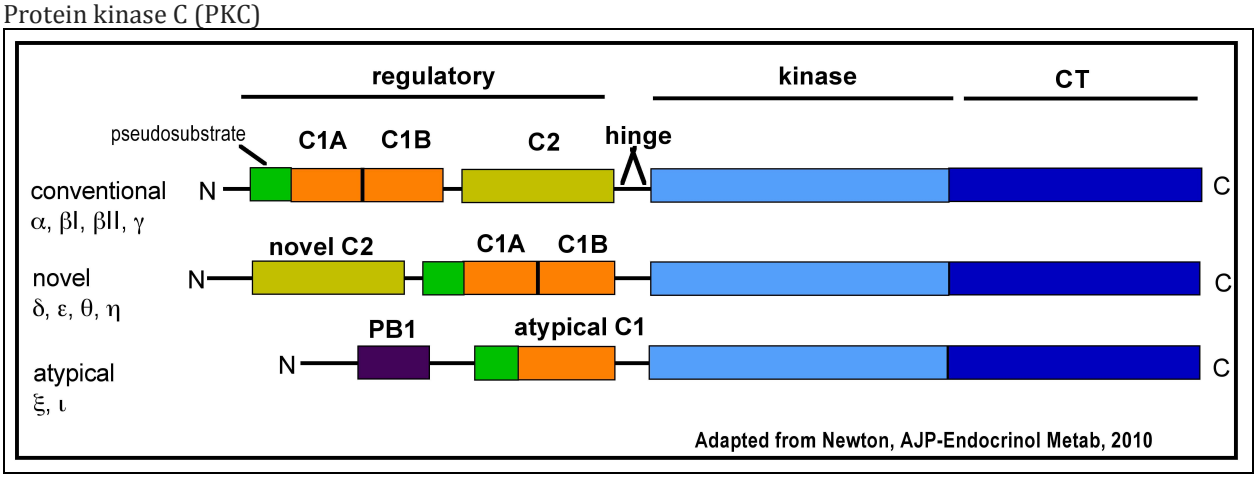

Figure 1. PKC Family. Domain composition of PKC isoforms: pseudosubstrate (green rectangle), C1 domain [orange rectangle; C1B domain binds diacylglycerol (DAG)], C2 domain [yellow rectangle; Ca^{2+} binding], hinge segment, kinase domain (light blue) and carboxyl-terminal tail (CT; dark blue rectangle).

DIFFERENTIAL REGULATION OF PKC ON VOLTAGE GATED CALCIUM CHANNELS

Ca_V channels respond differently to various activators of PKC. In the oocyte expression system, phorbol-12-myristate, 13-acetate (PMA) did not affect $Ca_V1.2c$ or 2.1 currents, whereas, $Ca_V2.2$ and 2.3 currents were potentiated. In contrast, acetyl-β-methylcholine (MCh) potentiated Ca_V 2.3 currents, decreased $Ca_V1.2c$ currents, and failed to modulate $Ca_V2.1$ or 2.2 currents[15]. The selectivity of PKC isozyme may allow for the inclusion of different ion channels in response to different agonists; and the possibility is raised that selective PKC isozyme inhibitors may be able to modify the action of specific members of the calcium channel family because of different isozyme-selective target sites on the channels[16].

Phosphorylation at specific PKC sites in the I-II linker and C-terminus would seem to be sufficient for the activation of Ca_V 2.2 channels, in the Ca_V 2.3 channels, these sites may be necessary but not sufficient. The experiments suggest that the PKCβII and PKCε isozymes may be involved in I-II linker phosphorylation events when cells are stimulated by PMA. Activation of the Ca_V 2.3 channels by MCh additionally requires unique sites in the II-III linker and one in the C-terminus[17,18]. The evidence that PKC α is required for the MCh but not the PMA effect suggests that PKC α may effect phosphorylation at the II-III linker sites[16].

Ca_V 2.2 channels are critical for pain transduction and we found the five phosphorylation sites (Ser-425, Ser-1757, Ser-2108, Ser-2132 and Thr-422) in the Ca_V 2.2 channels based on the PKC phosphorylation. The contribution of Ser-1757 to the increased PMA response may be biologically significant. The differential localization of Ser-2132 and Ser-1757 of Ca_V α1 2.2 subunit splice variants and the modulation of one of them by PKC phosphorylation may be relevant in the regulation of the signal transduction for pain. Thr-422, Ser-425, and Ser-2108 are homologous to Ca_V α_1 2.3 subunit Thr-365, Ser-369, and Ser-1995 of Ca_V α_1

PBMS
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2.3 subunit, and the latter set of Thr/Ser were responsible for PMA- or MCh-induced potentiation of Ca_V $\alpha_1$2.3[17]. The regulation at these sites appears to be complex. The availability or phosphorylation state of one site may determine the availability or role of another, i.e. the phosphorylation events may be ordered. It is possible that in the WT, Ser-425 becomes phosphorylated and blocks the phosphorylation of Thr-422 and the C-terminal sites leading to a limited response to PMA. The combined action of Thr-422 or Ser-425 of the I-II linker with Ser-2108 and not Ser-1757 or Ser-2132 of the C terminus suggests specific functional interaction, either direct or indirect, between the I-II linker and the C terminus. Such an action is analogous to the proposed interaction between the II-III linker and the C terminus in the Ca_V α_1 2.3 subunit[18].

The mutational analysis suggests that PKCβII may be better at phosphorylating C-terminal sites of Ca_V α1 2.2 subunits. Both PKCβII and PKCε can modulate the stimulatory Thr-422 in the I–II linker but PKCβII is better at regulating the inhibitory Ser-425 site. The lesser effect of PKC βII, and possibly PKCε, at the inhibitory site may be via indirect effects of those cPKCs on PKCεfunction. Thus, differential activation of PKC isozymes may permit separate regulation of different members of the Ca channel family. Even within one channel type, activation of different combinations of PKC isozymes may allow for graded levels of activation or inhibition and susceptibility or resistance of the channel to subsequent stimulatory events[19-21].

MCh or PKC isozymes a, βII or ε potentiated CaV2.2 currents expressed with Ca_V2.2α1 subunits. The potentiation of Ca_V2.2 currents by MCh or PKC isozymes is inhibited by the Ca_V β subunits. It is suggested that Ca_V β subunits compete with the PKC isozymes for the Ser/Thr PKC target sites on the CaV2.2α1 subunits, viz., Thr-422, Ser-425, Ser-1757, Ser-2108 and Ser-2132. Among these sites, Thr-422, Ser-1757 and Ser-2132 are the possible PKCα sites since Ser/Thr-Ala mutation of these sites inhibited MCh potentiation of Ca_V2.2α1 currents. On the contrary, Ser-2108 is a possible PKC βII and ε site since its mutation did not affect MCh response. Taken together it appears that CaV 2.2α1 subunits are responsible for the pore formation and the auxiliary Ca_V β subunits are for their susceptibility to neuromodulators22.

In differential affect of local anesthetic, isoflurane by itself inhibited Ca_V 2.1 and 2.2 currents in quantitatively similar manner, but the effect had a significant complexity in view of the activation of the PKC isozymes and their target sites. It is possible that the stimulatory PKC sites of Ca_V2.2 α_1 subunit, Thr-422, Ser-1757, Ser-2108 and Ser-2132 counter-acted the inherent depressant effect of isoflurane observed on a wide variety of voltage-gated channels. In contrast, the inhibitory site (Ser-425), when present augmented the effect of isoflurane. PKCδ potentiated Ca_V2.2 currents in the presence of isoflurane and similarly isoflurane was necessary for the potentiation of Ca_V2.1 currents by PMA or the combination of PKCβII and PKCε. This study demonstrates the complexity of combined direct and indirect actions of an anesthetic on an ion channel behavior. It seems likely that PKC isozyme-induced alteration of CaV currents by isoflurane may vary in different cell types and tissues depending upon the specific PKC isozymes that are present. In addition, it is possible that isoflurane-induced modulation of PKC activity could also have important consequences for other effecter proteins and intracellular cascades[20, 23].

Ca_V β subunits are members of membrane-associated guanylate kinase family, thereby suggesting a role in scaffolding multiple signaling pathways around the channel. The tridimensional structure of this subunit supports the above statement as it has large space for the interaction with putative partners[24]; PKCα may be one such partner. It has been reported by others also that PKC responsiveness of the ICa was modulated by Ca_V β subunits in a Ca_V α1 subunit dependent manner[17, 25-27]. Taken together, insulin secretion is the result of interaction between CaV α_1 subunits, their Ser/Thr sites, Cav β and PKC isozymes[22].

CONCLUSION

The selectivity of PKC isozyme may allow for the inclusion of different ion channels in response to different agonists; and the possibility is raised that selective PKC isozyme inhibitors may be able to modify the action of specific members of the calcium channel family because of different isozyme-selective target sites on the channels.

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