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Brain Research

Research Report

Intracellular calcium level is an important factor influencing ion channel modulations by PLC-coupled metabotropic receptors in hippocampal neurons

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ARTICLE INFO

Article history:
Accepted 19 March 2013
Available online 30 March 2013

Keywords:
Calcium dependence
Channel modulation
Phospholipase C
Hippocampal neuron
K^+ channel
Non-selective cation channel

ABSTRACT

Signaling pathways involving phospholipase C (PLC) are involved in various neural functions. Understanding how these pathways are regulated will lead to a better understanding of their roles in neural functions. Previous studies demonstrated that receptor-driven PLCβ activation depends on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), suggesting the possibility that PLCβ-dependent cellular responses are basically Ca^{2+} dependent. To test this possibility, we examined whether modulations of ion channels driven by PLC-coupled metabotropic receptors are sensitive to $[Ca^{2+}]_i$ using cultured hippocampal neurons. Muscarinic activation triggered an inward current at −100 mV (the equilibrium potential for K^+) in a subpopulation of neurons. This current response was suppressed by pirenzepine (an M_1-preferring antagonist), PLC inhibitor, non-selective cation channel blocker, and lowering $[Ca^{2+}]_i$. Using the neurons showing no response at −100 mV, effects of muscarinic activation on K^+ channels were examined at −40 mV. Muscarinic activation induced a transient decrease of the holding outward current. This current response was mimicked and occluded by XE991, an M-current K^+ channel blocker, suppressed by pirenzepine, PLC inhibitor and lowering $[Ca^{2+}]_i$, and enhanced by elevating $[Ca^{2+}]_i$. Similar results were obtained when group I metabotropic glutamate receptors were activated instead of muscarinic receptors. These results clearly show that ion channel modulations driven by PLC-coupled metabotropic receptors are dependent on $[Ca^{2+}]_i$, supporting the hypothesis that cellular responses induced by receptor-driven PLCβ activation are basically Ca^{2+} dependent.

1. Introduction

Phosphoinositide-specific phospholipase C (PLC) enzymes are involved in a number of cellular responses to receptor activation (Rebecchi and Pentyala, 2000; Rhee, 2001; Suh et al., 2008). Among six families of PLC enzymes (β, γ, δ, ε, ζ and η), PLCβ family members (PLCβ1–4) are driven by a wide range of $G_{q/11}$-coupled receptors including M_1/M_3 muscarinic

Abbreviations: $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; FFA, flufenamic acid; I-mGluR, Group I metabotropic glutamate receptor; NSC, non-selective cation; Oxo-M, oxotremorine M; PLC, phospholipase C; TTX, tetrodotoxin

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http://dx.doi.org/10.1016/j.brainres.2013.03.040

acetylcholine receptors, group I metabotropic glutamate receptors (I-mGluRs), α_1 adrenergic receptors, and the receptors for cholecystokinin, bradykinin, vasopressin, angiotensin II and thromboxane A_2 (Oude Weernink et al., 2007; Rebecchi and Pentyala, 2000; Rhee, 2001; Werry et al., 2003). The PLCβ enzymes play critical roles in neural functions, and PLCβ1 and PLCβ4-knockout mice develop epilepsy and ataxia, respectively (Kim et al., 1997). The $G_{q/11}$-coupled receptor-PLCβ signaling pathways contribute to neuronal functions through multiple second messengers that cause modulation of ion channels (Anwyl, 1999; Hernandez et al., 2008; Hughes et al., 2007; Suh and Hille, 2005), Ca^{2+} release from internal stores (Ross et al., 2005), endocannabinoid release (Kano et al., 2009) and activation of enzymes (Amadio et al., 2006; Delmas et al., 2004). Understanding how these signaling pathways are regulated will undoubtedly lead to a better understanding of their roles in brain functions. We previously found that $G_{q/11}$-coupled receptor-driven endocannabinoid release, which requires PLCβ activity, was influenced by intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Hashimotodani et al., 2005; Maejima et al., 2005), and that this Ca^{2+} dependence was attributable to the Ca^{2+} dependence of receptor-driven PLCβ activation (Hashimotodani et al., 2005). These observations have raised the question of whether cellular responses elicited by receptor-driven PLCβ activation are all Ca^{2+} dependent, which has not been fully elucidated.

Activation of $G_{q/11}$-coupled receptors induces activation of non-selective cation (NSC) channels and inhibition of K^+ channels (Delmas et al., 2004; Delmas and Brown, 2005; Moran et al., 2004), both of which contribute to depolarization, in various types of neurons including hippocampal neurons (Chiang et al., 2010; Congar et al., 1997; Gee et al., 2003). In the present study, we used these channel modulations as examples of cellular responses elicited by receptor-driven PLCβ activation, and examined whether they are Ca^{2+} dependent. Using cultured hippocampal neurons, we observed that ion channel modulations driven by muscarinic receptors and I-mGluRs are dependent on $[Ca^{2+}]_i$. Importantly, the Ca^{2+} dependence of muscarinic inhibition of K^+ channels was similar to that of muscarinic receptor-mediated PLCβ activation, indicating that the Ca^{2+} depencence of K^+ channel inhibition can be explained by the Ca^{2+} depencence of PLCβ activation. These data are consistent with the hypothesis that cellular responses induced by receptor-driven PLCβ activation are basically Ca^{2+} dependent.

2. Results

2.1. PLC dependence of muscarinic depolarization

First we examined effects of muscarinic receptor activation on the resting membrane potential in cultured hippocampal neurons. In the presence of the Na^+ channel blocker tetrodotoxin (TTX), the resting membrane potential was -64.0 ± 0.9 mV ($n=21$). When 10 μM oxotremorine M (oxo-M), a muscarinic agonist, was locally applied for 10 s, depolarization was induced in almost all neurons tested (Fig. 1A and C). The averaged peak amplitude of depolarization was 11.5 ± 2.0 mV ($n=21$) (Fig. 1B). The oxo-M-induced depolarization was markedly suppressed in the neurons that were pretreated with the PLC inhibitor U73122 (10 μM, 10–30 min) (Fig. 1). In these neurons, the averaged amplitude of depolarization was 1.3 ± 0.3 mV ($n=8$) (Fig. 1B). As mechanisms of muscarinic depolarization, potential candidates are inhibition of K^+ channels and activation of NSC channels. In the following experiments, these two components were separately examined by measuring current responses to oxo-M at −100 mV and −40 mV in voltage-clamp mode.

2.2. PLC and Ca^{2+} dependence of the current response to oxo-M at −100 mV

To minimize the contribution of K^+ channel-derived component to current responses, the membrane potential was clamped at −100 mV, which is close to the K^+ equilibrium potential. At this potential, the holding current is expected to be insensitive to K^+ conductance change. Out of 13 neurons that were dialyzed with pCa 7-solution, application of 3 μM oxo-M for 5 s induced an appreciable inward current (>25 pA) in only two neurons (Fig. 2C). At 10 μM, oxo-M application induced the inward current more often (16 out of 26 cells). The amplitude of current responses varied from neuron to neuron (Fig. 2C). Fig. 2B shows the time course of current response to 10 μM oxo-M, which was obtained by averaging 10 current responses larger than 50 pA after peak-normalization. The treatment with U73122, but not the inactive analog U73343, almost completely abolished the current response to 10 μM oxo-M (Fig. 2D). The M_1-preferring antagonist pirenzepine (1 μM) and NSC channel blocker flufenamic acid (FFA) also markedly suppressed the current response (Fig. 2E and H). These results indicate that activation of muscarinic receptors sensitive to 1 μM pirenzepine causes opening of NSC channels in a PLC-dependent manner.

Next we examined the Ca^{2+} dependency of this process, by using pCa 7- and pCa 9-pipette solutions. In this series of experiments, oxo-M was applied in the absence of external Ca^{2+} in order to minimize possible Ca^{2+} influx through Ca^{2+}-permeable NSC channels. In the neurons dialyzed with pCa 7-solution, application of 3 μM oxo-M in the absence of Ca^{2+} induced an appreciable inward current much more often (Fig. 3; pCa7) than in the presence of 2 mM Ca^{2+} (Fig. 2C; 3 oxo). This enhancement of current responses in the absence of external Ca^{2+} is the feature suggestive of NSC channels, as it is well known that the inward current through NSC channels is augmented when external Ca^{2+} ions are removed (Ishibashi et al., 2003; Lintschinger et al., 2000; Tsujino et al., 2005). In the neurons dialyzed with pCa 9-solution, no appreciable currents were induced by application of 3 μM oxo-M in the absence of Ca^{2+} (Fig. 3). These results indicate that the muscarinic activation of NSC channels requires intracellular Ca^{2+} ions. Further experiments were not performed to study the Ca^{2+} dependence of this process, because NSC channels are a diverse group of proteins including transient receptor potential (TRP) superfamily members and Ca^{2+} ions have been reported to exert variable effects on them (Blair et al., 2009; Lintschinger et al., 2000; Moran et al., 2004), which will make interpretation of experimental data difficult.