CaMKIIα, a modulator of M4 muscarinic acetylcholine receptors

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G protein-coupled receptors (GPCRs) are subject to the regulation by protein kinases. By controlling the phosphorylation-dephosphorylation balance, protein kinases actively modify GPCR expression and function. In a recent study, we have identified a novel phosphorylation-dependent regulation of Gαi/o-coupled muscarinic acetylcholine receptors. A synapse-enriched protein kinase, Ca2+/calmodulin-dependent protein kinase II (CaMKIIα), binds directly and selectively to second intracellular loops of muscarinic M4 receptors (M4Rs). This Ca2+-sensitive binding enables CaMKIIα to phosphorylate M4Rs at a selective threonine residue. In rat striatal neurons which abundantly express M4Rs, rapid cytoplasmic Ca2+ rises enhance the association of CaMKIIα with M4Rs and increase threonine phosphorylation of the receptor. This CaMKIIα-mediated phosphorylation results in a potentiation of M4R activity, which is critical for controlling cellular and behavioral responsivity to dopamine stimulation. In sum, our data identify a novel kinase-GPCR interaction. Through a Ca2+/activity-sensitive manner, CaMKIIα contributes to maintaining acetylcholine-dopamine homeostasis in the basal ganglia.

Key words: striatum, calcium, calmodulin, adenylyl cyclase, cAMP, phosphorylation, behavior, cocaine

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preferentially localized at postsynaptic sites.\textsuperscript{14,16} We thus targeted the striatum to define the protein-protein interaction between endogenous CaMKII\textalpha{} and M4Rs in vivo. In a complete set of coimmunoprecipitation experiments, physical interactions between the two partners in striatal neurons were established. This interaction and its specificity were confirmed by the lack of the interaction in M4R-deficient mice. Interestingly, in vivo CaMKII-M4R interactions are subject to a positive regulation by Ca\textsuperscript{2+}. This important property indicates a previously unrecognized Ca\textsuperscript{2+} route linking CaMKII to M4Rs in an activity-dependent manner. Nevertheless, coimmunoprecipitation data provide no clear indication about whether interacting proteins interact with each other directly or indirectly. Due to this reason, we expanded our study to include experiments more directly towards solving this issue. Based on the core CaMKII-binding sequence on M4R\textsubscript{\alpha{}} (YPARRTTKM) we identified, we developed an interaction-interfering peptide (M4R\textsubscript{\alpha{-}}) which directly inhibits CaMKII binding to a specific M4R site. This induced a CaMKII-mediated and M4R\textsubscript{\alpha{-}}-dependent phosphorylation of M4Rs. Of note, an actual level of phosphorylation at the specific Thr145 site has not been examined. A site- and phospho-specific antibody or a proteomic tandem mass spectrometry analysis is needed, if feasible, to portray accurate responses of M4R\textsubscript{\alpha{-}} phosphorylation at Thr145 to Ca\textsuperscript{2+} signals.

The in vitro interaction between recombinant CaMKII and M4Rs needs to be validated for native proteins in vivo. In rodent brains, M4Rs are most abundantly present in the striatum and are bound to M4Rs and (2) among second loops from all five muscarinic subtypes, CaMKII\textalpha{} to M4Rs. Synergistic activation of M4Rs by an intracellular mechanism involving the Ca\textsuperscript{2+}-regulated CaMKII association with M4Rs potentiates the efficacy of M4Rs in inhibiting cAMP signaling. Ca\textsuperscript{2+} activates CaMKII to increase its binding to the C-terminal region of the second intracellular loop of M4Rs (M4R\textsubscript{\alpha{-}}). This enhances the inhibitory tone of M4Rs on cAMP responses to dopamine. Other abbreviations: AC, adenylyl cyclase; CaM, calmodulin; DA, dopamine; PKA, cAMP-dependent protein kinase A.

In fact, evidence shows that M4RIL2 serves as a preferred substrate of CaMKII.\textsuperscript{13} This, therefore, potentiates the efficacy of the CaMKII-M4R interaction. In rodent brains, M4Rs are most abundantly present in the striatum and are bound to M4Rs and (2) among second loops from all five muscarinic subtypes, CaMKII\textalpha{} to M4Rs. Synergistic activation of M4Rs by an intracellular mechanism involving the Ca\textsuperscript{2+}-regulated CaMKII association with M4Rs potentiates the efficacy of M4Rs in inhibiting cAMP signaling. Ca\textsuperscript{2+} activates CaMKII to increase its binding to the C-terminal region of the second intracellular loop of M4Rs (M4R\textsubscript{\alpha{-}}). This enhances the inhibitory tone of M4Rs on cAMP responses to dopamine. Other abbreviations: AC, adenylyl cyclase; CaM, calmodulin; DA, dopamine; PKA, cAMP-dependent protein kinase A.

Biochemical binding of CaMKII\textalpha{} to M4R\textsubscript{\alpha{-}} may or may not lead to phosphorylation of M4R\textsubscript{\alpha{-}}. Sequence analysis of the CaMKII\textalpha{}-binding site in M4R\textsubscript{\alpha{-}} reveals a consensus substrate recognition motif (RXSTT/S) for CaMKII.\textsuperscript{13} This, together with the finding that the catalytic domain is the part of the kinase binding to M4R\textsubscript{\alpha{-}}, suggests M4R\textsubscript{\alpha{-}} to be a potential phosphorylation substrate of the kinase. In fact, evidence shows that M4R\textsubscript{\alpha{-}} serves as a preferred substrate of CaMKII\textalpha{}. An accurate phosphorylation site (Thr145) resides within the consensus phosphorylation motif of M4R\textsubscript{\alpha{-}}. The in vitro interaction between recombinant CaMKII and M4Rs needs to be validated for native proteins in vivo. In rodent brains, M4Rs are most abundantly present in the striatum and are bound to M4Rs and (2) among second loops from all five muscarinic subtypes, CaMKII\textalpha{} to M4Rs. Synergistic activation of M4Rs by an intracellular mechanism involving the Ca\textsuperscript{2+}-regulated CaMKII association with M4Rs potentiates the efficacy of M4Rs in inhibiting cAMP signaling. Ca\textsuperscript{2+} activates CaMKII to increase its binding to the C-terminal region of the second intracellular loop of M4Rs (M4R\textsubscript{\alpha{-}}). This enhances the inhibitory tone of M4Rs on cAMP responses to dopamine. Other abbreviations: AC, adenylyl cyclase; CaM, calmodulin; DA, dopamine; PKA, cAMP-dependent protein kinase A.

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to activate adenyl cyclase and increase cAMP formation in D1-bearing neurons. At these same neurons, the M4R efficacy in inhibiting adenyl cyclase and cAMP formation is potentiated simultaneously by CaMKII. This provides a Ca\(^{2+}\)/activity-dependent mechanism to prevent overstimulation of D1 signaling or to bring in a heterologous desensitization of D1 receptor responses. Behaviorally, the CaMKII-regulated dopamine-acetylcholine balance controls movement. By balancing D1 and M4R activity in striatonigral efferent neurons, the kinase participates in the regulation of the ‘direct pathway’ outflow and motor activity. In the case of cocaine stimulation, elevated dopamine activates D1 receptors on striatonigral neurons to increase cAMP levels and activate downstream protein kinase A, which in turn upregulates excitability of these neurons and stimulates motor activity.\(^1\) Similar to dopamine, acetylcholine responds to cocaine by showing an increased local release.\(^2\)\(^\text{12}\) Cellular CaMKII activity in striatonigral neurons may also be enhanced by a Ca\(^{2+}\) entry to be identified. Through both increased extracellular acetylcholine levels and augmented intracellular CaMKII activity, M4R efficacy is upregulated to match an enhanced D1 activity. This ultimately contributes to restricting motor responses to cocaine and balancing an unbalanced dopamine-acetylcholine level. Given the fact that the dopamine-acetylcholine system is linked to the pathogenesis of psychiatric disorders such as addiction and neurodegenerative disorders such as Parkinson’s disease, future interesting studies will be needed to elucidate the accurate role of the CaMKII-M4R coupling in the progressive development of these disorders.

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