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CaMKII hunkers down on the muscarinic M4 receptor to help curb cocaine-induced hyperlocomotion

Ivar S Stein and Johannes W Hell*

Department of Pharmacology, University of California, Davis, CA, USA
*Correspondence to: jwhell@ucdavis.edu

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The dopaminergic receptor D1 activates and the muscarinic acetylcholine receptor (mAChR) M4 inhibits adenylyl cyclase (AC) and thereby cAMP production through the trimeric $G\alpha_s$ and $G\alpha_{i/o}$ proteins, respectively (Wess *et al*, 2007). D1 and M4 are coexpressed in striatal output neurons, where M4 antagonizes D1 signalling as recently pinpointed by conditional M4 knockout in D1-expressing neurons (Jeon *et al*, 2010). Closely following on the heels of this article, Guo *et al* (2010) report in this issue that CaMKII binds and phosphorylates M4 upon Ca^{2+} influx to augment the antagonistic action of M4 on D1 signalling and on D1-mediated, cocaine-triggered hyperlocomotion.

The Ca^{2+}- and calmodulin (CaM)-dependent protein kinase CaMKII is omnipresent throughout the brain: it is a huge complex of 12 identical or closely related subunits. It constitutes ~1% of the total brain protein. It converts Ca^{2+} influx into protein phosphorylation that regulates numerous signalling pathways. Moreover, it acts as a molecular memory through its Ca^{2+}/CaM-triggered autophosphorylation of T286, which keeps the kinase active until dephosphorylation (Strack and Hell, 2008).

Guo *et al* (2010) now show that CaMKII binds with its catalytic domain (residues L91–S272) to the second intracellular loop (IL2; residues D128–M147) of M4 (Figure 1A) but not the closely related M2. The $M2_{IL2}$ core-binding site differs only in two residues from $M4_{IL2}$, pinpointing Ala141 and Arg142 as critical for the CaMKII–M4 interaction. This interaction requires Ca^{2+}/CaM binding to CaMKII or autophosphorylation of CaMKII on T286, indicating that M4 interacts with the so-called T site in the catalytic domain of CaMKII. The T site anchors the unphosphorylated T286 in the inactive kinase, thereby positioning the pseudosubstrate region (PS) downstream of T286 for effective interaction with the substrate site (S site) in the catalytic centre. Ca^{2+}/CaM binding downstream of the PS displaces PS from the S site and T286 from the T site. T286 can then be phosphorylated by a neighbouring subunit, preventing reassociation of T286 with the T site, which remains available for binding other targets including M4.

In striatal slices, ionomycin-induced Ca^{2+} influx stimulated coimmunoprecipitation of endogenous M4 and CaMKII. This coprecipitation was inhibited by a membrane-permeant peptide that mimicked the CaMKII-binding site in $M4_{IL2}$. This tat-$M4_{IL2}$ peptide did not disrupt ionomycin-induced CaMKII association with the NMDA-type glutamate receptor (NMDAR) in striatal slices even though residues A1290–Q1311 in the GluN2B subunit also bind to the T site (Bayer *et al*, 2001). One possible explanation is that CaMKII also interacts with the NMDAR GluN1 subunit but perhaps not through its T site (Strack and Hell, 2008). In addition, $M4_{IL2}$ binds to the T site in a manner that apparently differs from the GluN2B interaction, as the I205K mutation in the T site inhibits GluN2B but not M4 binding. The $M4_{IL2}$ peptide might thus not strongly affect the CaMKII–GluN2B association. GluN2B binding to the T site hinders its reassociation with T286, thereby keeping CaMKII in a constitutively active state beyond dissipation of Ca^{2+}/CaM and dephosphorylation of T286 (Bayer *et al*, 2001; Strack and Hell, 2008). Whether association of native M4 with the T site also extends CaMKII activity remains an interesting question the future will have to answer.

In striatal slices, forskolin-induced stimulation of AC and cAMP production was inhibited by the general muscarinic agonist oxotremorin-M and the M4 selective antagonist MT3, as expected. This effect was enhanced by ionomycin-triggered Ca^{2+} influx. The ionomycin effect was inhibited by two CaMKII inhibitors (KN93 and Tat-CaMKIINtide) and the tat-$M4_{IL2}$ peptide. In slices from M4 knockout mice, oxotremorin-M had no effect on forskolin-stimulated cAMP production, whether applied by itself or together with ionomycin. Finally, forskolin-induced cAMP production was modestly reduced by oxotremorin-M in HEK293 cells transfected with WT or T145A mutant M4 (Guo *et al* (2010) identify T145 as the CaMKII phosphorylation site in M4). This reduction was enhanced by ionomycin in WT but not T145A mutant M4 in HEK293 cells. In striatal slices, activation of AC through the G_s-coupled dopamine receptor D1 was blunted by oxotremorin-M. This effect was enhanced by ionomycin. The enhancing ionomycin effect, but not basal oxotremorin-M effect, was prevented by KN93 and the tat-$M4_{IL2}$ peptide. Accordingly, CaMKII activation and its consequent binding to M4 and phosphorylation of T145 augment the M4-mediated inhibition of AC activation by D1 (Figure 1B).

Cocaine induces release of dopamine and acetylcholine in the striatum. Gao *et al* show that the stimulation of locomotive and stereotypic activity in rats by cocaine or the D1

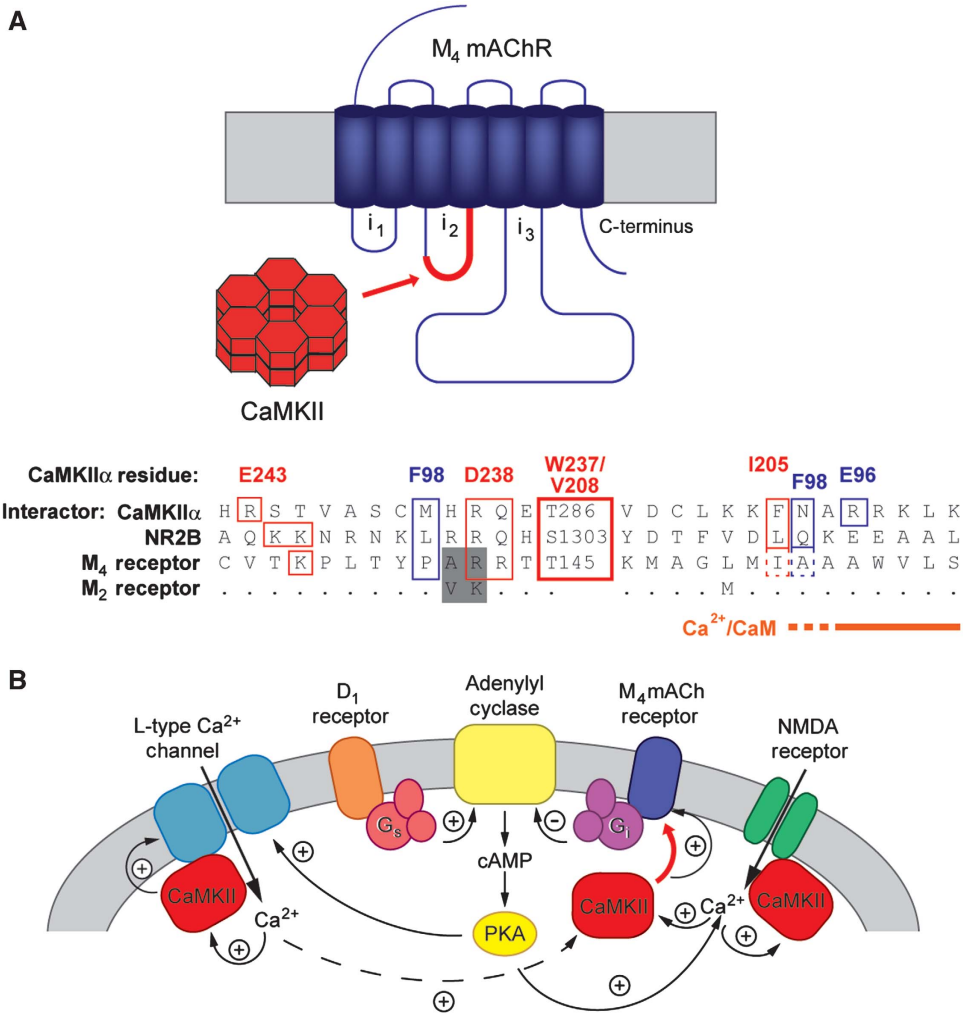

Figure 1 The CaMKII–M4 interaction. (**A**) Binding of CaMKII (red dodecamer) binds to the IL2 (red segment) of M4 (blue). Similar to the T286 segment (top in sequence alignment), the CaMKII-binding segments of GluN2B (middle) and M4 (bottom) can associate with the T site. M2 IL2, which does not bind CaMKII, differs in only two positions from the core-binding region of M4 IL2 (shadowed area). Residues shown in red and blue above the alignment are part of the functionally defined T and S sites of CaMKII, respectively. The T and S sites are in immediate proximity to each other and interact with the residues in the autoinhibitory domain and in GluN2B that are marked by red and blue boxes, respectively (because M4 does not depend on I205 in CaMKII and A153 is not a conservative substitution for F98-interacting residues, I152 and A153 are framed with dashed lines). The red box in bold depicts (auto) phopshorylation sites. In the inactive state, the T-site binding segment of the kinase is associated with the T site fostering binding of the pseudosubstrate segment (PS) to the S site. Ca^{2+}–CaM binds immediately downstream of PS and R297 (and, upon T286 phosphorylation, N294; solid and dashed orange line) within PS to displace PS from the S site and thereby T286 from the T site. T286 can then bind the S site of a neighbouring Ca^{2+}–CaM-activated kinase subunit with M281 interacting with F98 in the neighbouring subunit (blue box). The corresponding residue in GluN2B (L1298) is critical for the GluN2B–CaMKII association, suggesting that constitutive CaMKII association requires initial binding of GluN2B to the S site (Merrill *et al*, 2005) (see also Bayer *et al*, 2006). (**B**) Inhibition of D1 signalling by CaMKII-stimulated M4 and hypothesized Ca^{2+} influx and negative feedback loops. Ca^{2+} influx induces binding of CaMKII to M4 (red arrow). The ensuing phosphorylation of M4 augments the inhibitory effect of M4 through $G_{i/o}$ on AC, which is otherwise stimulated by D1 through G_s. We propose NMDARs and L-type channels as likely candidates for the Ca^{2+} influx that activates CaMKII.

agonist SKF81297 was significantly stronger when rats had been pre-treated with tat-$M4_{IL2}$. Cocaine also induced co-immunoprecipitation of CaMKII with M4 and phosphorylation of M4. The locomotive response to cocaine was enhanced in M4 knockout versus WT mice. Tat-$M4_{IL2}$ increased the response to cocaine in WT but not KO mice. Accordingly, activation of CaMKII enhances the M4-mediated dampening of cocaine- and D1-triggered hyperlocomotion.

Mice in which M4 was specifically abrogated in D1-expressing neurons showed an increase in amphetamine- and cocaine-induced locomotor activity as well as in sensitization towards both drugs, further implicating M4 in counteracting these D1-mediated physiological responses (Jeon *et al*, 2010). It will thus be of interest to evaluate whether CaMKII also enhances the negative M4 effect in sensitization.

CaMKII also binds and phosphorylates the dopaminergic receptor D3 in striatal slices (Liu *et al*, 2009). D3 is another $G_{i/o}$-coupled antagonist to D1-mediated cAMP signalling, but in this case, CaMKII reduces this D3 activity and disruption of the CaMKII–D3 interaction enhances the cocaine-induced hyperlocomotion (Liu *et al*, 2009). Although this effect occurs in the nucleus accumbens, which is part of the striatum, it is unclear whether D1, D3, and M4 are expressed by the same neurons.

Another obvious question now is, which mechanisms activate CaMKII in the striatum to augment the inhibitory effect of M4. Given that Ca^{2+} influx through NMDARs and L-type Ca^{2+} channels triggers CaMKII binding to D3 (Liu *et al*, 2009) and that both anchor themselves CaMKII (Dai *et al*, 2009), we propose those as likely candidates (Figure 1B). As the Ca^{2+} influx through NMDARs and L-type channels is increased by cAMP/PKA signalling (Skeberdis *et al*, 2006; Dai *et al*, 2009), both passages could participate in a complex network of negative feedback through CaMKII/M4 and positive feedback through CaMKII/D3 on D1 signalling if D1, D3, and M4 are present within the same neurons, as the case might be in the nucleus accumbens (Figure 1B).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bayer KU, De Koninck P, Leonard AS, Hell JW, Schulman H (2001) Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature* **411:** 801–805

Bayer KU, LeBel E, McDonald GL, O'Leary H, Schulman H, De Koninck P (2006) Transition from reversible to persistent binding of CaMKII to postsynaptic sites and NR2B. *J Neurosci* **26:** 1164–1174

Dai S, Hall DD, Hell JW (2009) Supramolecular assemblies and localized regulation of voltage-gated ion channels. *Physiol Rev* **89:** 411–452

Guo M-L, Fibuch EE, Liu X-Y, Choe ES, Buch S, Mao L-M, Wang JQ (2010) CaMKIIα interacts with M4 muscarinic receptors to control receptor and psychomotor function. *EMBO J* **29:** 2070–2081

Jeon J, Dencker D, Wortwein G, Woldbye DP, Cui Y, Davis AA, Levey AI, Schutz G, Sager TN, Mork A, Li C, Deng CX, Fink-Jensen A, Wess J (2010) A subpopulation of neuronal M4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *J Neurosci* **30:** 2396–2405

Liu X-Y, Mao L-M, Zhang G-C, Papasian CJ, Fibuch EE, LacKamp A, Lan H-X, Zhou H-F, Xu M, Wang JQ (2009) Activity-dependent modulation of limbic dopamin D3 receptors by CaMKII. *Neuron* **61:** 425–438

Merrill MA, Chen Y, Strack S, Hell JW (2005) Activity-driven postsynaptic translocation of CaMKII. *Trends Pharmacol Sci* **26:** 645–653

Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Suadicani SO, Lin Y, Bennett MV, Yuste R, Castillo PE, Zukin RS (2006) Protein kinase A regulates calcium permeability of NMDA receptors. *Nature Neurosci* **9:** 501–510

Strack S, Hell JW (2008) Postsynaptic targeting of kinases and phosphatases. In *Structural and Functional Organization of the Synapse*, Hell JW, Ehlers MD (eds) Heidelberg: Springer

Wess J, Eglen RM, Gautam D (2007) Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nat Rev Drug Discov* **6:** 721–733