JGP Journal of General Physiology

RESEARCH NEWS

How calcium helps α7 nicotinic acetylcholine receptors fulfill their potential

Ben Short

Study reveals that, at low acetylcholine concentrations, calcium enhances channel opening by binding to a novel motif near the ligand binding site.

Kathiresan Natarajan, Nuriya Mukhtasimova, Cecilia Bouzat, Steven Sine (left to right), and colleagues demonstrate that, at low ACh levels, the activity of α7 nAChRs is potentiated by extracellular calcium, which binds to a pair of anionic residues at the entrance to the ligand-binding pocket. Ribbon diagrams show the position of this binding site on an α7/AChBP chimera used as a surrogate in MD simulations.

The α7 nicotinic acetylcholine receptor (nAChR) is one of the most abundant types of nicotinic receptor in the brain and is also expressed in a variety of peripheral tissues. It has been implicated in a wide range of physiological processes and is an emerging therapeutic target for a number of neurodegenerative and psychiatric disorders as well as inflammatory disease. α7 binds ACh with low affinity, and yet, even within the brain, many α7 receptors are located outside of cholinergic synapses at sites where, due to dilution and enzymatic degradation, the ACh concentration is reduced (1). In this issue of *JGP*, Natarajan et al. reveal how extracellular calcium potentiates the ability of low ACh levels to activate α7 receptors (2).

α7's abundance at extra-synaptic sites isn't the only unusual feature of this receptor. Unlike other heteromeric nAChRs that contain two to three ligand-binding sites, α7 is a homopentamer containing five identical sites, each capable of binding acetylcholine. In 2013, a team led by Steven Sine at the Mayo Clinic and Cecilia Bouzat at Universidad Nacional del Sur surprisingly found that ACh binding to a single site was sufficient to fully stabilize the open state of α7 receptors (3). Realizing that calcium was present in those experiments, the team did experiments in the absence of calcium and observed markedly reduced channel opening at low but not high ACh concentrations.

Calcium has been linked to α7 activity before (4, 5), but its effects on the full-length channel have never been assessed in mammalian cells. Sine and colleagues, including co–first author Nuriya Mukhtasimova, made high resolution single-channel recordings of human α7 coexpressed in fibroblasts with the α7 chaperone NACHO. The researchers found that, at low levels of ACh, α7 activity is potentiated by calcium, which increases both the frequency and duration of channel opening (2). At higher ACh concentrations, in contrast, calcium had no effect on channel activity. Meanwhile, Bouzat's team in Argentina confirmed these findings, using outside-out patches and rapid solution exchange to show that calcium potentiation of α7 activity is fully reversible.

There is, as yet, no crystal structure of full-length α7, so, to investigate how calcium might interact with the receptor, co-first author Kathiresan Natarajan performed MD simulations of a chimeric protein containing sequences from both α7 and ACh binding protein. These simulations identified a single binding site for calcium at the entrance to the ligand-binding pocket on each subunit of the α7 pentamer. "There are two anionic residues that frame the bound calcium ion at the entrance to each ligand-binding site," Sine explains.

Mutating either of these anionic residues, which are conserved across all vertebrate α7 proteins, abolished the ability of calcium to potentiate α7 activity. Sine notes that, though this calcium-binding site is required for potentiation, how it influences gating, and whether it works in conjunction with additional binding sites that might be present in the full-length protein, remains unclear.

Regardless of the mechanism, calcium expands the range at which ACh can activate α7 receptors. Moreover, the study raises the possibility that local changes in extracellular calcium levels might modulate α7 activity. Sine and colleagues are now interested in understanding the stoichiometry with which calcium and

Ben Short: bshort@rockefeller.edu.

ACh occupy their respective binding sites. “This is something we’ll need to know if we’re going to design drugs that target α7 for therapeutic benefit,” Sine says.

References

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