Visualizing the dynamics of ionotropic glutamate receptors using atomic force microscopy

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Summary

Glutamate is the major excitatory neurotransmitter in the mammalian brain. It binds to three different subclasses of ionotropic glutamate receptors (iGluRs): AMPA, kainate and NMDA receptors, and triggers a cation influx that generates synaptic currents crucial to brain function. Significantly, iGluRs are implicated in various neurological disorders, such as depression, schizophrenia, Alzheimer's and Parkinson's diseases, autism, seizure, and stroke. The iGluR subunits are composed of modular domains - the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain, and carboxy-terminal domain - and assemble into tetramers to form ligand-gated ion channels.

Several crystal structures for intact iGluRs in various functional states (i.e. closed, activated and desensitized) have now been reported. The receptors have also been studied using single-particle cryo-electron microscopy. Together, these studies provide fascinating 'snap-shots' of the receptors as they transition between different states. What is lacking, so far, is information about the kinetics underlying these structural transitions, because the techniques used lack time resolution.

I have used fast-scan atomic force microscopy (AFM), in some cases in combination with UV photolysis of caged L-glutamate, to study activation-induced structural changes in GluK2 kainate receptors and GluA2 AMPA receptors. AFM provides single-molecule resolution under fluid, permitting the imaging of proteins 'in action'. Receptors were purified from transfected cells by immunoaffinity chromatography and imaged after integration into supported lipid bilayers. Activation of both receptors caused a rapid ~1-nm vertical compression of the receptor. In both cases, the height reduction did not occur in the presence of receptor antagonists. Further, the D776K mutant of the kainate receptor, which does not desensitize, did not undergo the height change, and cyclothiazide, which blocks desensitization of the AMPA receptor, also blocked the height change. I conclude, therefore, that the vertical compression is associated with receptor desensitization, and suggest that it may reflect a weakening of the interaction between receptor subunits at the LBD dimer interface.

When imaged from the 'top' by AFM, the receptors appeared as double-blob structures, with each blob representing a pair of ATDs. By measuring the distance between the centres of the blobs in successive AFM images, I was able to monitor the mobility of the ATDs relative to each other before and during receptor stimulation. I found that for both kainate and AMPA receptors, the relative mobility of the ATDs became greater after stimulation. Further, at low glutamate concentrations, the ATDs of the (rapidly desensitizing) flop splice variant of the AMPA receptor were more mobile than those of the (more slowly desensitizing) flip splice variant. I suggest that the greater mobility of the flop splice variant might be connected with its more short-lived functional response to activation.

In a final series of experiments, in collaboration with two other groups, I used AFM to measure conformational changes induced by allosterically-bound halide ions. We found that anion substitution (i.e. chloride to bromide, or chloride to iodide) produced vertical compression of AMPA receptors prior to agonist binding, and also (in electrophysiological experiments conducted by collaborators) altered the duration of agonist-evoked channel activity. The anion binding site was identified (in X-ray crystal structures obtained by collaborators) within the ligand binding domain, where flip-flop alternative splicing occurs. Interestingly both anion effects were isoform-dependent. Together, these results demonstrate that resting-state allosteric interactions can 'prime' AMPA receptors for their eventual response to agonists.

Overall, my results obtained using fast-scan AFM imaging provide fascinating new information about the global dynamics of these key neurotransmitter receptors.