Chapter 8

Functional Analysis of Recombinant Channels in Host Cells Using a Fast Agonist Application System

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Abstract

A reduced recombinant system provides a unique opportunity to study the biophysical properties of NMDAR channels with known subunit compositions, by using a point mutation approach to analyze the structural determinants of receptor function (Wollmuth and Sobolevsky, Trends Neurosci 27:321–328, 2004). However, in addition to the well-developed repertoire of molecular biological techniques, these types of studies also require electrophysiological methods that allow a wide range of receptor activation protocols that can adequately assess desensitization, inactivation, ion permeability, and other properties of the channels. Currently, one of the most well-developed techniques suitable for addressing these issues is use of the fast agonist application system for rapid activation of ligand gated ion-channels (Colquhoun et al., J Physiol 458:261–287, 1992; Jonas and Sakmann, J Physiol 455:143–171, 1992).

Key words Recombinant NMDA Receptors, Kinetics, Calcium permeability, Magnesium block, Fast agonist application

1 Introduction

The kinetics of ligand gated ion-channel responses depends crucially on the concentration time course of agonist administration. In most of the central synapses mediator concentration rises within a few microseconds and declines in the submillisecond range [4]. Thus to mimic "physiological" activation of recombinant channels expressed in host cells lines like HEK-293, CHO, and HeLa cells, one needs to develop an application system that can provide rapid wash in and washout of the compounds to be tested. Over the last few decades there have been multiple attempts to design an experimental approach allowing channel activation with on- and offset times comparable to those measured in native synapses. Previously proposed techniques ranged from single capillary high pressure applications and U-tube perfusion to piezo-driven multicapillary applications [5–7]. Although these methods could produce consistent ligand/drug applications for 10–100 ms, all of them failed to

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