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Research Report

Methodology for rapid measures of glutamate release in rat brain slices using ceramic-based microelectrode arrays: Basic characterization and drug pharmacology

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ABSTRACT

Excessive excitability or hyperexcitability of glutamate-containing neurons in the brain has been proposed as a possible explanation for anxiety, stress-induced disorders, epilepsy, and some neurodegenerative diseases. However, direct measurement of glutamate on a rapid time scale has proven to be difficult. Here we adapted enzyme-based microelectrode arrays (MEA) capable of detecting glutamate *in vivo*, to assess the effectiveness of hyperexcitability modulators on glutamate release in brain slices of the rat neocortex. Using glutamate oxidase coated ceramic MEAs coupled with constant voltage amperometry, we measured resting glutamate levels and synaptic overflow of glutamate after K^+ stimulation in brain slices. MEAs reproducibly detected glutamate on a second-by-second time scale in the brain slice preparation after depolarization with high K^+ to evoke glutamate release. This stimulus-evoked glutamate release was robust, reproducible, and calcium dependent. The K^+-evoked glutamate release was modulated by ligands to the $\alpha_2\delta$ subunit of voltage sensitive calcium channels (PD-0332334 and PD-0200390). Meanwhile, agonists to Group II metabotropic glutamate (mGlu) receptors (LY379268 and LY354740), which are known to alter hyperexcitability of glutamate neurons, attenuated K^+-evoked glutamate release but did not alter resting glutamate levels. This new MEA technology provides a means of directly measuring the chemical messengers involved in glutamate neurotransmission and thereby helping to reveal the role multiple glutamatergic system components have on glutamate signaling.

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Abbreviations: VSCC, voltage sensitive calcium channels; mGlu, metabotropic glutamate; MEA, microelectrode array; GluOx, glutamate oxidase; S_1, first stimulus; S_2, second stimulus; aCSF, artificial cerebrospinal fluid

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1. Introduction

Glutamate neurotransmission contributes to normal neural function in the central nervous system. Normally, glutamate release and signaling are tightly regulated by an ensemble of presynaptic and postsynaptic receptors in association with glutamate transporters. However, when glutamate neurotransmission falters, an aberrant functioning glutamatergic system may develop that becomes associated with several neural and psychiatric disorders. Disorders such as anxiety and stress involve an alteration in communication among neurons in various regions of the brain including the thalamus, amygdala, hippocampus, and the neocortex (Holmes and Wellman, 2009; Rodrigues et al., 2009; Shin and Liberzon, 2009). Aberrant glutamate neurotransmission can take the form of excessive excitability as increased or uncontrolled excitation alters the normal communication among a neural network (Swanson et al., 2005). The need to identify clinically effective approaches to modulating glutamate neurotransmission remains challenging and largely unmet, in part, because of the temporal and spatial constraints of existing analytical methodologies (Timmerman and Westerink, 1997; Burmeister et al., 2002; Juranyi et al., 2003; van der Zeyden et al., 2008).

One common aspect of neuronal signaling is that calcium entry into the presynaptic terminals is critical for the release of neurotransmitters from synaptic vesicles into the synaptic cleft. With this in mind, ligands, such as gabapentin and pregabalin, to the $\alpha_2\delta$-auxiliary subunit of voltage-sensitive calcium channels (VSCC) provide a new alternative to modulating glutamate release (Dooley et al., 2000; Dooley et al., 2007). There are four variants of the $\alpha_2\delta$ subunits that interact with the channel-forming α_1 subunit and other subunits to form a functional VSCC (Catterall et al., 2005; Wolf et al., 2003). In particular, the $\alpha_2\delta$-type1 subunit appears to be the major binding protein for gabapentin and pregabalin (Bian et al., 2006; Thorpe and Offord, 2010). Ligands acting on the $\alpha_2\delta$ subunit appear to have the added attribute that while normal, resting levels of neurotransmitters are not affected, stimulus-evoked release is.

An additional regulatory point for glutamate neurotransmission is the role of metabotropic glutamate receptors. Glutamate signaling is mediated by two families of glutamate receptors: the ionotropic glutamate receptors and the G-protein coupled metabotropic glutamate (mGlu) receptors. The eight subtypes of the mGlu receptor family are divided among three groups (Group I, Group II, Group III) with members of each group found both on the presynaptic and postsynaptic terminals of glutamatergic synapses. In particular, the $mGlu_2$ receptors are located perisynaptically, on presynaptic neurons and thus are activated only when glutamate overflows the synapse, such as in cases of hyperexcitability or interruptions to the glutamate transporters (Swanson et al., 2005). The $mGlu_{2/3}$ receptors are distributed throughout the brain including areas linked to anxiety and stress disorder such as the frontal cortex area (Ohishi et al., 1993a, 1993b; Petralia et al., 1996; Shigemoto et al., 1997). mGlu Group II receptor agonists can inhibit excitatory postsynaptic potentials (Kilbride et al., 1998) and long-term potentiation induction (Huang et al., 1997) through a negative feedback action on glutamate release. In addition, $mGlu_{2/3}$ receptor agonists show an anxiolytic effect in the treatment of generalized anxiety disorder (Schoepp et al., 2003).

Here we report the methodology using enzyme-based microelectrode arrays (MEA) to directly measure, on a second-by-second basis, glutamate release from living brain slices of the rat neocortex. These MEAs have been shown to selectively detect glutamate, have a rapid response time (~600 ms), and measure TTX-dependent glutamate release in anesthetized or freely-moving rodents (Day et al., 2006; Hascup et al., 2010). We first characterized the basic properties of glutamate release in brain slices of rat neocortex, determining the basic characteristics, reproducibility, and calcium dependence. Next, we examined the role two components of the glutamatergic system, VSCC and $mGlu_{2/3}$ receptors, have on glutamate neurotransmission, especially stimulus-evoked glutamate release, in order to assess their potential effectiveness at modulating excessive or uncontrolled excitation.

2. Results

2.1. *Using glutamate oxidase-based ceramic microelectrodes to measure glutamate*

Ceramic-based MEAs capable of measuring glutamate have been used in anesthetized and freely moving preparations (Burmeister et al., 2002; Rutherford et al., 2007) but have not been tested in the *in vitro* conditions of a perfusion slice chamber or in brain slices. The ceramic-based MEAs (130 μm thick) used for these studies consisted of four, 50 μm × 150 μm platinum recording sites arranged in a row with 50 μm spacing between sites with an approximate cross sectional dimension of 0.015 mm^2 for the MEA (Fig. 1A). To verify the effectiveness of the MEA to identify glutamate in the chamber, we placed a glutamate-oxidase (GluOx) coated MEA in the chamber and superfused a solution of 60 μM glutamate dissolved in artificial cerebral spinal fluid (aCSF) through the chamber. The enzyme-coated electrode site showed a I_{+GluOx} (current on GluOx-coated MEA site) response to the addition of glutamate to the chamber (Fig. 1C). Meanwhile, an uncoated MEA electrode site did not show a change in I_{-GluOx} (current on MEA site without GluOx) response during a glutamate addition to the chamber. Therefore, MEA sites coated with glutamate-oxidase show a response to glutamate that is absent in the uncoated electrode site.

2.2. *K^+-evoked glutamate release in brain slices*

High, extracellular K^+ stimulation is commonly used to depolarize neurons and evoke neurotransmitter release. Previously, we have used high potassium stimulation to evoke glutamate release in anesthetized animals (Day et al., 2006; Quintero et al., 2007). To verify the MEA's capability to detect dynamic glutamate neurotransmission, we superfused a high K^+ (70 mM) aCSF solution to depolarize the neural network and stimulate glutamate release. Delivery of high K^+ repeatedly evoked glutamate release from brain slices of the neocortex (Fig. 2) with a difference in amplitude between the