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Mechanisms of bi-directional modulation of thalamocortical transmission in barrel cortex by presynaptic kainate receptors

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ABSTRACT

Presynaptic kainate receptors play an important role in synaptic transmission and short-term plasticity to profoundly regulate network activity in many parts of the mammalian brain. In primary sensory neocortex, where short-term synaptic plasticity is important for receptive field structure and information processing, kainate receptors are highly expressed and regulate thalamocortical inputs, particularly during development. However, the mechanisms of the kainate receptor-dependent presynaptic regulation of thalamocortical transmission are unclear. We therefore investigated this issue using electrophysiology in neonatal thalamocortical slices of barrel cortex combined with pharmacology and biochemical analyses. We show that presynaptic kainate receptors can both facilitate or depress synaptic transmission depending on the extent of their activation. This bi-directional regulation is mediated in part by kainate receptors that directly influence thalamocortical axonal excitability, but also likely involves receptors acting at thalamocortical terminals to regulate transmitter release. The efficacy of kainate in regulating thalamocortical transmission is low compared to that reported for other inputs. Consistent with this low efficacy, our biochemical analyses indicate that the presynaptic kainate receptors regulating neonatal thalamocortical inputs likely lack the high kainate affinity GluK4 and 5 subunits. Thus thalamocortical transmission can be bi-directionally regulated by low affinity kainate receptors through two mechanisms. Such presynaptic regulation provides a potentially powerful mechanism to influence sensory processing during development of barrel cortex.

1. Introduction

Kainate-type glutamate receptors are widely expressed in the mammalian brain and play important roles in synaptic transmission and neuronal excitability (Wisden and Seeburg, 1993; Bahn et al., 1994; Bettler and Mulle, 1995). Presynaptic kainate receptors are best characterized in hippocampus where they can up- or down-regulate both glutamatergic and GABAergic transmission (Kullmann, 2001; Lerma et al., 2001; Isaac et al., 2004), profoundly impacting circuit function. For example, kainate receptors regulate network activity in hippocampal CA3 and CA1 (Kullmann, 2001; Fisahn et al., 2004; Lauri et al., 2005) and contribute to epileptiform activity in hippocampus (Smolders et al., 2002). Presynaptic kainate receptors also play a role in long-term synaptic plasticity, being required for the induction of mossy fiber LTP (Bortolotto et al., 1999; Contractor et al., 2001; Lauri et al., 2001, 2003; Schmitz et al., 2003) and the expression of neonatal hippocampal CA1 LTP (Lauri et al., 2006).

Kainate receptors are tetramers made up of combinations of GluK1-5 subunits (Bettler and Mulle, 1995; Collingridge et al., 2009). GluK1-3 subunits can form functional ion channels either as homomers or heteromers; however, GluK4 or 5 homomers are not functional and are not surface expressed in neurons (Gallyas et al., 2003; Ren et al., 2003; Isaac et al., 2004; Nasu-Nishimura

Abbreviations: LTP, long-term potentiation (LTP); EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; VB, ventrobasal complex of thalamus; DC, direct current; AMPAR, AMPA receptor; NMDAR, NMDA receptor; GluK1, kainate-type glutamate receptor subunit 1 (GluR5 in previous nomenclature); GluK2, kainate-type glutamate receptor subunit 2 (GluR6 in previous nomenclature); GluK3, kainate-type glutamate receptor subunit 3 (GluR7 in previous nomenclature); GluK4, kainate-type glutamate receptor subunit 4 (KA1 in previous nomenclature); GluK5, kainate-type glutamate receptor subunit 5 (KA2 in previous nomenclature).

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et al., 2006). Kainate receptors can act both as ionotropic and metabotropic receptors (Lerma et al., 2001) and both modes of their signaling for presynaptic regulation by kainate receptors have been described (Frerking et al., 2001; Kidd et al., 2002; Lauri et al., 2003; Lerma, 2003; Pinheiro and Mulle, 2008). The mechanisms by which kainate receptors regulate presynaptic function are diverse (Kullmann, 2001; Isaac et al., 2004). Kainate receptors located at presynaptic terminals have been shown to up-regulate or down-regulate glutamate or GABA release in a dose-dependent manner when activated by exogenous agonist and also by synaptically-released glutamate e.g. (Jiang et al., 2001; Lauri et al., 2001; Schmitz et al., 2001; Semyanov and Kullmann, 2001; Kidd et al., 2002). In addition to acting at presynaptic locations, kainate receptors have also regulate synaptic function by altering axonal excitability as shown in hippocampus for the mossy fibre input to CA3 (Kamiya and Ozawa, 2000; Schmitz et al., 2000) and for GABAergic interneuronal input to CA1 pyramidal neurons (Semyanov and Kullmann, 2001; Maingret et al., 2005). Importantly such axonal receptors have been shown to be physiologically activated by synaptically-released glutamate to regulate synaptic transmission (Schmitz et al., 2000; Semyanov and Kullmann, 2001; Contractor et al., 2003). Therefore, these studies show that presynaptic kainate receptors physiologically regulate neurotransmitter release through at least two distinct mechanisms and further that studying these receptors using exogenously applied agonist is a useful approach in understanding the mechanisms of their action.

Kainate receptors are highly expressed and developmentally regulated in somatosensory pathways (Bettler and Mulle, 1995; Kerchner et al., 2001; Daw et al., 2007b). In layer 4 of neonatal barrel cortex presynaptic kainate receptors are found at thalamocortical inputs where they can be synaptically-activated during brief trains of activity to mediate a short-term depression (Kidd et al., 2002). Moreover, short-term plasticity plays an important role for in information processing in primary sensory neocortex (Abbott and Regehr, 2004). However, little is known about the properties or mechanisms of action of kainate receptors that regulate thalamocortical transmission. We therefore probed the mechanisms by which presynaptic kainate receptors can regulate thalamocortical transmission in neonatal barrel cortex. We show that kainate receptors can both up- and down-regulate neonatal thalamocortical transmission by at least two mechanisms. We find that presynaptic kainate receptors strongly influence axonal excitability; however, kainate receptors also act independently of this mechanism likely through direct regulation of glutamate release at terminals. In addition, we show that the efficacy of kainate in modulating thalamocortical transmission is low compared to other inputs in the brain, and consistent with this our biochemical analyses indicate that the presynaptic kainate receptors likely lack the high kainate affinity GluK4 and 5 subunits. This bi-directional regulation of thalamocortical transmission is predicted to be important in determining the dynamic properties of thalamocortical inputs hence influencing sensory processing in developing layer 4.

2. Materials and methods

2.1. Ethical approval

All experiments were performed in accordance with the guidelines of the National Institutes of Health Animal Care and Use Committee guidelines.

2.2. Electrophysiology

Thalamocortical slices (Agmon and Connors, 1991) were prepared from C57Bl/6 or 129SV/EV mouse pups aged between postnatal day (P) 3 and 7 (day of birth is P0), as previously described (Bannister et al., 2005; Daw et al., 2006). Animals were anaesthetised by inhalation of isofluorane and then decapitated with sharp scissors. The brain was rapidly removed and placed in ice-cold extracellular solution. Thalamocortical slices were then prepared as previously described (Daw et al., 2006). The data from slices prepared from C57Bl/6 mice are shown in Figs. 1–4. The 129SV/EV mice were used for the histoblot experiments (Fig. 5). For electrophysiological recordings the extracellular solution contained: 119 mM NaCl, 2.5 mM KCl, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM glucose, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, saturated with 95% O_2/5%CO_2, pH 7.4. 50 μM picrotoxin and 50 μM D-AP5 were routinely included to block $GABA_A$ and NMDA receptors (NMDARs), respectively, in all experiments except those monitoring NMDAR-mediated EPSCs. To monitor NMDAR EPSCs, D-AP5 was omitted and 10 mM BAPTA was included in the intracellular solution. All recordings were performed at room temperature. Whole-cell patch-clamp recordings were made from visually-identified stellate cells in layer 4 barrel cortex (Bannister et al., 2005; Daw et al., 2006) using 4–7 MΩ electrodes (Fig. 1A–C). For whole-cell voltage-clamp recordings the intracellular solution was as follows: 135 mM $CsMeSO_4$, 8 mM NaCl, 10 mM HEPES, 5 mM QX-314, 0.5 mM EGTA, 4 mM Mg-ATP, 0.3 mM Na-GTP, pH 7.2, 285 mOsm. For current-clamp recordings the intracellular solution was as follows: 130 mM $KMeSO_4$, 8.5 mM NaCl, 5 mM HEPES, 0.5 mM EGTA, 4 mM Mg-ATP, 0.5 mM Na-GTP, pH 7.2, 285 mOsm. The intracellular solution also contained 20 μM AlexaFluor488, which was used for visualizing the recorded neurons (Fig. 1B, C). Fluorescent signals were visualized using a cooled CCD camera (Hamamatsu Orca ER). Thalamocortical EPSCs were evoked by electrical stimulation of the VB thalamus (Agmon and Connors, 1991; Bannister et al., 2005; Daw et al., 2006) at a frequency of 0.2 Hz. Cells were voltage-clamped at −70 mV except when recording pharmacologically-isolated NMDAR-mediated EPSCs for which holding potential was +30 mV. During most whole-cell recordings, fibre volley was also simultaneously recorded using a patch electrode filled with extracellular solution placed in the internal capsule (Fig. 1A) (Crair and Malenka, 1995). In other experiments the fibre volley in layer 4 was recorded; in these experiments an extracellular field potential elicited by stimulation in VB was first recorded and then 0 mM Ca^{2+} superfused onto the slice to isolate the fibre volley component. Signals were amplified by a Multiclamp 700B (Axon Instruments), filtered at 4 KHz, digitized at 10 KHz and analyzed using Signal software (CED). Input resistance, series resistance, DC and EPSC amplitude were displayed on-line. Series resistance was estimated as previously described (Daw et al., 2000) and if it varied by >20% during the recording, the cell was discarded.

2.3. Analysis

For EPSCs recorded at −70 mV peak EPSC amplitude was measured, which is mediated predominately by AMPA receptors (Kidd and Isaac, 1999, 2001). The fibre volley amplitude was measured as the amplitude of the negative going deflection (Crair and Malenka, 1995). For statistical comparisons an averaged response amplitude over a 1.5 min period immediately before drug application was compared to the average over a 1.5 min period at the peak of the effect. Statistical analysis was performed using the Student's *t*-test unless otherwise noted, paired or unpaired as appropriate.

2.4. Histoblot experiments

Region-specific changes in distribution of GluK1/2 and GluK5 immunoreactivities were analyzed with previously characterized antibodies (Wenthold et al., 1994; Molnar et al., 1995) in 10 μm thick thalamocortical sections of P3, 5, 7 and adult brains of mice using an *in situ* blotting technique ('histoblot') (Tonnes et al., 1999; Gallyas et al., 2003; Jo et al., 2006). Nissl staining was performed on adjacent sections to confirm brain regions. Mean pixel density of histoblots was analyzed using previously established image quantification procedures (Kopniczky et al., 2005; Jo et al., 2006, $n = 4$ (n = one animal)).

3. Results

3.1. Kainate causes a dose-dependent depression or facilitation of thalamocortical transmission in layer 4 of developing barrel cortex

Our previous work shows that a presynaptic GluK1-containing kainate receptor exists at thalamocortical inputs to neonatal layer 4 barrel cortex that can be synaptically-activated during brief trains of activity to cause a short-term depression (Kidd et al., 2002). To investigate the mechanisms for this presynaptic kainate receptor-dependent regulation, we studied the effects of bath application of kainate at different concentrations in thalamocortical slices prepared from mice aged postnatal day (P) 3–7 (Agmon and Connors, 1991; Crair and Malenka, 1995; Daw et al., 2006). In thalamocortical slices, barrels in layer 4 could be visualized under low power magnification with infra-red illumination (Fig. 1A, B). Whole-cell recordings were made from visually-identified stellate cells, which make up ~80% of neurons in layer 4 (Feldmeyer et al., 1999;