Neuropharmacology 56 (2009) 131–140

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Antagonism of recombinant and native GluK3-containing kainate receptors

David Perrais [a,1], Paulo S. Pinheiro [a,1], David E. Jane [b], Christophe Mulle [a,*]

[a] Laboratoire Physiologie Cellulaire de la Synapse, CNRS UMR 5091, Bordeaux Neuroscience Institute, University of Bordeaux, 33077 Bordeaux, France
[b] Department of Physiology and Pharmacology, MRC Centre for Synaptic Plasticity, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK

ARTICLE INFO

Article history:
Received 29 April 2008
Received in revised form 31 July 2008
Accepted 1 August 2008

Keywords:
Ligand-gated ion channel
Glutamate receptor
Kainate receptor
Hippocampus
Mossy fibre
Presynaptic receptor

ABSTRACT

A number of kainate receptor antagonists have shown selectivity for receptors containing the GluK1 subunit. Here, we analyze the effects of these GluK1 antagonists on currents mediated by recombinant homomeric GluK3 and heteromeric GluK2/3 receptors expressed in HEK 293 cells and activated by fast application of glutamate. We show that, amongst these compounds, UBP302, UBP310 and UBP316 effectively block recombinant homomeric GluK3 receptors. However, these antagonists are ineffective in blocking homomeric GluK2 or heteromeric GluK2/3 receptors. In addition, these antagonists do not affect presynaptic kainate receptors at mouse hippocampal mossy fibre synapses, which are thought to be composed of GluK2 and GluK3 subunits. Moreover, the AMPA receptor-selective non-competitive antagonist GYKI 53655 blocks, at high concentrations, GluK3-containing receptors and decreases short-term plasticity at mossy fibre synapses. These results expand the range of targets of kainate receptor antagonists and provide pharmacological tools to study the elusive mechanisms of neurotransmitter control by presynaptic kainate receptors.

1. Introduction

At many brain synapses presynaptic ionotropic glutamate receptors play a role in the control of neurotransmitter release (Pinheiro and Mulle, 2008). At hippocampal mossy fibre synapses onto CA3 pyramidal cells, presynaptic kainate receptors acting as autoreceptors greatly facilitate synaptic transmission thus contributing to short- and long-term forms of synaptic plasticity (Bortolotto et al., 1999; Contractor et al., 2001; Pinheiro et al., 2007; Schmitz et al., 2001). Progress in the understanding of the mechanisms of this control requires the identification of the subunits composing these presynaptic autoreceptors, the characterization of their biophysical and pharmacological properties, and the possible links between these receptors and components of the presynaptic release machinery.

Kainate receptors are tetramers composed of diverse combinations of five subunits: GluK1–3 (or GluK2) and GluK4–5. Comparison of short-term plasticity in wild type and knockout mice has clearly identified GluK2 and GluK3 as part of these presynaptic kainate receptors (Contractor et al., 2001; Pinheiro et al., 2007). Short-term plasticity, such as paired pulse facilitation or low frequency facilitation (LFF) is also reduced by the AMPA/kainate receptor antagonists CNQX and philanthotoxin-433 (the latter blocking non-edited, calcium-permeable glutamate receptors), in wild type animals (Lauri et al., 2003; Pinheiro et al., 2007; Schmitz et al., 2001) but not in GluK2$^{-/-}$ and GluK3$^{-/-}$ mice (Pinheiro et al., 2007). Intriguingly, induction of LTP and short-term plasticity are also impaired by antagonists against GluK1-containing receptors (Bortolotto et al., 1999; Lauri et al., 2003; More et al., 2004; but see Breustedt and Schmitz, 2004), despite no change in synaptic plasticity in GluK1$^{-/-}$ mice (Breustedt and Schmitz, 2004; Contractor et al., 2001).

It would be greatly advantageous to find selective antagonists for presynaptic kainate receptors. Since GluK2 is also implicated in postsynaptic kainate receptors (Mulle et al., 1998), we targeted GluK3 to find potential antagonists. A number of groups have used a binding assay to test the affinity of glutamate receptor antagonists on GluK3 (Bortolotto et al., 1999; Dolman et al., 2006, 2005; Loscher et al., 1999; Weiss et al., 2006) but functional studies have not yet been performed. This may be, in part, because of the difficulty in studying GluK3; the only efficient way to activate GluK3-containing receptors is to use fast application of high concentrations of glutamate or kainate on lifted expressing cells or on outside-out patches (Pinheiro et al., 2007; Schiffer et al., 1997). In this study, we have found that recombinant homomeric GluK3 receptors, but not GluK2/3 heteromeric receptors, are sensitive to several competitive antagonists previously characterized as selective for GluK3. Accordingly, these antagonists did not affect short-term plasticity in mossy fibre synapses. However, we found that the non-competitive AMPA receptor antagonist GYKI 53655 also blocked GluK3 homomeric and GluK2/3 heteromeric receptors and affected

* Corresponding author. Tel.: +33 5 57 57 40 86; fax: +33 5 57 57 40 82.
E-mail address: mulle@u-bordeaux2.fr (C. Mulle).
[1] Equal contribution.

0028-3908/$ – see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2008.08.002

short-term mossy fibre synaptic plasticity. These results reinforce the idea that presynaptic kainate receptors at mossy fibre synapses are composed of GluK2 and GluK3 subunits.

2. Methods

2.1. Electrophysiological recordings

HEK 293 cells were transfected and recorded as previously described (Coussen et al., 2005; Pinheiro et al., 2007). Cells were cotransfected with GFP and GluK2a(Q), GluK3a or GluA1 at a cDNA ratio of 1:3, or with GFP, GluK2b(R) and GluK3a at a ratio of 1:1.5:1.5. One to three days after transfection, whole-cell recordings were performed on brightly fluorescent cells. Pipette solution contained (in mM): 130 $CsCH_3SO_3$, 2 NaCl, 2 $MgCl_2$, 10 EGTA, 10 HEPES, 4 Na_2ATP, 0.1 spermine (pH 7.2, 315 mOsm per liter). Currents were evoked by application of 30 mM glutamate for 100 ms every 20 s. Antagonists were applied for at least 3 min at any given concentration. Exchange of solutions was complete in less than 1 min. Dose-dependent effects on current amplitude were fitted with the Hill equation: $R = 1 - 1/(1 + (IC_{50}/C)^h)$, where R is the ratio of current amplitudes, and C is the antagonist concentration. IC_{50} and h are free parameters. To determine current decays, single exponential functions were fitted to the decay, starting after the inflexion point after the peak current, until the end of glutamate application. Fitting was performed using IGOR software (Wavemetrics).

Electrophysiological recordings of CA3 pyramidal cells were performed using parasagittal hippocampal slices from 14 to 21 days old WT and $GluK3^{-/-}$ mice or Wistar rats. Briefly, whole-cell voltage-clamp recordings (3.5–5 MΩ electrodes, −70 mV or +40 mV holding potential) were made at room temperature (22–24 °C) from pyramidal cells of the hippocampal CA3 field visualized by infrared video-microscopy. Slices were perfused with extracellular solution composed of (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO4, 26 $NaHCO_3$, 2.3 $CaCl_2$, 1.3 $MgCl_2$, 17 glucose and saturated with 95% O_2/5% CO_2. Bicuculline (10 μM) and D-AP5 (50 μM) were added to the bath to block $GABA_A$ and NMDA receptors, respectively, unless stated otherwise. The intracellular solution was composed of (in mM): 122 $CsCH_3SO_3$, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 2 NaCl and 4 Na_2ATP, pH 7.3. EPSCs were evoked in CA3 pyramidal cells by stimulation of mossy fibres using a glass electrode filled with extracellular solution and placed in the hilus of the dentate gyrus. Minimal intensities of stimulation were used to limit indirect activation of non-mossy fibres. To confirm that only mossy fibres were stimulated, we routinely checked that application of the group II mGluR agonist L-CCG-I (10 μM) resulted in a near complete inhibition of synaptic transmission. When bath-applying drugs, these were allowed at least 10 min to equilibrate in the recorded slice although, when noticeable, full effects were observed within 3 min. Recordings were made using an EPC9 or EPC10 amplifier (HEKA) and analyzed with IGOR Pro. Data are presented as mean ± SEM of n experiments. Statistical significance was evaluated using a paired or unpaired t-test, as appropriate.

2.2. Chemicals

UBP310 and UBP316 were synthesized as previously described (Dolman et al., 2007; Mayer et al., 2006). UBP302 was purchased from Tocris. D-AP5, NBQX and bicuculline were from Ascent Scientific (North Sommerset, UK). GYKI 53655 was synthesized on demand by ABX GmbH (Radeberg, Germany). Aliquots from a second batch of GYKI 53655 were kindly provided by Juan Lerma (Alicante, Spain). LY382884 was a kind gift from David Lodge (Eli Lilly, Indianapolis, IN). All other chemicals were from Sigma.

3. Results

3.1. Homomeric GluK1 receptors are blocked by GluK3 competitive antagonists

We have tested the effect of UBP302, 310 and 316 on GluK3-containing recombinant receptors. These molecules have been previously characterized as potent and selective GluK1 receptor antagonists (Dolman et al., 2007; Mayer et al., 2006; More et al., 2004). Application of glutamate (30 mM) for 100 ms, every 20 s, to GluK3-expressing HEK 293 cells activated an inward current that was stable over minutes. When UBP310 (1 μM) was added to the perfusion lines, it almost completely and reversibly blocked the response (1.6 ± 0.4% of control amplitude, $n = 9$; Fig. 1A). Lower concentrations, down to 10 nM, partially blocked the currents evoked by glutamate. We fitted the dose-dependent effect on current amplitude with the Hill equation and calculated an IC_{50} of 23 ± 2 nM (Fig. 1B). As previously shown on receptors expressed in *Xenopus* oocytes (Mayer et al., 2006), UBP310 did not block GluK2-mediated currents at concentrations of up to 10 μM (97 ± 1% of control amplitude, $n = 3$; Fig. 1A, B). We also tested the commercially available antagonist UBP302 that blocks GluK1 receptors with micromolar affinity (More et al., 2004). Likewise, it blocked GluK3 receptors with an IC_{50} of 4.0 ± 0.2 μM (Fig. 1B), similar to the one reported for GluK1. Finally, we tested a newly characterized antagonist, UBP316, or ACET (Dolman et al., 2007) and found that it also blocked GluK3-mediated currents with an IC_{50} of 92 ± 9 nM. These two compounds were similarly ineffective in blocking GluK2-mediated currents (Fig. 1B).

We wondered whether all antagonists showing selectivity for GluK1 receptors also acted on GluK3. However, some such as LY382884, do not significantly bind to GluK3 receptors (Bortolotto et al., 1999; Weiss et al., 2006). Accordingly we found that LY382884 (10 μM) only marginally blocked GluK3-mediated currents (90 ± 5% of control amplitude, $n = 6$; Fig. 1C). Thus, selectivity for GluK1 over GluK3 can be obtained for some antagonists.

Since the UBP compounds blocked GluK3 receptors, leaving GluK2 receptors unaffected, we also wanted to know if they were effective against GluK2/GluK3 heteromeric receptors, which are probably composing presynaptic kainate receptors at mouse hippocampal mossy fibre synapses (Pinheiro et al., 2007). To isolate currents mediated by heteromeric receptors we cotransfected GluK3 and the edited form of the b splice variant of GluK2, GluK2b(R). Homomeric receptors composed of this subunit produce only very small currents because they are of small conductance and, in addition, are poorly targeted to the plasma membrane (Coussen et al., 2005; Jaskolski et al., 2004). However, GluK2b assembled with another subunit, such as GluK3a, could reach the membrane and give rise to a membrane conductance with outward rectification. On the other hand, activation of GluK3 homomeric receptors evokes large currents at negative potentials which show very strong inward rectification, such that no current is recorded at positive potentials (Pinheiro et al., 2007). Therefore, in cells cotransfected with GluK2b(R) and GluK3a, currents recorded at −80 mV are due to the activation of both GluK3 homomeric and GluK2/3 heteromeric receptors. In contrast, at +80 mV, currents arise mainly from heteromeric receptors, with a very minor component from GluK2 homomeric receptors. In cells cotransfected with GluK2b(R) and GluK3a application of UBP310 (1 μM) partially blocked currents recorded at −80 mV (34 ± 9% of control amplitude, $n = 9$, Fig. 2A, D). However, it did not affect current amplitude when recorded at +80 mV (94 ± 7% of control amplitude, $n = 9$) indicating that the partial block observed at −80 mV is due to block of GluK3 homomeric receptors. To confirm this, we recorded currents at voltages from −100 mV to +100 mV in 20 mV increments (Fig. 2B). At membrane voltages above −20 mV, there was no difference between control and during UBP310 application. The subtracted currents have a current–voltage relationship that mimics the one of GluK3 alone (Pinheiro et al., 2007). Furthermore, there was a very strong correlation between the ratio of currents at +80 mV and −80 mV, a parameter that is proportional to the amount of heteromeric receptors, and the remaining current in UBP310 (correlation coefficient of 0.97; Fig. 2C). These results clearly show that 1 μM UBP310 does not block heteromeric GluK2/3 receptors. At 10 μM, UBP310 slightly blocks currents at +80 mV (Fig. 2D). We also tested the effect of UBP316 and UBP302 on heteromeric receptors. At +80 mV, 1 μM UBP316 or 100 μM UBP302, which almost completely block GluK3 homomeric receptors, reduced the recorded currents to about 68 ± 3% ($n = 5$) and 72 ± 6% ($n = 3$) of control values, respectively (Fig. 2D). Thus, these two latter antagonists show some effect on heteromeric receptors. Despite the lack of effect of UBP310 on current amplitude at +80 mV, it greatly affected current kinetics, increasing their decay time about twofold (Fig. 2A, E). This shows that this compound