Neuropharmacology 67 (2013) 213–222

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Potentiation of mGlu7 receptor-mediated glutamate release at nerve terminals containing N and P/Q type Ca^{2+} channels

José Javier Ferrero, David Bartolomé-Martín, Magdalena Torres, José Sánchez-Prieto*

Departamento de Bioquímica, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 7 June 2012
Received in revised form
18 September 2012
Accepted 6 October 2012

Keywords:
mGlu7 receptor
Glutamate release potentiation
N and P/Q Ca^{2+} channels
Hippocampal nerve terminal
Cerebrocortical nerve terminals
N-type Ca^{2+} channel KO mice

ABSTRACT

Calcium channels that mediate glutamate release (N-type and P/Q-type) are expressed in distinct populations of cerebrocortical nerve terminals in adult mice. mGlu7 receptors are exclusively expressed in nerve terminals containing N-type Ca^{2+} channels, which are less tightly coupled to glutamate release than P/Q-type Ca^{2+} channels. We recently reported that in addition to inhibit, mGlu7 receptors can also potentiate glutamate release via phosphatidyl inositol (4,5)-bisphosphate hydrolysis and activation of the non-kinase diacylglycerol binding protein Munc13-1, a protein that primes synaptic vesicles for exocytosis. Here, we assessed whether mGlu7 receptor-mediated potentiation of glutamate release is restricted to nerve terminals expressing N-type Ca^{2+} channels to compensate for their weak coupling to release. In the hippocampus, mGlu7 receptors are expressed both in nerve terminals containing N-type Ca^{2+} channels and in nerve terminals containing P/Q-type Ca^{2+} channels. When analyzed, we observed potentiation of mGlu7 receptor mediated release in wild type hippocampal nerve terminals at physiological (1.3 mM) and low (0.1 mM) concentrations of external Ca^{2+}. By contrast, in nerve terminals from mice lacking the α1B subunit of N-type channels ($Ca_v2.2$), in which evoked release is mediated by P/Q-type channels only, no release potentiation was observed at 1.3 mM Ca^{2+}. We conclude that release potentiation at 1.3 mM $[Ca^{2+}]_e$ occurs in nerve terminals expressing N-type channels, whereas that which occurs at low 0.1 mM $[Ca^{2+}]_e$ represents the release from nerve terminals containing P/Q-type Ca^{2+} channels. Although, mGlu7 receptor mediated potentiation is independent of Ca^{2+} channel activity, as it was induced by the Ca^{2+} ionophore ionomycin, release potentiation is influenced by the Ca^{2+} channel type and/or the associated release machinery.

1. Introduction

At central synapses, neurotransmitter release is initiated by the opening of voltage-dependent Ca^{2+} channels that is mediated by action potentials, triggering synaptic vesicle exocytosis at the presynaptic active zone. Presynaptic neurotransmitter release depends primarily on P/Q-type ($Ca_v2.1\alpha1$) and N-type ($Ca_v2.2\alpha1$) Ca^{2+} channels, although a small contribution of R-type ($Ca_v2.3\alpha1$) has been described at some synapses (Luebke et al., 1993; Takahashi and Momiyama, 1993; Vázquez and Sánchez-Prieto, 1997). While these channels are heterogeneously distributed among immature synapses, neurotransmitter release becomes more dependent on P/Q-type channels in mature synapses (Wu et al., 1999; Millán et al., 2002b; Fedchyshyn and Wang, 2005; Gónzalez-Inchauspe et al., 2007). By contrast, substantial neurotransmitter release at cerebrocortical synapses is mediated by N-type Ca^{2+} channels in adult animals (Iwasaki et al., 2000; Vázquez and Sánchez-Prieto, 1997; Millán et al., 2002a, 2003).

Ca^{2+} imaging of single nerve terminals has demonstrated the segregation of N- and P/Q-type Ca^{2+} channels in distinct subpopulations of nerve terminals (Millán et al., 2002a, 2003). Moreover, in central synapses these two channel types mediate glutamate release with different degrees of efficiency (Luebke et al., 1993; Ahmed and Siegelbaum, 2009). In cerebrocortical nerve terminals, N-type Ca^{2+} channels support glutamate release less efficiently than P/Q-type channels, as witnessed by the greater sensitivity of N-type channel mediated release to cytoplasmic Ca^{2+} buffers and decreases in the extracellular Ca^{2+} concentrations (Millán et al.,

Abbreviations: mGlu7 receptor, metabotropic glutamate receptor 7; L-AP4, L(+) phosphonobutyrate; PLC, phospholipase C; PKC, protein kinase C; HBM, HEPES buffer medium; BSA, bovine serum albumin; NGS, normal goat serum; TBS, Tris buffer saline; $[Ca^{2+}]_e$, extracellular free Ca^{2+} concentration; GPCR, G protein coupled receptor; PTx, pertussis toxin; PICK1, protein interacting with Cα-kinase 1; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonol[4,5-c]pyridine-4(5H)-one.

* Corresponding author. Tel.: +34 91 394 3891; fax: +34 91 394 3909.
E-mail address: jsprieto@vet.ucm.es (J. Sánchez-Prieto).

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2012.10.032

2003; Ladera et al., 2009). Another distinct property of glutamate release supported by N- and P/Q-type Ca^{2+} channels is the sensitivity to G protein-coupled receptors (GPCRs: Zhang et al., 1996; Currie and Fox, 1997; Gónzalez-Inchauspe et al., 2007).

In cerebrocortical nerve terminals of adult rats, the metabotropic glutamate receptor 7 (mGlu7) is exclusively expressed in nerve terminals containing N-type Ca^{2+} channels, where it inhibits glutamate release via Gi/o-mediated inhibition of this channel type (Millán et al., 2002a, 2003). This mGlu7 receptor is co-expressed with adenosine A_1 and $GABA_B$ receptors at these nerve terminals, the latter also participating in the inhibition of glutamate release (Ladera et al., 2007; Martín et al., 2008). We recently demonstrated that the mGlu7 receptor also contributes to signaling pathways that enhance glutamate release. Indeed, potentiation of glutamate release is observed only after prolonged exposure of this receptor to the agonist L-AP4, and it is a process that involves phospholipase C (PLC) activation via a pertussis toxin (PTx) insensitive G protein and the subsequent hydrolysis of phosphatidyl inositol (4,5)-bisphosphate. This mGlu7 receptor-dependent potentiation enhances glutamate release via a mechanism that is dependent on release machinery, as parallel translocation of active zone Munc13-1 protein from the soluble to particulate fractions is observed upon receptor activation (Martín et al., 2010; Ferrero et al., 2011). Furthermore, mGlu7 receptor-mediated release potentiation occludes that induced by phorbol esters, suggesting that similar intracellular mechanisms underlie both processes (Martín et al., 2011) However, it remains unknown whether mGlu7 receptor-mediated release potentiation is restricted to nerve terminals expressing N-type Ca^{2+} channels to compensate for their weak coupling to glutamate exocytosis and/or their strong inhibition by GPCRs.

To determine whether mGlu7 receptors also potentiate release at nerve terminals containing P/Q-type Ca^{2+} channels, we analyzed hippocampal nerve terminals in which mGlu7 receptors are co-expressed with P/Q-type channels (Martín et al., 2008). Similarly, we analyzed nerve terminals from mice lacking N-type Ca^{2+} channels (Ino et al., 2001) in which evoked release is entirely dependent on P/Q-type Ca^{2+} channels. The mGlu7 receptor mediated glutamate release was observed in nerve terminals containing P/Q-type Ca^{2+} channels, but only at low (0.1 mM) extracellular Ca^{2+} concentrations ($[Ca^{2+}]_e$), while release potentiation in nerve terminals containing N-type Ca^{2+} channels was observed at only 1.3 mM $[Ca^{2+}]_e$. Finally, mGlu7 receptor-mediated potentiation of glutamate release was resistant to N and P/Q channel blockade with ω-conotoxin-GVIA and ω-agatoxin-IVA, respectively, demonstrating that it occurs independently of Ca^{2+} channel activity. Taken together these findings demonstrate that up-regulation of glutamate release by mGlu7 receptors is influenced by Ca^{2+} channel type and/or the associated release machinery.

2. Materials and methods

2.1. Animals

Mice lacking the α_{1B} subunit ($Ca_v2.2$) of N-type calcium channels were generated by homologous recombination, as described previously (Ino et al., 2001). Heterozygous male and female mice were interbred to generate homozygous mutant mice lacking the α_{1B} subunit of voltage-dependent Ca^{2+} channels. The genotypes of the offspring were identified by polymerase chain reaction (PCR) analysis of tail DNA biopsies. The primers used to screen the α_{1B} genotypes were NP6 (5′-TGGCACCTTATGCCTTGCACGGTGCCTGCG-3′), NP8 (5′-GGTCGAGATGGCTTGCGGGACCCGTTGGGA-3′) and AGN2 (5′-GCCTGCTTGCCGAATATCATGGTGGAAAAT-3′), corresponding to the nucleotides of the cytoplasmic repeat II–III linker of the α_{1B} subunit and the inserted phosphoglycerol kinase promoter neomycin resistance gene (Ino et al., 2001). The predicted sizes for the wild type and mutant fragments were 500 and 600 bp, respectively. Mutant and wild-type mice were housed together with *ad libitum* access to food and water. Experiments were performed on 2–6 month old homozygous (KO) and wild type (WT) animals. All procedures for the handling and sacrifice of animals were carried out in accordance with the European Commission guidelines (86/609/CEE) and they were approved by the Animal Research Committee of the Complutense University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Synaptosomal preparation

Synaptosomes were purified on discontinuous Percoll gradients (Amersham Pharmacia Biotech, Uppsala, Sweden) as described previously (Millán et al., 2002a). Briefly, the cerebral cortex and hippocampus from wild type and mutant mice were homogenized in medium containing 0.32 M sucrose [pH 7.4]. The homogenate was centrifuged for 2 min at 2000 × *g* and 4 °C, and the supernatant was spun again at 9500 × *g* for 12 min. From the pellets formed, the loosely compacted white layer containing the majority of the synaptosomes was gently resuspended in 8 ml of 0.32 M sucrose [pH 7.4]. Subsequently, 2 ml of this synaptosomal suspension was placed onto a 3 ml Percoll discontinuous gradient containing 0.32 M sucrose, 1 mM EDTA, 0.25 mM DL-dithiothreitol and 3%, 10% and 23% Percoll [pH 7.4]. After centrifugation at 25,000 × *g* for 10 min at 4 °C, the synaptosomes were recovered from between the 10% and 23% Percoll bands, and they were diluted in a final volume of 30 ml HEPES buffer medium (HBM; 140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES, pH 7.4). Following further centrifugation at 22,000 × *g* for 10 min, the synaptosome pellet was resuspended in 6 ml of HBM medium and the protein content was determined by the Biuret method. Finally, 0.75 mg of the synaptosomal suspension was diluted in 2 ml of HBM and spun at 3000 × *g* for 10 min. The supernatant was discarded and the pellets containing the synaptosomes were stored on ice. Under these conditions the synaptosomes remain fully viable for at least 4–6 h, as determined by the extent of KCl evoked glutamate release.

2.3. Glutamate release

Glutamate release was assayed by on-line fluorometry as described previously (Millán et al., 2002a). Synaptosomal pellets were resuspended in HBM (0.67 mg/ml) and preincubated at 37 °C for 1 h in the presence of 16 μM bovine serum albumin (BSA) to bind any free fatty acids released from synaptosomes during preincubation (Herrero et al., 1991). A 1-ml aliquot was then transferred to a stirred cuvette containing 1 mM $NADP^+$, 50 U glutamate dehydrogenase (Sigma, St. Louis, MO), and 1.33, 0.5 or 0.1 mM $CaCl_2$. Subsequently, NADPH fluorescence was monitored in a Perkin–Elmer LS-50 luminescence spectrometer at excitation and emission wavelengths of 340 and 460 nm, respectively. The traces were calibrated by adding 2 nmols of glutamate at the end of each assay and the data was obtained at 2 s intervals. The ionomycin-induced glutamate release was calculated by subtracting the release observed during a 5 min period in the absence of the Ca^{2+} ionophore (basal) from that observed in the presence of ionomycin.

2.4. Immunocytochemistry

The affinity-purified rabbit polyclonal antisera against mGlu7a used here has been described previously (Shigemoto et al., 1997). Polyclonal goat antisera against P/Q-type (α1A) and N-type (α1B) channel subunits were obtained from Santa Cruz Biotechnology Inc., Europe. The Santa Cruz Biotechnology antibody against N Ca^{2+} channels recognizes an epitope mapping near the C-terminus of N-type Ca^{2+} channels and therefore not included in the truncated protein expressed in the KO in which the N-type Ca^{2+} channel α_{1B} gene was disrupted in the central portion of the cytoplasmic repeat II–III linker (Ino et al., 2001). The specificity of the N-type Ca^{2+} channel antibody was confirmed by the very low "non- specific" labeling observed in synaptosomes from N-type Ca^{2+} channels KO mice. Unfortunately, P/Q-type Ca^{2+} channels KO mice are not available for a similar control of P/Q-type Ca^{2+} channel antibody specificity. As a control for the immunochemical reactions, primary antibodies were omitted from the staining procedure, resulting in the absence of any immunoreactivity resembling that obtained with specific antibodies.

2.5. Immunocytochemical procedures

Synaptosomes (0.67 mg/ml) were added to medium containing 0.32 M sucrose [pH 7.4] at 37 °C, allowed to attach to polylysine coated coverslips for 1 h and then fixed for 4 min in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature. Following several washes with 0.1 M PB [pH 7.4], the synaptosomes were pre-incubated for 1 h in 10% normal donkey serum (NDS) diluted in 50 mM Tris buffer [pH 7.4] containing 0.9% NaCl (TBS) and 0.2% Triton X-100. Subsequently, they were incubated for 12 h with the appropriate primary antiserum diluted in TBS with 1% NDS and 0.2% Triton X-100: mGluR7a rabbit polyclonal antiserum (1 μg/ml); P/Q-type (α1A) goat polyclonal antiserum (1:300); or N-type (α1B) goat polyclonal antiserum (1:500). After washing in TBS, the synaptosomes were incubated with the appropriate secondary antibodies diluted in TBS for 2 h: Alexa Fluor 594 donkey anti-rabbit IgG (1:200) and Alexa fluor 488 Donkey anti-goat IgG (1:200), both obtained from Molecular Probes (Eugene, OR, USA). After several washes in TBS, the coverslips were mounted with Prolong Antifade Kit (Molecular Probes, Eugene, OR, USA) and the synaptosomes were examined on a Nikon Diaphot