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Cross-talk between metabotropic glutamate receptor 7 and beta adrenergic receptor signaling at cerebrocortical nerve terminals

José Javier Ferrero [a, b], Jorge Ramírez-Franco [a], Ricardo Martín [a, b], David Bartolomé-Martín [a, b], Magdalena Torres [a, b], José Sánchez-Prieto [a, b, *]

[a] Departamento de Bioquímica, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain
[b] Instituto de Investigación Sanitaria del Hospital Clínico San Carlos, Hospital Clínico San Carlos, C/Profesor Martín Lagos s/n, Madrid 28040, Spain

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ABSTRACT

The co-existence of presynaptic G protein coupled receptors, GPCRs, has received little attention, despite the fact that interplay between the signaling pathways activated by such receptors may affect the neurotransmitter release. Using immunocytochemistry and immuhistochemistry we show that mGlu7 and β-adrenergic receptors are co-expressed in a sub-population of cerebrocortical nerve terminals. mGlu7 receptors readily couple to pathways that inhibit glutamate release. We found that when mGlu7 receptors are also coupled to pathways that enhance glutamate release by prolonged exposure to agonist, and β-adrenergic receptors are also activated, a cross-talk between their signaling pathways occurs that affect the overall release response. This interaction is the result of mGlu7 receptors inhibiting the adenylyl cyclase activated by β adrenergic receptors. Thus, blocking Gi/o proteins with pertussis toxin provokes a further increase in release after receptor co-activation which is also observed after activating β-adrenergic receptor signaling pathways downstream of adenylyl cyclase with the cAMP analog Sp8Br or 8pCPT-2-OMe-cAMP (a specific activator of the guanine nucleotide exchange protein directly activated by cAMP, EPAC). Co-activation of mGlu7 and β-adrenergic receptors also enhances PLC-dependent accumulation of IP1 and the translocation of the active zone protein Munc13-1 to the membrane, indicating that release potentiation by these receptors involves the modulation of the release machinery.

1. Introduction

Neurotransmitter release is initiated by the activation of voltage dependent Ca^{2+} channels and the fusion of the readily releasable pool of synaptic vesicles at the active zone. Many pre-synaptic G protein coupled receptors (GPCRs) inhibit release by reducing the activity of pre-synaptic Ca^{2+} channels (Choi and Lovinger, 1996; Millan et al., 2002, 2003), yet GPCRs also modulate neurotransmitter release downstream of Ca^{2+} channels by targeting proteins in the release machinery (Sakaba and Neher, 2003; Gerachshenko et al., 2005; Bauer et al., 2007; Pelkey et al., 2008; Nakajima et al., 2009; Martin et al., 2010; Zhang et al., 2011; Ferrero et al., 2013a).

The metabotropic glutamate receptor 7 (mGlu7 receptor) located in the presynaptic active zone mediates feedback inhibition of glutamate release by activating a pertussis toxin (PTx) sensitive Gi/o protein, subsequently dampening Ca^{2+} channel activity (Millan et al., 2002, 2003; Pelkey et al., 2006; Martin et al., 2007). However, mGlu7 receptors also activate phospholipase C (PLC: Perroy et al., 2000; Martin et al., 2010). Thus, prolonged exposure of mGlu7 receptors to the agonist L-AP4 enhances glutamate release in cerebrocortical nerve terminals by activating a PTx insensitive G protein and through the subsequent hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP_2: Martin et al., 2010; Ferrero et al.,

Abbreviations: β-AR, β-adrenergic receptor; mGlu7 receptor, metabotropic glutamate receptor 7; GPCRs, G protein coupled receptors; SNARE, soluble NSF attachment protein receptor; L-AP4, L-2-amino-4-phosphonobutyric acid; Sp8Br, Sp-8-Br-cAMPS; HTRF, Homogeneous Time Resolved Fluorescence; PB, phosphate buffer; NDS, normal donkey serum; AC, adenylate cyclase; PLC, phospholipase C; PIP_2, phosphatidylinositol (4,5)-bisphosphate; IP_3, inositol trisphosphate; DAG, diacylglycerol; TTx, tetrodotoxin; HBM, HEPES buffered medium; BSA, bovine serum albumin; IP1, inositol monophosphate; TBS, Tris-buffered saline; PKA, cAMP-dependent protein kinase; Rim, Rab3-interacting molecule; EPAC, exchange protein directly activated by cAMP; IBMX, 3-isobutyl-1-methylxanthine; PDBu, phorbol dibutyrate; 8-pCPT, 8-(4-chlorophenylthio)-2′-O-methyladenosine 3′,5′-cyclic monophosphate monosodium hydrate; AP, action potential.

* Corresponding author. Departamento de Bioquímica, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain.
E-mail address: jsprieto@vet.ucm.es (J. Sánchez-Prieto).

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2011, 2013b). This enhancement of neurotransmitter release by mGlu7 receptors is associated with the translocation of the active zone protein Munc13-1 from the soluble to particulate fraction, suggesting that this signaling targets the release machinery (Martin et al., 2010). The mGlu7 mediated potentiation of release occludes that induced by phorbol esters, suggesting a similar mechanism of action (Martin et al., 2011). Moreover, an increase in glutamate release after prolonged mGlu7 receptor activation was observed in hippocampal synapses (Pelkey et al., 2008). Interestingly, release potentiation after prolonged activation of mGlu7 receptors does not result from a switch in signaling as the receptor continues to inhibit Ca^{2+} channels (Martin et al., 2010) but rather, from receptor coupling to PLC-dependent signaling cascades. This phenomenon is consistent with the well established capacity of GPCRs to simultaneously activate several signaling pathways.

Beta adrenergic receptors (β-ARs) are also expressed presynaptically in axons that establish asymmetric, putative glutamatergic synapses (Ferrero et al., 2013a), consistent with their role as heteroreceptors that enhance glutamate release (Huang et al., 1996; Kobayashi et al., 2009; Ferrero et al., 2013a). β-ARs activate Gs proteins and increase cAMP levels. However, cAMP-mediated enhancement of spontaneous release is not mediated by PKA, the classic presynaptic cAMP target (Herrero and Sanchez-Prieto, 1996; Castillo et al., 2002), but rather by the guanine nucleotide exchange factor, EPAC, which has emerged as a new alternative cAMP effector (Huang and Hsu, 2006; Gekel and Neher, 2008; Ferrero et al., 2013a). Indeed, EPAC activation mimics and occludes the isoproterenol-induced increase in spontaneous release. Interestingly, β-AR and EPAC activation increases PIP_2 hydrolysis, and it provokes Munc13-1 translocation to the membrane (Ferrero et al., 2013a). In addition, EPAC also activates phospholipase C_ϵ (PLC_ϵ), which can transduce signals from small GTPases through its Ras binding site (Schmidt et al., 2001; Branham et al., 2009; Dzhura et al., 2011). Accordingly, Munc13-1 can not only integrate PLC-coupled receptor signaling through its DAG binding site but also, that mediated by Gs/adenylyl-cyclase coupled GPCRs.

The co-existence of presynaptic GPCRs at nerve terminals has received little attention (Manzoni et al., 1995; Ladera et al., 2007; Marchi and Grilli, 2010; Partovi and Frerking, 2006), even though the interplay between GPCR signaling pathways may affect neurotransmitter release. While there is currently little evidence that mGlu7 and β adrenergic receptors co-localize, both receptors are expressed at the active zone of glutamatergic nerve terminals in the cerebral cortex (Shigemoto et al., 1996, 1997; Kinoshita et al., 1998; Ohishi et al., 1995; Ferrero et al., 2013a). Although mGlu7 receptors inhibit adenylyl cyclase (Okamoto et al., 1994), mGlu7 receptor-induced inhibition of release is unrelated to changes in the levels of cAMP in the absence of adenylyl cyclase activity (Herrero et al., 1996), yet it is strongly dependent on cAMP when adenylyl cyclase is activated (Millan et al., 2002; Martin et al., 2007). Thus, activation and inhibition of adenylyl cyclase by β-ARs and mGluR7, respectively, would anticipate a functional interaction between their signaling pathways if these receptors were co-expressed.

By studying mGlu7 and β-adrenergic receptors in synaptosomes, we found that these receptors are indeed co-expressed in a subpopulation of cerebrocortical nerve terminals. When the mGlu7 receptor is coupled to the signaling pathway that enhances release by prolonged agonist exposure, the ensuing activation of β-ARs further enhances glutamate release. However, the overall effects on glutamate release by co-activation of mGlu7 and β-adrenergic receptors involve a cross-talk between the signals that result from mGlu7 receptor-mediated inhibition of β-AR-activated adenylyl cyclase. Thus, blocking Gi/o proteins with pertussis toxin results in a supra-additive enhancement of release, which also occurs when the β-AR associated signaling is activated downstream of adenylyl cyclase with the cAMP analog Sp8Br or an EPAC activator. Co-activation of mGlu7 and β adrenergic receptors also enhances the PLC-dependent accumulation of IP_1 and the membrane translocation of Munc13-1, indicating that release potentiation by these receptors is a result of modulating the release machinery.

2. Materials and methods

2.1. Synaptosome preparation

All animal handling was performed in accordance with European Commission guidelines (2010/63/UE) and was approved by the Animal Research Committee at the Complutense University. Synaptosomes from the cerebral cortex of adult C57BL/6 mice (2–3 months old) were purified on discontinuous Percoll gradients (GE Healthcare, Uppsala, Sweden) as described previously (Millan et al., 2002). Briefly, the tissue was 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 then centrifuged again for 12 min at 9500 × *g*. From the pellets obtained, the loosely compacted white layer containing the majority of the synaptosomes was gently resuspended in 0.32 M sucrose [pH 7.4] and an aliquot of this synaptosomal suspension (2 ml) 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 or 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 the 23% Percoll bands, and they were diluted in a final volume of 30 ml of HEPES buffered 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 re-suspended in 0.5–1 ml of HBM medium and the protein content was determined by the Biuret method. Finally, 0.75–1 mg of the synaptosomal suspension was diluted in 2 ml HBM and centrifuged at 10,000 × *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 4–5 h.

2.2. Glutamate release

Glutamate release was assayed by on-line fluorimetry as described previously (Millan et al., 2002). 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). Adenosine deaminase (1.25 U/mg: Roche Diagnostics, Barcelona, Spain) was added for 30 min, and the synaptosomes were then washed by centrifugation for 30 s at 16,000 × *g* and resuspended in HBM medium. A 1 ml aliquot of the synaptosomes was transferred to a stirred cuvette containing 1 mM $NADP^+$, 50 U glutamate dehydrogenase (Sigma, St. Louis, MO, USA) and 1.33 mM $CaCl_2$, and the fluorescence of NADPH was measured in a Perkin Elmer LS-50 luminescence spectrometer at excitation and emission wavelengths of 340 and 460 nm, respectively. Data were obtained at 0.8 s intervals and the fluorescence traces were calibrated by the addition of 2 nmols of glutamate at the end of each assay. Plotted traces are the mean of all individual traces corresponding to a given condition. Smoothing protocols were applied to reduce the noise using OriginPro 8 and the Savitzky-Golay method. Glutamate release was induced with the Ca^{2+} ionophore ionomycin, which inserts into the membrane and delivers Ca^{2+} to the release machinery independently of Ca^{2+} channel activity. In addition, ionomycin was added in the presence of the Na^+-channel blocker tetrodotoxin (1 μM: Abcam, Cambridge, UK),