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ScienceDirect
Neuropharmacology 54 (2008) 804–814
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated

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Received 11 September 2007; received in revised form 5 December 2007; accepted 14 December 2007

Abstract

Group III metabotropic glutamate receptors (mGluRs) reduce synaptic transmission at the Schaffer collateral-CA1 (SC-CA1) synapse in rats by a presynaptic mechanism. Previous studies show that low concentrations of the group III-selective agonist, L-AP4, reduce synaptic transmission in slices from neonatal but not adult rats, whereas high micromolar concentrations reduce transmission in both age groups. L-AP4 activates mGluRs 4 and 8 at much lower concentrations than those required to activate mGluR7, suggesting that the group III mGluR subtype modulating transmission is a high affinity receptor in neonates and a low affinity receptor in adults. The previous lack of subtype selective ligands has made it difficult to test this hypothesis. We have measured fEPSPs in the presence of novel subtype selective agents to address this question. We show that the effects of L-AP4 can be blocked by LY341495 in both neonates and adults, verifying that these effects are mediated by mGluRs. In addition, the selective mGluR8 agonist, DCPG, has a significant effect in slices from neonatal rats but does not reduce synaptic transmission in adult slices. The mGluR4 selective allosteric potentiator, PHCCC, is unable to potentiate the L-AP4-induced effects at either age. Taken together, our data suggest that group III mGluRs regulate transmission at the SC-CA1 synapse throughout development but there is a developmental regulation of the subtypes involved so that both mGluR7 and mGluR8 serve this role in neonates whereas mGluR7 is involved in regulating transmission at this synapse throughout postnatal development.
Published by Elsevier Ltd.

Keywords: Metabotropic glutamate receptor; Group III; Hippocampus; DCPG; L-AP4

1. Introduction

Glutamate is the major excitatory neurotransmitter in the brain and can exert its effects through activation of both ionotropic and metabotropic glutamate receptors (mGluRs). The mGluRs are members of the family C G protein-coupled receptors (GPCRs). Eight mGluR subtypes have been identified from mammalian brain and are grouped into three groups based on their sequence homologies, ligand selectivity, and downstream effector molecules. Group I mGluRs include mGluR1 and mGluR5 which couple to G_q and activate PLCβ. mGluRs 2 and 3 comprise Group II and mGluRs 4, 6, 7, and 8 comprise Group III. The members of Groups II and III couple to $G_{i/o}$ which results in modulation of multiple effector systems, including various ion channels and inhibition of adenylyl cyclase (see Conn and Pin, 1997 for review).

One of the most common physiological effects of mGluR activation that is consistent throughout the central nervous system is a role as presynaptic autoreceptors involved in reducing transmission at glutamatergic synapses. For instance, activation of mGluRs reduces transmission at each of the major glutamatergic synapses in the hippocampal formation. Interestingly, evidence suggests that different mGluR subtypes

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0028-3908/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2007.12.009

serve this role in each major hippocampal synapse (Gereau and Conn, 1995b; Kamiya and Ozawa, 1999; Koerner and Cotman, 1981, 1982; Lanthorn et al., 1984; Macek et al., 1996). One of the most important synapses in the hippocampal circuit that is often implicated in learning and memory is the synapse between CA3 pyramidal cell axons, termed Schaffer collaterals (SC), and CA1 pyramidal cells (SC-CA1 synapse). Previous studies suggest that Group III mGluRs attenuate transmission at the SC-CA1 synapse by a presynaptic mechanism (Baskys and Malenka, 1991; Gereau and Conn, 1995a,b; Vignes et al., 1995). Baskys and Malenka (1991) found that 50 μM L-AP4, a selective agonist of group III mGluRs, attenuates transmission at this synapse in slices from neonatal but not adult rats. These data suggested that group III mGluRs may only participate in regulation of transmission at the SC-CA1 synapse at early stages of postnatal development. However, subsequent studies revealed that higher concentrations of L-AP4 (500 μM) have similar effects on transmission at this synapse in slices from neonatal and adult animals (Gereau and Conn, 1995a), suggesting that group III mGluRs regulate SC-CA1 transmission throughout postnatal development. A difference in the effect of 50 μM versus 500 μM L-AP4 raises the possibility of a developmental regulation of the specific group III mGluR subtype(s) that serve this role in neonatal versus adult animals. For instance, L-AP4 has nanomolar potencies at mGluR4 and mGluR8 but micromolar potency at mGluR7 (Schoepp et al., 1999). Thus, mGluR4 or 8 could predominate at early postnatal stages while mGluR7 could serve as the predominant receptor mediating this response in adults. However, the reduction in sensitivity to L-AP4 could also be due to the presence of high receptor reserve in neonatal animals and a developmental decrease in expression of a single group III mGluR subtype, leading to loss of receptor reserve and reduced agonist potency. In support of the possibility that mGluR7 is the only group III mGluR subtype involved in regulating transmission at this synapse in adults, immunohistochemical analysis of Group III mGluRs in the adult rat hippocampus revealed that mGluR4a is primarily localized to the inner third of the molecular layer and mGluR8 is found in the lateral perforant path terminal zone. In contrast, mGluR7a is distributed in all dendritic layers throughout the hippocampus (Bradley et al., 1996; Corti et al., 2002; Kosinski et al., 1999; Shigemoto et al., 1997). While immunohistochemical analysis has not been performed for all group III mGluR subtypes in neonates, antibodies to mGluR7a in the neonatal (p7) rat hippocampus revealed that this receptor is not highly expressed in the stratum radiatum of the CA1 region (Bradley et al., 1998). Thus, it is unlikely that mGluR7 expression and receptor reserve are higher in neonates than adults. Therefore, it is possible that another mGluR subtype is expressed in neonates and is responsible for the higher potency of L-AP4.

Unfortunately, pharmacological reagents that clearly differentiate between the group III mGluR subtypes have not been available, making it impossible to directly determine the mechanism for this difference. However, recent efforts have provided novel pharmacological tools that provide greater selectivity between group III mGluR subtypes (Marino et al., 2003a,b; Mitsukawa et al., 2005; Thomas et al., 2001; Zhai et al., 2002) than previously available compounds. We have used these newer reagents to rigorously test the hypothesis that there is a developmental regulation of the presynaptic mGluR subtypes involved in regulating transmission at this synapse and to determine which mGluR subtypes mediate this effect at the different stages of postnatal development.

2. Materials and methods

2.1. Compounds

L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4) and *N*,*N*′-dibenzhydrylethane-1,2-diamine (AMN082) were purchased from Ascent Scientific (Weston-Super-Mare, UK) or Tocris Bioscience (Ellisville, MO). (*S*)-3,4-Dicarboxyphenylglycine ((*S*)-DCPG), *N*-Phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxamide (PHCCC), and 2*S*-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-xanth-9-yl) propanoic acid (LY341495), were purchased from Tocris Bioscience (Ellisville, MO). *Z*-Cyclopentyl AP4 ((*Z*)-1(*RS*)-amino-3(*RS*)-phosphonocyclopentanecarboxylic acid) (Crooks et al., 1986) was supplied by Dr. Rodney Johnson (University of Minnesota).

2.2. Animals

All animals used in these studies were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental protocols were in accordance with all applicable guidelines regarding the care and use of animals. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) International approved facility with free access to food and water. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Electrophysiology

Nine- to 11-day-old (neonatal) or >80-day-old (adult) male Sprague–Dawley rats (Charles River, Wilmington, MA) were anesthetized with isofluorane, decapitated and the brains were quickly removed and submerged into ice cold cutting solution (in mM: 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 5 glucose, 0.6 (+)-sodium-L-ascorbate, 0.5 $CaCl_2$, 7 $MgCl_2$) continuously bubbled with 95% O_2/5% CO_2. The brains were then hemisected and 400 μm transverse slices were made using a vibratome (Leica VT100S). Individual hippocampi were removed from the slice and transferred to a room temperature mixture containing equal volumes of cutting solution and artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 2 $CaCl_2$, 1 $MgCl_2$) where they were allowed to equilibrate for 30 min. The hippocampi were then placed into a holding chamber containing room temperature ACSF continuously bubbled with 95% O_2/5% CO_2 and allowed to recover for at least 1 h.

Following recovery, slices were moved to a submersion chamber continuously perfused with oxygenated ACSF at a rate of 1.5 ml/min. ASCF temperature was maintained between 24 and 25 °C. Field excitatory postsynaptic potentials (fEPSPs) were evoked at 0.05 Hz by placing a bipolar nichrome stimulating electrode in the stratum radiatum near the CA3–CA1 border in order to stimulate the Schaffer collaterals. Baseline intensities that evoked half-maximal fEPSPs were chosen for all experiments. Recording electrodes were pulled on a Flaming/Brown micropipette puller (Sutter Instruments) to a resistance of 4–5 MΩ, filled with ACSF and placed in the stratum radiatum of area CA1. Field potentials were recorded using either an Axoclamp 2 (Molecular Devices, Sunnyvale, CA) or a Microelectrode AC Amplifier Model 1800 (A-M Systems) and analyzed using Clampex 9.2. Sampled data was analyzed off-line using Clampfit 9.2. Three sequential fEPSPs were averaged and their slopes calculated. All fEPSP slopes were normalized