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Immunocytochemical and pharmacological characterization of metabotropic glutamate receptors of the vestibular end organs in the frog

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Abstract

Using immunocytochemistry and multiunit recording of afferent activity of the whole vestibular nerve, we investigated the role of metabotropic glutamate receptors (mGluR) in the afferent neurotransmission in the frog semicircular canals (SCC). Group I (mGluR1α) and group II (mGluR2/3) mGluR immunoreactivities were distributed to the vestibular ganglion neurons, and this can be attributed to a postsynaptic locus of metabotropic regulation of rapid excitatory transmission. The effects of group I/II mGluR agonist (*1S*,*3R*)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (ACPD) and antagonist (*R*,*S*)-α-methyl-4-carboxyphenylglycine (MCPG) on resting and chemically induced afferent activity were studied. ACPD (10–100 μM) enhanced the resting discharge frequency. MCPG (5–100 μM) led to a concentration-dependent decrease of both resting activity and ACPD-induced responses. If the discharge frequency had previously been restored by L-glutamate (L-Glu) in high-Mg^{2+} solution, ACPD elicited a transient increase in the firing rate in the afferent nerve suggesting that ACPD acts on postsynaptic receptors. The L-Glu agonists, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and *N*-methyl-D-aspartate (NMDA), were tested during application of ACPD. AMPA- and NMDA-induced responses were higher in the presence than absence of ACPD, implicating mGluR in the modulation of ionotropic glutamate receptors. These results indicate that activation of mGluR potentiates AMPA and NMDA responses through a postsynaptic interaction. We conclude that ACPD may exert modulating postsynaptic effects on vestibular afferents and that this process is activity-dependent.

Keywords: Metabotropic receptors; Neuromodulation; Afferent synapse; Semicircular canal; Frog

Abbreviations: mGluR, metabotropic glutamate receptors; iGluR, ionotropic glutamate receptors; SCC, semicircular canals; mGluR1α, group I metabotropic glutamate receptors; mGluR2/3, group II metabotropic glutamate receptors; ACPD, (1*S*,3*R*)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid; MCPG, (*R*,*S*)-α-methyl-4-carboxyphenylglycine; L-Glu, L-glutamate; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; NMDA, *N*-methyl-D-aspartate; KA, kainate; DHPG, (*S*)-3,5-dihydroxyphenylglycine; CPG, (*S*)-4-carboxyphenylglycine; AIDA, (*R*,*S*)-1-amino-indian-1,5-dicarboxilic acid; MS 222, 3-aminobenxoic acid ethyl ester

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1. Introduction

Among the large number of possible neurotransmitter candidates, an excitatory amino acid, most probably L-glutamate (L-Glu), is believed to be the afferent neurotransmitter in the peripheral vestibular system both in mammals and frogs (Bledsoe et al., 1998; Zucca et al., 1992; Akoev and Andrianov, 1993; Rabejac et al., 1997; Guth et al., 1998a). The effects of glutamate are mediated by ionotropic receptors (iGluR) including *N*-methyl-D-aspartate (NMDA)-type, kainate (KA)-type and α-amino-3-hydroxy-5-methyl-4-isoxazol-propionate (AMPA)-type receptors, and also by the metabotropic glutamate receptors (mGluR) (Zucca et al., 1993; Prigioni et al., 1994; Andrianov and Ryzhova, 2000; Guth et al., 1998b). Eight mGluR subtypes have been cloned, and are categorized into three groups according to sequence similarities, coupling to second messenger systems and pharmacological characteristics (Nakanishi, 1994). There is evidence that mGluR modulate excitatory synaptic transmission and are determinant in regulating the excitability of neurons in various regions of the brain (Pin and Duvoisin, 1995; Conn and Pin, 1997; Anwyl, 1999).

Involvement of group I mGluR in vestibular afferent neurotransmission has been demonstrated in the frog semicircular canal organs (SCC) (Guth et al., 1998b; Hendricson and Guth, 2002a,b). In these studies, the group I mGluR agonists (*1S,3R*)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (ACPD) and (*S*)-3,5-dihydroxyphenylglycine (DHPG) were shown to increase the afferent firing rates of the ampullar nerve; and the group I mGluR antagonists (*S*)-4-carboxyphenylglycine (CPG) and (*R,S*)-1-aminoindian-1,5-dicarboxilic acid (AIDA) blocked the ACPD facilitatory effect. Interestingly, when synaptic transmission of the SCC was blocked by low Ca^{2+}-high Mg^{2+} solution, ACPD and DHPG failed to restore afferent firing. This suggests that the receptors activated by ACPD are presynaptic on the hair cells and function as autoreceptors. Activation of presynaptic mGluR generally facilitates glutamatergic transmission due to intracellular Ca^{2+} release from both IP_3-sensitive and ryanodine/caffeine-sensitive intracellular Ca^{2+} stores. This process may have an important role in producing a positive feedback augmentation of evoked but not resting transmitter release.

Valli et al. (1985) suggested that exogenous L-Glu has at least two sites of action, one presynaptic and one postsynaptic. The presynaptic action is responsible for increased release of the neurotransmitter, which in turn produces a substantial increase in the firing rate in afferent fibers. L-Glu also produces a depolarization of the postsynaptic membrane of afferent fibers. Detailed analysis of the effects of quisqualate, KA and NMDA in the frog vestibular system showed that L-Glu agonists act both pre and postsynaptically (Prigioni and Russo, 1995). The authors believe that presynaptic amino acid receptors may function as autoreceptors controlling afferent transmitter release by a positive feedback mechanism, and thereby increase the gain of the mechanoelectrical transduction in the hair cells. The involvement of mGluR in modulation of the postsynaptic response has not been analyzed in the vestibular end organs.

In cochlear afferent neurotransmission, studies using microionophoretic techniques demonstrated that group I mGluR participates in the synaptic transmission of inner hair cells of the guinea pig (Kleinlogel et al., 1999). The group I mGluR agonist, DHPG, induces excitatory responses in afferent fibers, which are antagonized by the group I mGluR specific antagonist, AIDA. In contrast to findings for the frog SCC (Guth et al., 1998b), AIDA also diminished spontaneous activity and slightly affected the AMPA- and NMDA-induced responses. The evidence directly implicating group I mGluR in cochlear excitatory neurotransmission was obtained by molecular biology, immunolabeling and patch clamp methods (Peng et al., 2004).

In this study, we use specific antibodies against mGluR1α (group I) and mGluR2/3 (group II) to characterize their immunocytochemical expression in the frog vestibular ganglion. To determine whether mGluR are postsynaptic in the frog SCC and whether activation of mGluR modulates excitatory glutamatergic transmission, we tested the effect of mGluR agonists and antagonists on resting and chemically induced activities of the SSC afferent fibers.

2. Materials and methods

2.1. Animals

Adult frogs, *Rana temporaria* (Janvier, Le Genest St Isle, France) were used. The protocols for animal experiments conformed to the guidelines of the French Department of Agriculture, Forestry and Fisheries.

2.2. Antibodies and western blot analysis

Rabbit antibodies directed against mGluR1α and mGluR2/3 (Chemicon, Temecula, CA) were used at a dilution of 1:300. The specificity of these antibodies has been well documented in various species including human, rat, and mouse but no immunocytochemical data was available in the frog tissue. To characterize the specificity of the antibodies in frog tissues, we used western blotting analysis with frog and rat brain extracts. The frog and rat tissues were homogenized in a homogenization buffer (2 mM EDTA, 10% glycerol, 2.3% SDS, 62 mM Tris pH 6.8 and protease inhibitor cocktail from Boehringer). The resulting lysates were boiled for 5 min, centrifuged and protein concentration was determined