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Group III metabotropic glutamate receptor-mediated, chemically induced long-term depression differentially affects cell viability in the hippocampus

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Abstract

In vivo, activation of group III metabotropic glutamate (mGlu) receptors leads to a reduction of basal synaptic transmission in the hippocampus, and depending on the experimental conditions in vitro, leads to neuroprotection or neurotoxicity. Here, the cellular response to cerebral application of L(+)-2-amino-4-phosphonobutanoic acid (AP4) was investigated in the CA1 region and dentate gyrus of freely moving rats. Drugs were applied via the lateral ventricle, and electrophysiological measurements were obtained via chronically implanted electrodes. AP4 produced a slowly developing depression of evoked responses in both hippocampal regions which lasted for over 4 h. Effects could be reversed by application of high frequency tetanus. Histological evaluation, 4 h or 7 d, following a single, acute AP4 injection into the lateral cerebral ventricle showed that AP4-mediated synaptic depression either amplified (CA1) or attenuated (dentate gyrus) excitotoxic neuronal death, strongly depending on the sub-region investigated. Effects were long-lasting, being still evident 7 days after AP4 application. In both hippocampal areas, the effects obtained were subtle, however, with the CA1 region being more potently affected. Interestingly, effects in the dentate gyrus comprised a slight enhancement of live cell number coupled with deterioration in cell area, suggesting that cell proliferation triggered by group III mGlu receptor activation may have masked neurotoxic effects mediated by activation of this receptor. These results show that although AP4 induces a slow-onset synaptic depression in both sub-regions, cell viability is differentially influenced by activation of group III mGlu receptors in the CA1 region and dentate gyrus.

Keywords: AP4 (L(+)-2-amino-4-phosphonobutanoic acid); LTD (Long-term depression); Histology; Hippocampus; In vivo

1. Introduction

The adenylyl cyclase-coupled group III metabotropic glutamate (mGlu) receptors comprise mGlu4, mGlu6, mGlu7 and mGlu8 (Nakanishi, 1992). With the exception of mGlu6, all are expressed in the hippocampus, and immunocytochemical studies have demonstrated that mGlu4 and 7 are localized presynaptically (Bradley et al., 1996; Shigemoto et al., 1997, 1996). In response to application of the group III mGlu receptor agonist, L-2-amino-4-phosphonobutyric acid (AP4), a suppression of glutamatergic transmission occurs within the hippocampus via a presynaptic mechanism (Koerner and Cotman, 1981; Baskys and Malenka, 1991) that involves inhibition of voltage-gated calcium entry (Trombley and Westbrook, 1992). An autoreceptor function for group III mGlu receptors has been proposed (Gereau and Conn, 1994; Macek et al., 1996; Thomsen, 1997). Moreover, group III mGlu receptors have been shown to mediate inhibition of *N*-methyl-D-aspartate (NMDA) receptors (Toms et al., 1997).

A possible role for group III receptors in both hippocampal neuroprotection and neurodegeneration has been postulated.

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Thus, a neuroprotective role has been reported in cell cultures (Gasparini et al., 1999; Bruno et al., 2000) and transgenic mice which are deficient in mGlu7 are prone to epileptic seizures (Sansig et al., 2001). In addition, both anticonvulsant and proconvulsant effects following agonist activation of mGlu receptors has been reported in mice and rats in vivo (Abdul-Ghani et al., 1997; Ghauri et al., 1996; Gasparini et al., 1999). Agonist activation of mGlu receptors in vivo leads to alterations in synaptic strength (Manahan-Vaughan and Reymann, 1995a,b,c). Furthermore, application of the group I and group II mGlu receptor agonist (1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) results in the induction of a slowly developing potentiation in the hippocampus in vivo (Manahan-Vaughan and Reymann, 1995a,b), which is associated with the onset of cell death (Manahan-Vaughan et al., 1999). Currently it is unclear if group III mGlu receptor-mediated modulation of synaptic strength has any influence on cell viability in the intact hippocampus. The present study therefore assessed hippocampal neuronal populations in the hippocampal CA1 region and dentate gyrus following induction of synaptic depression by pharmacological activation of group III mGlu receptors in freely moving rats.

2. Materials and methods

2.1. Experimental procedures

Seven- to eight-week old male Wistar rats underwent implantation of a monopolar recording, and a bipolar stimulating electrode into the dentate gyrus granule cell layer and perforant path, respectively, to enable evoked recordings from the dentate gyrus, as described previously (Manahan-Vaughan et al., 1998b; Naie and Manahan-Vaughan, 2004). Alternatively, a monopolar recording electrode was implanted in the stratum radiatum of hippocampal area CA1 and a bipolar stimulating electrode was implanted in the CA1 Schaffer collateral–commissural pathway to enable evoked recordings from the CA1 region as previously described (Manahan-Vaughan and Reymann, 1997). In each case a cannula was implanted into the lateral cerebral ventricle, through which drug application was made. The animals were allowed between 7 and 10 days to recover from surgery before experiments were conducted. Throughout experiments the animals could move freely. Experiments were carried out in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Measurement of evoked potentials

Responses were evoked by stimulating at low frequency (0.025 Hz). For each time-point, five evoked responses were averaged. Both field excitatory post-synaptic potential slope and population spike amplitude were monitored. In the dentate gyrus, the population spike amplitude was measured from the peak of the first positive deflection of the evoked potential to the peak of the following negative potential. For CA1 region and dentate gyrus, field excitatory post-synaptic potential slope was measured as the maximal slope through the five steepest points obtained on the first negative or on the positive deflection of the evoked potential, respectively. By means of input/output curve determination the maximum population spike amplitude was found, and all potentials employed as baseline criteria were evoked at a stimulus intensity which produced 40% of this maximum.

High frequency tetanisation comprised 200 Hz stimulation (10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval) given 2 h after injection of AP4. Cortical electroencephalogram (EEG) activity was constantly monitored throughout all electrophysiological experiments, by means of the silver wire attached to the reference and earth electrodes. This was done to exclude that seizure activity or EEG disturbances influenced the outcome of the experiments.

2.3. Compounds and drug treatment

L(+)-2-amino-4-phosphonobutanoic acid (AP4, Biotrend Germany) was first dissolved in 5 µl sodium hydroxide solution (1 mM), and then made up to a 100 µl volume with 0.9% sodium chloride. AP4 was injected in a 5 µl volume over a 6 min period via a Hamilton syringe.

Throughout the experiments, injections were administered following measurement of the baseline for 30 min with measurements then taken at $t=5$, 10, 15 and then 15 min intervals (after injection) for up to 4 h. Further measurements were taken 24 h later.

The osmolarity of the AP4 solution was determined as being 335 mOsm/l, compared to 308 mOsm/l for the saline solution. These solutions were slowly administered in a 5 µl volume into the lateral cerebral ventricle (ca. 2.5–3 mm^3; (Bramlett and Dietrich, 2002)) and thus should have undergone osmotic buffering within the ventricle (ca. 320 mOsm/l) before diffusing to the hippocampus. These small osmolarity differences (a variation of −3.75% for the vehicle solution and of +4.69% for the AP4 solution from cellular osmolarity) would therefore not be expected to contribute to cell viability.

2.4. Data analysis

The baseline field excitatory post-synaptic potential slope or population spike amplitude data were obtained by averaging the response to stimulating the medial perforant path or the Schaffer collateral–commisural pathway, to obtain five sweeps at 0.025 Hz, every 5 or 15 min as described above. The data were then expressed as mean percent pre-injection baseline reading ± standard error of the mean. Statistical significance was estimated using analysis of variance (ANOVA) with repeated measures, followed by post hoc Student's *t*-tests if applicable. The probability level interpreted as statistically significant was $P<0.05$. To estimate the significance of drug effects we computed the function of the sums of (deviation) squares at different points in time *on the same subjects*, this factor is a *repeated measures factor* referred to as *"within-factor"*. In addition we analysed *"between-factor"* effects, which is