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MAJOR DORSOVENTRAL DIFFERENCES IN THE MODULATION OF THE LOCAL CA1 HIPPOCAMPAL NETWORK BY NMDA, mGlu5, ADENOSINE A_{2A} AND CANNABINOID CB_1 RECEPTORS

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Abstract—Recent research points to diversification in the local neuronal circuitry between dorsal (DH) and ventral (VH) hippocampus that may be involved in the large-scale functional segregation along the long axis of the hippocampus. Here, using CA1 field recordings from rat hippocampal slices, we show that activation of N-methyl-D-aspartate receptors (NMDARs) reduced excitatory transmission more in VH than in DH, with an adenosine A_1 receptor-independent mechanism, and reduced inhibition and enhanced postsynaptic excitability only in DH. Strikingly, co-activation of metabotropic glutamate receptor-5 (mGluR5) with NMDAR, by CHPG and NMDA respectively, strongly potentiated the effects of NMDAR in DH but had not any potentiating effect in VH. Furthermore, the synergistic actions in DH were occluded by blockade of adenosine A_{2A} receptors (A_{2A}Rs) by their antagonist ZM 241385 demonstrating a tonic action of these receptors in DH. Exogenous activation of A_{2A}Rs by 4-[2-[[6-amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride (CGS 21680) did not change the effects of mGluR5–NMDAR co-activation in either hippocampal pole. Importantly, blockade of cannabinoid CB_1 receptors (CB_1Rs) by their antagonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide (AM 281) restricted the synergistic actions of mGluR5–NMDARs on excitatory synaptic transmission and postsynaptic excitability and abolished their effect on inhibition. Furthermore, AM 281 increased the excitatory transmission only in DH indicating that CB_1Rs were tonically active in DH but not VH. Removing the magnesium ions from the perfusion medium neither stimulated the interaction between mGluR5 and NMDAR in VH nor augmented the synergy of the two receptors in DH. These findings show that the NMDAR-dependent modulation of fundamental parameters of the local neuronal network, by mGluR5, A_{2A}R and CB_1R, markedly differs between DH and VH. We propose that the higher modulatory role of A_{2A}R and mGluR5, in combination with the role of CB_1Rs, provide DH with higher functional flexibility of its NMDARs, compared with VH.

Key words: hippocampus, dorsoventral, NMDA receptor, mGlu5 receptor, A_{2A} receptor, CB_1 receptor.

INTRODUCTION

The segregation of functions along the longitudinal axis of the hippocampus is a rather well established concept (Small, 2002; Fanselow and Dong, 2010; Bannerman et al., 2014). In addition, recently observed differences in the functioning of the local neuronal circuitry between the opposite poles of the hippocampus have attracted the attention of researchers leading to a gradually accumulating body of evidence and to the emergent concept of diversification of the intrinsic neuronal network between the dorsal (DH) and the ventral (VH) hippocampus. These observations have been made at several levels of organization including cell properties (Liagkouras et al., 2008; Dougherty et al., 2012; Honigsperger et al., 2015), neurochemical markers (Gage and Thompson, 1980; Verney et al., 1985; Sotiriou et al., 2005; Pandis et al., 2006), synaptic transmission (Papatheodoropoulos et al., 2002; Petrides et al., 2007; Georgopoulos et al., 2008; Maggio and Segal, 2009), synaptic plasticity (Papatheodoropoulos and Kostopoulos, 2000a,b; Maruki et al., 2001; Colgin et al., 2004; Maggio and Segal, 2007; Grigoryan et al., 2012; Kenney and Manahan-Vaughan, 2013; Keralapurath et al., 2014; Pofantis and Papatheodoropoulos, 2014), gene expression profiles (Thompson et al., 2008; Dong et al., 2009), and network electrographic activity (Gilbert et al., 1985; Bragdon et al., 1986; Papatheodoropoulos et al., 2005; Sabolek et al., 2009; Mikroulis and Psarropoulou, 2012; Patel et al., 2012; Papatheodoropoulos, 2015a). This diversification is expected to have important implications for the information processing and the functional roles performed by the two hippocampal segments. Revealing functional

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Abbreviations: A_{2A}R, adenosine A_{2A} receptor; AM 281, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide; CB_1R, cannabinoid CB_1 receptor; CGS, 4-[2-[[6-amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride; CHPG, (*RS*)-2-chloro-5-hydroxyphenylglycine sodium salt; CPP, 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; DH, dorsal hippocampus; DMSO, dimethyl-sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EPSP, excitatory postsynaptic potential; Fv, fiber volley; mGluR5, metabotropic glutamate receptor-5; MTEP, 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride; NMDAR, *N*-methyl-D-aspartate receptor; PS, population spike; VH, ventral hippocampus; ZM 241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol.

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differences at the synaptic level, including the modulatory actions of the plethora of receptors and their interactions, between DH and VH will help us understand how the circuitries of the two hippocampal segments process information. Consequently, we will be able to make links between the operations of the small-scale circuit and the large-scale functional segregation along the long axis of the hippocampus.

The glutamate N-methyl-D-aspartate receptors (NMDARs) and metabotropic receptors (mGluRs) as well as the adenosine receptors are important modulators of the neuronal activity (Anwyl, 1999; Mukherjee and Manahan-Vaughan, 2013; Sebastiao and Ribeiro, 2014). The family of mGluRs comprises three groups of receptors (Sheffler et al., 2011), with those belonging to the group I mGluRs having particularly important role in modulating the activity of hippocampal pyramidal cells (Charpak et al., 1990; Desai and Conn, 1991; Pedarzani and Storm, 1993; Gereau and Conn, 1995b; Mannaioni et al., 1999; Mannaioni et al., 2001). NMDARs (Monaghan and Cotman, 1985) and group I metabotropic glutamate receptor-5 (mGluR5) are especially abundant in the CA1 field of the hippocampus (Shigemoto et al., 1992; Romano et al., 1995). Notably, mGluR5 and NMDARs interact synergistically to potentiate NMDAR-mediated responses (Doherty et al., 1997; Anwyl, 1999; Mannaioni et al., 2001; Tebano et al., 2005). Furthermore, recent observations have demonstrated that this synergistic action of the two glutamate receptors is under the control of adenosine A_{2A} receptor ($A_{2A}R$) (Tebano et al., 2005). $A_{2A}Rs$ have a prominent modulatory role in the brain (Cunha et al., 2008; Sebastiao and Ribeiro, 2009) and they are involved in hippocampus-dependent processes (Costenla et al., 2010).

In this study, using recordings of local field potentials from the CA1 area of adult rat hippocampal slices we show that pharmacological manipulation of the three receptors, i.e. NMDAR, mGluR5 and $A_{2A}R$, has remarkably different actions on synaptic transmission, postsynaptic excitability and paired-pulse inhibition between DH and VH. In addition it is shown that a considerable portion of these actions require the activity of CB_1 cannabinoid receptors.

EXPERIMENTAL PROCEDURES

Animals and slice preparation

Hippocampal slices were prepared from forty eight adult, 2–4 month-old male Wistar rats. All experimental treatment and procedures were conducted in accordance with the European Communities Council Directive Guidelines (86/609/EEC, JL 358, 1, December, 12, 1987) for the care and use of Laboratory animals and they have been approved by the Prefectural Animal Care and Use Committee (No: EL 13BIO04). In addition, all efforts have been made to minimize the number and the suffering of animals used. Animals were housed in our Institution under controlled conditions of temperature (20–22 °C), light–dark cycle (12/12 h) and free access to food and water. Hippocampal slices from the DH and the VH were prepared as previously described (Papatheodoropoulos and Kostopoulos, 2000a). Specifically, animals were decapitated after deep anesthesia with diethyl-ether. The brain was removed and placed in chilled (2–4 °C) standard artificial cerebrospinal fluid where the two hippocampi were excised free. The standard medium contained 124 mM NaCl, 4 mM KCl, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM glucose, equilibrated with 95% O_2 and 5% CO_2 gas mixture at pH = 7.4. Transverse slices 500–550-μm thick were prepared from the regions extending more than 1 and less than 4 mm from the dorsal (septal) and the ventral (temporal) ends of the hippocampus using a McIlwain tissue chopper. In order to maintain an orthogonal cut plane during sectioning of the two poles a turn of the plate supporting the structure was required. From each animal 1–4 slices from each pole were selected for experimentation. Immediately after sectioning, slices were transferred and maintained to an interface type recording chamber continuously perfused with standard medium of the same composition as above described and humidified with a mixed gas containing 95% O_2 and 5% CO_2 at a constant temperature of 31 ± 0.5 °C. Slices were left to equilibrate for at least one and a half hour after their preparation before starting recordings. In a set of experiments, slices were bathed in medium containing no magnesium ions (magnesium-free medium, Mg^{2+} = 0 mM). Furthermore, in some experiments, slices containing the CA1 but not the CA3 field, after disconnecting the two fields by a knife cut of the Schaffer collaterals, were also used (specified in Results).

Recordings, data processing and analysis

Field potentials consisting of presynaptic fiber volley (Fv), excitatory postsynaptic potential (EPSP) and population spike (PS) were evoked by delivering electrical pulses (of varying amplitude and stable duration of 100 μs) at Schaffer collaterals using a bipolar platinum–iridium electrode (25-μm wire-diameter, at an inter-wire distance of 100 μm, World Precision Instruments, USA) and recorded from the CA1 stratum radiatum (the EPSP and Fv) and stratum pyramidale (the PS) using carbon fiber electrodes (diameter 7 μm, Kation Scientific, Minneapolis, USA). Stimulation and recording electrodes were placed at a distance of 350 μm from each other. Signals were acquired with a Neurolog amplifier (Digitimer Limited, UK), band-pass filtered at 0.5 Hz–2 kHz, digitized at 10 kHz and stored in a computer disk using the CED 1401-plus interface and the Signal6 software (Cambridge Electronic Design, Cambridge, UK) for off-line analysis. Single electrical pulses were delivered at the frequency of 0.033 Hz. However, stimulation frequency was increased to 0.01 Hz during the short periods of drug-induced rapid changes in the evoked response (noted in Results). Only slices which displayed stable EPSP and PS for at least 10 min were selected for further experimentation. Input/output curves between stimulation intensity and evoked response were made in control conditions and during drug application. In conditions of strong drug-induced