Neuroscience 218 (2012) 89–99

IDENTIFICATION OF mRNA FOR ENDOCANNABINOID BIOSYNTHETIC ENZYMES WITHIN HIPPOCAMPAL PYRAMIDAL CELLS AND CA1 STRATUM RADIATUM INTERNEURON SUBTYPES USING QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

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Abstract—The hippocampus is required for short-term memory and contains both excitatory pyramidal cells and inhibitory interneurons. These cells exhibit various forms of synaptic plasticity, the mechanism underlying learning and memory. More recently, endocannabinoids were identified to be involved in synaptic plasticity. Our goal was to describe the distribution of endocannabinoid biosynthetic enzymes within CA1 stratum radiatum interneurons and CA3/CA1 pyramidal cells. We extracted mRNA from single interneurons and pyramidal cells and used real-time quantitative polymerase chain reaction (RT-PCR) to detect the presence of 12-lipoxygenase, N-acyl-phosphatidylethanolamine-specific phospholipase D, diacylglycerol lipase α, and type I metabotropic glutamate receptors, all known to be involved in endocannabinoid production and plasticity. We observed that the expression of endocannabinoid biosynthetic enzyme mRNA does occur within interneurons and that it is coexpressed with type I metabotropic glutamate receptors, suggesting interneurons have the potential to produce endocannabinoids. We also identified that CA3 and CA1 pyramidal cells express endocannabinoid biosynthetic enzyme mRNA. Our data provide the first molecular biological evidence for putative endocannabinoid production in interneurons, suggesting their potential ability to regulate endocannabinoid-mediated processes, such as synaptic plasticity.

Key words: mGluR, LTD, CCK, calbindin, calretinin, eicosanoid.

INTRODUCTION

The hippocampus is the brain region involved in learning and declarative memory. The process of learning and memory formation is thought to occur through synaptic plasticity. Long-term potentiation is the strengthening of a synapse (Bliss and Lomo, 1973), while long-term depression is the weakening of a synapse (Dudek and Bear, 1992). Within the hippocampus there are fairly homogeneous excitatory pyramidal cells and heterogeneous interneurons, which can both exhibit various types of plasticity.

Recently, some types of synaptic plasticity have been reported to either be modulated by or require endocannabinoids (Feinmark et al., 2003; Abush and Akirav, 2010; for review see Oudin et al., 2011; Alger, 2012). Endocannabinoids are a group of lipid soluble molecules, often arachidonic acid metabolites, that can function in retrograde neurotransmission (Alger and Pitler, 1995). 2-Arachidonylglycerol (2-AG), synthesized by diacylglycerol lipase α (DAGLα) (Tanimura et al., 2010), binds cannabinoid receptor 1 (CB1) (Hill et al., 2007; Ludanyi et al., 2011). 12-(S)-Hydroperoxyeicosa-5Z, 8Z, 10E, 14Z-tetraenoic acid (12-HPETE), which is synthesized by 12-lipoxygenase (12-LO) (Hwang et al., 2000), can activate transient receptor potential vanilloid 1 (TRPV1) receptors. Anandamide (AEA) is produced by N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) (Di Marzo et al., 1994; Ueda et al., 2005) and can bind TRPV1 (Smart et al., 2000; De Petrocellis and Di Marzo, 2005) or CB1 (Fig. 1). Importantly, while most studies have examined the role of endocannabinoids in pyramidal cell synaptic plasticity, few have investigated their role in hippocampal interneuron plasticity.

However, a recent paper suggested CA1 stratum radiatum interneurons do indeed produce endocannabinoids (Gibson et al., 2008). In this example, endocannabinoids mediated a novel interneuron long-term depression at the CA3 pyramidal cell-CA1 stratum radiatum interneuron

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Abbreviations: 2-AG, 2-arachidonylglycerol; 12-HPETE, 12-(S)-hydroperoxyeicosa-5Z, 8Z, 10E, 14Z-tetraenoic acid; 12-LO, 12-lipoxygenase; AEA, anandamide; CCK, cholecystokinin; CB, calbindin; CB1, cannabinoid receptor 1; DAGLα, diacylglycerol lipase α; EGTA, ethylene glycol tetraacetic acid; FAM-TAMRA, 6-carboxyfluorescein-tetramethylrhodamine; HEPES, hydroxyethyl piperazinethanesulfonic acid; LTD, long-term depression; NAPE-PLD, N-acyl-phosphatidylethanolamine-specific phospholipase D; RT-qPCR, real-time quantitative polymerase chain reaction; TRPV1, transient receptor potential vanilloid 1; VGlut1, vesicular glutamate transporter 1.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.05.012

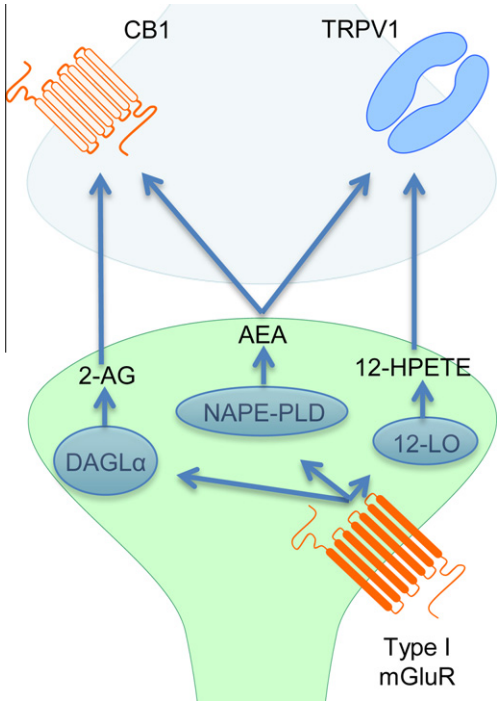

Fig. 1. Endocannabinoid biosynthetic pathways and receptor targets. Postsynaptic type I metabotropic glutamate receptor activation commonly produces metabolites used in endocannabinoid and eicosanoid synthesis. Endocannabinoid biosynthetic enzymes such as diacylglycerol lipase α (DAGLα), N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and 12-lipoxygenase (12-LO) produce the endocannabinoids 2-arachidonylglycerol (2-AG), anandamide (AEA), and 12-(S)-hydroperoxyeicosa-5Z, 8Z, 10E, 14Z-tetraenoic acid (12-HPETE), respectively. Endocannabinoids are lipophilic substances that can act retrogradely on presynaptic terminals to modulate neurotransmitter release via 2-AG or AEA activating cannabinoid receptor 1 (CB1), or 12-HPETE or AEA activating transient receptor potential vanilloid 1 (TRPV1).

synapse. This was identified to be elicited by retrograde endocannabinoid signaling. It was proposed that postsynaptic type I metabotropic glutamate receptor (mGluR) activation induced formation of arachidonic acid, which was then converted to the endocannabinoid 12-HPETE by 12-LO. 12-HPETE retrogradely activated TRPV1 receptors, decreasing neurotransmitter release onto the interneuron. The data suggested that the interneuron itself produced 12-HPETE. However, whether interneurons or various interneuron subtypes have the capability to synthesize endocannabinoids or express endocannabinoid biosynthetic enzymes is unclear and remains controversial as no molecular data has been presented to provide evidence for the presence of endocannabinoid-synthesizing enzymes in hippocampal interneurons.

As there are many interneuron subtypes, various classification schemes have been developed to distinguish between them. These schemes are based on gene expression, physiology or anatomy (Ascoli et al., 2008). Classified subtypes include axo-axonic, basket, bistratified, and interneuron-selective subtypes, based on the innervation patterns of their axons. Using the expression of calcium binding proteins such as parvalbumin, calbindin (CB) and calretinin, as well as neuropeptides such as cholecystokinin (CCK), neuropeptide Y and somatostatin one can generally categorize interneurons into these anatomical subtypes. Parvalbumin-positive cells are generally axo-axonic cells or basket cells present in stratum pyramidale. Another population of basket cells present in the stratum radiatum expresses CCK and can coexpress CB. Many bistratified cells express CB (Freund and Buzsáki, 1996), as well as other subtype markers (Fuentealba et al., 2008; Klausberger, 2009). Interneuron-selective cells are identified by the expression of calretinin and these cells may express CB (Gulyas et al., 1996; Ferraguti et al., 2004).Because of the remarkable heterogeneity of interneurons, it is plausible that different subtypes could produce different varieties of endocannabinoids, and therefore express different endocannabinoid-synthesizing enzymes.

Pyramidal cells are the other major cell type involved in CA3–CA1 hippocampal circuitry. Pyramidal cells are mostly homogeneous in gene expression, morphology, and electrophysiological properties. In pyramidal cells, endocannabinoid involvement in mediating plasticity has been noted physiologically (Edwards et al., 2006; Heifets and Castillo, 2009; Abush and Akirav, 2010) and endocannabinoid biosynthetic enzymes have been identified using immunocytochemistry (Cristino et al., 2008). However, none of these studies have utilized real-time quantitative polymerase chain reaction (RT-qPCR) to describe the distribution of endocannabinoid biosynthetic enzyme mRNA expression in pyramidal cells.

Our first goal was to use RT-qPCR to determine if CA1 stratum radiatum interneurons possess the cellular machinery to synthesize endocannabinoids and to correlate this with interneuron subtype if possible. Our second goal was to examine CA3 and CA1 pyramidal cells for the presence of endocannabinoid biosynthetic enzyme mRNA. To date, there are no studies published using this technique to examine endocannabinoid biosynthetic enzyme mRNA in hippocampal neurons. Our data clearly suggests that CA1 stratum radiatum interneurons indeed express the enzymes necessary for endocannabinoid synthesis, which appear to be fairly widespread in different interneuron subtypes, with the exception of calretinin interneuron-selective cells. Also, our data demonstrate the expression of endocannabinoid biosynthetic enzymes within hippocampal pyramidal cells. Collectively, this suggests that interneurons have the putative capacity to produce endocannabinoids and thus could directly be involved in endocannabinoid signaling, including modulating synaptic plasticity, and even possibly regulating their own plasticity independent of pyramidal cell endocannabinoid production. This is the first molecular study to suggest the potential involvement of interneurons in endocannabinoid signaling.

EXPERIMENTAL PROCEDURES

Preparation of slices

All experiments were performed in accordance with Institutional Animal Care and Use Committee protocols and followed the NIH guidelines for the care and use of laboratory animals. These guidelines include minimizing animal suffering and the number of animals used to perform the required experiments. Sprague–Dawley