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Colocalization of cannabinoid receptor 1 with somatostatin and neuronal nitric oxide synthase in rat brain hippocampus

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ABSTRACT

Somatostatin (SST), a growth hormone inhibitory peptide, is expressed in different parts of the brain and functions as a neurotransmitter and neuromodulator. In the central nervous system (CNS), SST inhibits Ca^{2+} influx and regulates neuronal excitability in the hippocampus, the brain region which plays a major role in seizure, as well as cognitive and memory function. Much like SST, cannabinoid receptor 1 (CB1 receptor) is also widely distributed in the CNS, associated with memory function ad exerts inhibitory effects on seizure. It is unknown whether overlapping functional activities of SST and CB1 receptor are also associated with coexpression in the hippocampus. In the present study, we determined the colocalization between SST and CB1 receptor in adult rat brain hippocampus. In the CNS, the majority of SST positive interneurons coexpress neuronal nitric oxide synthase (nNOS). Accordingly, colocalization studies were also performed to determine whether nNOS positive neurons display comparable colocalization with CB1 receptor. The findings suggested that SST and nNOS are expressed in most interneurons whereas CB1 receptor is present in both interneurons and projection neurons in hippocampal regions. The distinct neuronal populations either expressing CB1 receptor, SST and nNOS alone or colocalization were observed in a region specific manner. Taken together, the observations described here anticipate the possibility of crosstalk between somatostatin subtypes and CB1 receptor in regulation of physiological activities in the hippocampus.

1. Introduction

Cannabinoid receptors are prominent members of the G-protein coupled receptor (GPCR) family. They are expressed in different parts of the brain at distinct densities. Two different isoforms of cannabinoid receptors have been cloned and characterized pharmacologically, namely cannabinoid receptor 1 (CB1) and 2 (CB2). The widespread distribution of cannabinoid receptors in central and peripheral tissues is associated with various functions including learning, memory, cognition, as well as regulation of pain and behavior. In the central nervous system (CNS), CB1 receptors function as

Abbreviations: CB1 receptor, cannabinoid receptor 1; CNS, central nervous system; DG, dentate gyrus; GABA, γ-aminobutyric acid; GL, granular layer; GPCR, G-protein coupled receptor; ML, molecular layer; NGS, normal goat serum; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; PML, polymorphic layer; SO, stratum oriens; SP, stratum pyramidal layer; SR, stratum radiatum; SST, somatostatin; SSTR, somatostatin receptor

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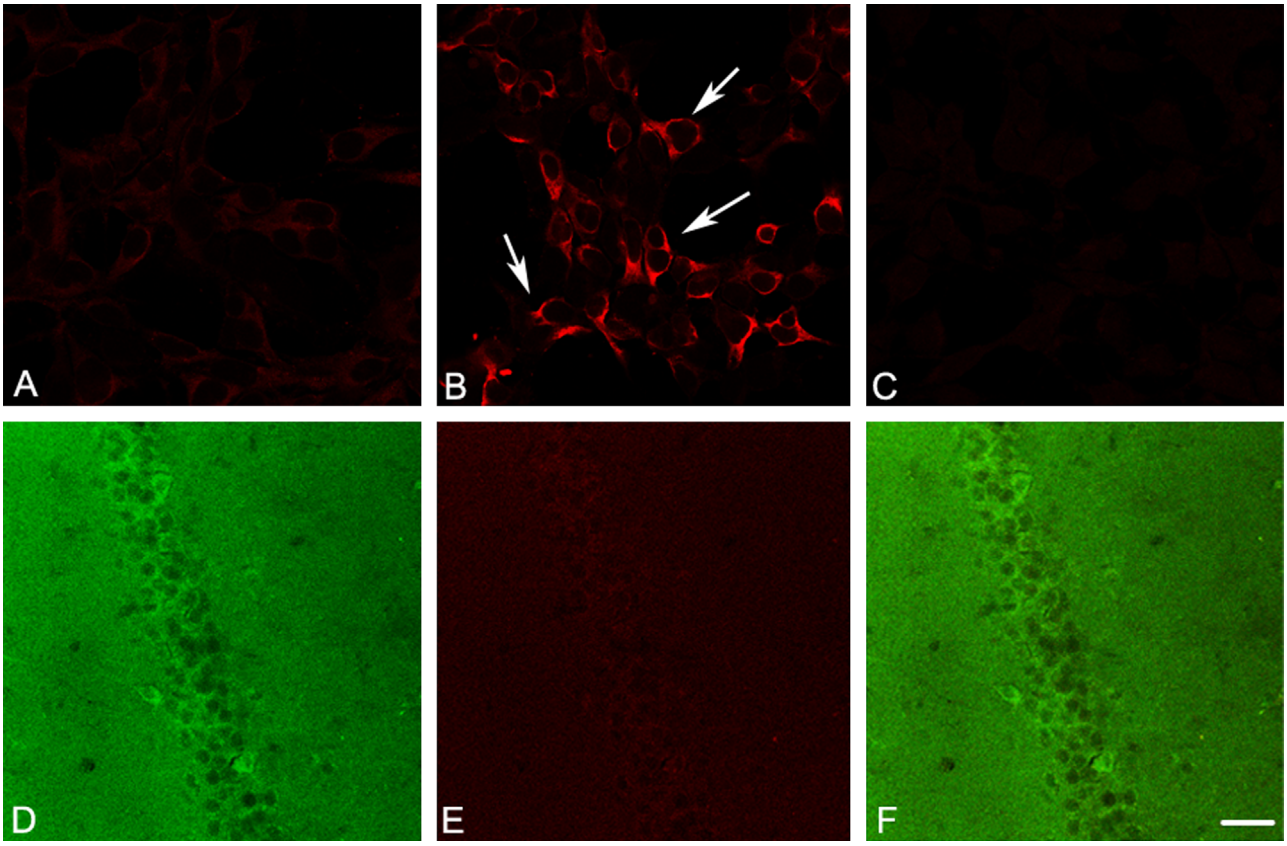

Fig. 1 – Photomicrographs illustrating the specificity of CB1 receptor antibody. Wild-type and CB1 receptor transfected HEK-293 cells were incubated with CB1 receptor antibody at a dilution of 1:500 overnight at 4 °C, followed by Cy3-conjugated secondary antibody (dilution 1:800) for 2 h at room temperature. In wild-type cells (A) no receptor like immunoreactivity was detected whereas transfected cells exhibited strong cell surface immunoreactivity for CB1 receptor (B, arrows). CB1 receptor transfected cells probed only with secondary antibody in the absence of primary antibody were devoid of specific fluorescence (C). Note that no bleach through was detected when hippocampal section stained for SST using FITC conjugated secondary antibody showed only green fluorescence. Scale bar = 20 μm (A–C) and 40 μm (D–F) respectively.

neuromodulator, regulating neurotransmitter release in a retrograde route (Marsicano et al., 2003). The localization of CB1 receptors has been observed on both excitatory and inhibitory synapses in many brain regions including the hippocampus (Katona et al., 1999, Kawamura et al., 2006). Notably, despite GABAergic interneurons carry the majority of central CB1 receptors, evidence indicates that only the CB1 subpopulation expressed on glutamatergic neurons is responsible for many if not all cannabinoid-related physiological effects, including neuroprotection against excitotoxicity (Chiarlone et al., 2014, Monory et al., 2006). The hippocampus is a critical component of the limbic system and is directly associated with regulation of learning and memory as well as several other endocrine functions (Bliss and Collingridge, 1993). A dysfunctional hippocampus not only results in impaired memory but is also associated with several prominent neurological disorders. The hippocampal tissue from epileptic patients displays decreased CB1 receptor density, especially in the dentate gyrus (Ludanyi et al., 2008). In the hippocampus, neurogenesis triggered by high chronic doses of cannabinoids is mediated via CB1 receptors (Jiang et al., 2005).

As with cannabinoid, somatostatin (SST) functions as a neurotransmitter and neuromodulator and has been linked to seizures, memory and cognitive function (Liang and Wu, 1990). SST is expressed in glutamic acid decarboxylase-containing neurons and colocalizes with a subpopulation of GABAergic interneurons in rat dentate gyrus (Tallent, 2007). The loss of SST-positive interneurons in the dentate gyrus has been recognized as a hallmark of epilepsy and in neuropsychological disorders such as schizophrenia and bipolar disorder (Konradi et al., 2011a, Konradi et al., 2011b, Tallent and Qiu, 2008). Furthermore, SST-mediated suppression of long-term potentiation (LTP) further attests its role in both epileptogenesis and memory formation in the hippocampus (Baratta et al., 2002).

Nitric oxide (NO) is a gaseous retrograde messenger synthesized by three different nitric oxide synthases (NOS). Neuronal NOS (nNOS), the predominant form in neurons, is abundantly expressed in the hippocampus (Vincent and Kimura, 1992, Harooni et al., 2009). Studies have shown that nNOS at post-synapse produces NO to conduct retrograde signaling in GABAergic neurons in hippocampal pyramidal cells (Szabadits et al., 2007). nNOS participates in LTP in cultured rat hippocampal neurons while nNOS inhibitor depresses LTP in rats (Bohme et al., 1993, Arancio et al., 1996).