Please cite this article in press as: Zou S et al. Somatostatin receptor 5 is a prominent regulator of signaling pathways in cells with coexpression of Cannabinoid receptors 1. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.10.056

Neuroscience xxx (2016) xxx–xxx

SOMATOSTATIN RECEPTOR 5 IS A PROMINENT REGULATOR OF SIGNALING PATHWAYS IN CELLS WITH COEXPRESSION OF CANNABINOID RECEPTORS 1

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Abstract—Endocannabinoids and somatostatin (SST) play critical roles in several pathophysiological conditions via binding to different receptor subtypes. Cannabinoid receptor 1 (CB1R) and somatostatin receptors (SSTRs) are expressed in several brain regions and share overlapping functions. Whether these two prominent members of G-protein-coupled receptor (GPCR) family interact with each other and constitute a functional receptor complex is not known. In the present study, we investigated the colocalization of CB1R and SSTR5 in rat brain, and studied receptor internalization, interaction and signal transduction pathways in HEK-293 cells cotransfected with human cannabinoid receptor 1 (hCB1R) and hSSTR5. Our results showed that CB1R and SSTR5 colocalized in rat brain cortex, striatum, and hippocampus. CB1R was expressed in SSTR5 immunoprecipitate prepared from the brain tissue lysate, indicating their association in a system where these receptors are endogenously expressed. In cotransfected HEK-293 cells, SSTR5 and CB1R existed in a constitutive heteromeric complex under basal condition, which was disrupted upon agonist treatments. Furthermore, concurrent receptor activation led to preferential formation of SSTR5 homodimer and dissociation of CB1R homodimer. We also discovered that second messenger cyclic adenosine monophosphate and downstream signaling pathways were modulated in a SSTR5-dominant and concentration-dependent manner in the presence of receptor-specific agonist. In conclusion, with predominant role of SSTR5, the functional consequences of crosstalk between SSTR5 and CB1R resulting in the regulation of receptor trafficking and signal transduction pathways open new therapeutic avenue in cancer biology and excitotoxicity.

Key words: somatostatin receptor 5, cannabinoid receptor 1, G protein-coupled receptor, heterodimerization, signaling.

INTRODUCTION

Cannabinoid receptor 1 (CB1R) belongs to G-protein-coupled receptor (GPCR) family and is the major cannabinoid receptor (CBR) that is well-expressed in both central and peripheral nervous systems. In central nervous system (CNS), most of the physiological effects of Δ^9-tetrahydrocannabinol, the main psychoactive component in *cannabis*, are mediated by CB1R (Matsuda et al., 1990). CB1R is widely expressed in the CNS, and plays a crucial role in neurotransmission, neuromodulation, and synaptic plasticity upon activation by its endogenous ligand, endocannabinoids (Howlett et al., 2002). Endocannabinoids bind to CB1R and inhibit the release of several neurotransmitters from presynaptic terminals and exert neuroprotective effects against excitotoxicity (Marsicano et al., 2003). Given that endocannabinoids exert such predominant role in synaptic communication, any interference in this system could initiate severe pathophysiological conditions. Previous studies have reported decreased expression of CB1R in Alzheimer's disease (AD), Parkinson's disease and Huntington's disease (HD) Bisogno and Di Marzo, 2010. CB1R knockout mice display an increased susceptibility to excitotoxin along with sustained neurodegeneration, indicating the pivotal role of CB1R in neuroprotection (Mievis et al., 2011). The neuroprotective role of CB1R has been reported both *in vivo* and *in vitro* (Ramirez et al., 2005; Liu et al., 2009). In peripheral tissues, CB1R is associated with several pathophysiological functions, including cardiac functions, energy metabolism and bone formation (Pacher et al., 2006). Despite these promising therapeutic benefits, the use of cannabinoids in clinical practice is limited, due to undesired psychoactive effects and increased incidence of schizophrenia (Mackie, 2006).

Like cannabinoids, somatostatin (SST) plays a critical role in many pathological conditions through binding to five different receptor subtypes, namely somatostatin

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Abbreviations: AD, Alzheimer's disease; cAMP, cyclic adenosine monophosphate; CB1R, Cannabinoid receptor 1; CNS, central nervous system; co-IP, co-immunoprecipitation; Cy3, cyanine 3; D2R, dopamine receptor 2; ERK1/2, extracellular signal-regulated kinase 1/2; FITC, fluorescein isothiocyanate; FSK, forskolin; GPCR, G protein-coupled receptor; HA, hemagglutinin; hCB1R, human cannabinoid receptor 1; HD, Huntington's disease; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hSSTR5, human somatostatin receptor 5; IBMX, 3-isobutyl-1-methylxanthine; NGS, normal goat serum; PAGE, polyacrylamide gel electrophoresis; pbFRET, photobleaching fluorescence resonance energy transfer; PBS, phosphate-buffered saline; PI3K, phosphoinositide-3 kinase; PKA, protein kinase A; RIPA, radioimmune precipitation assay; RT, room temperature; SP, stratum pyramidal layer; SST, somatostatin; SSTRs, somatostatin receptors; TBS, Tris-buffered saline.

http://dx.doi.org/10.1016/j.neuroscience.2016.10.056
0306-4522/

receptor 1–5 (SSTR1–5). SSTR subtypes are well-expressed in the CNS in a region- and receptor-specific manner (Schindler et al., 1996; Patel, 1999; Ramirez et al., 2004; Kumar, 2007). Although the expression of SSTR5 in the CNS is relatively low in comparison to other SSTR subtypes, its pathophysiological significance in neurological disorders has been supported by several studies (Craft et al., 1999; Stroh et al., 1999; Kumar, 2005; Watson et al., 2009). In AD patients, decreased expression of SSTR5-like immunoreactivity has been observed in cortical brain regions (Kumar, 2005). SSTR5 is suggested to play a role in SST analog-mediated memory facilitation in both AD patients and healthy adults (Craft et al., 1999; Watson et al., 2009). Furthermore, our recent study has demonstrated comparable neurochemical changes in the striatum of HD transgenic R6/2 mice and mice deficient in SSTR1/5 (Rajput et al., 2011). In addition, SSTR5 is involved in protection of rat retina against α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-induced excitotoxicity (Kiagiadaki et al., 2010; Chen et al., 2014). Taken together, these observations suggest a potential beneficial role of SSTR5 in neurological disorders, including excitotoxicity.

At present, it is well established that GPCRs function as dimers or even higher-order oligomers in a manner distinct from the native receptor (Kumar and Grant, 2010; Ferre et al., 2014; Gomes et al., 2016). Numerous studies have shown that SSTRs, as well as CBRs, form homo- and/or heterodimers either within the family or with other GPCRs including dopamine, opioid, orexin and adenosine receptors and members of receptor tyrosine kinase family (Kearn et al., 2005; Ellis et al., 2006; Rios et al., 2006; Carriba et al., 2007; Hudson et al., 2010; Somvanshi and Kumar, 2012). There is growing evidence that GPCR heterodimerization not only modulates pharmacological properties of receptors, but also brings novel signaling to the interacting protomers in a receptor-specific fashion. For instance, interaction between dopamine receptor 2 (D2R) and CB1R leads to a switch of preferential G-protein coupling from G_i to G_s, whereas heterodimerization of D2R with either SSTR2 or SSTR5 increases dopamine affinity and augments D2R-mediated signaling with significant clinical implication in pituitary tumor treatment (Glass and Felder, 1997; Rocheville et al., 2000a; Kearn et al., 2005; Tichomirowa et al., 2005; Baragli et al., 2007; Saveanu and Jaquet, 2009).

CB1R and SSTR5 share a number of overlapping properties at molecular level; both receptors are coupled to $G_{i/o}$ to inhibit adenylyl cyclase, activate mitogen-activated protein kinase pathways, inhibit voltage-dependent calcium channel and activate inwardly-rectifying potassium channel, thus playing critical roles in physiological responses of neuronal cells (Patel, 1999; Howlett et al., 2002). Neuroprotective role of SST and cannabinoids in excitotoxicity, oxidative stress, and traumatic and ischemic brain injury are well-established and associated with modulation of signaling pathways including extracellular signal-regulated kinase (ERK1/2) and phosphoinositide 3-kinase (PI3K) Molina-Holgado et al., 2005; Hu et al., 2010; Kumar and Grant, 2010. However, nothing is currently known whether CB1R interacts with any SSTR subtypes and if such interaction exists, what the functional consequences are. We recently have reviewed that SSTR5 is one of the most dynamic receptors that displays significant diversity upon heterodimerization with other members of GPCR family (Somvanshi and Kumar, 2012). CB1R heterodimerizes with D2R and tends to switch its coupling from G_i to G_s (Glass and Felder, 1997). Our previous study demonstrated D2R heterodimerization with SSTR5 (Rocheville et al., 2000a). It is tempting to determine whether this phenomenon is unique for CB1R in the combination with D2R or a common feature of CB1R. Accordingly, in the present study, we employed multiple techniques to determine the possible interaction between SSTR5 and CB1R in rat brain with endogenous expression and HEK-293 cells stably transfected with hSSTR5 and/or human cannabinoid receptor 1 (hCB1R). Our results show that SSTR5 and CB1R are coexpressed in rat brain cortex, striatum and hippocampus and CB1R is expressed in SSTR5 immunoprecipitate. In cotransfected HEK-293 cells, SSTR5 and CB1R constituted a functional complex and displayed novel properties in modulation of downstream signaling. At present, no molecular mechanism is on place that could help to minimize undesired side effect of cannabinoids while enhancing its potential medical use. Results described here showing complex formation between CB1R and SSTR5 provide possibility of developing a new therapeutic in drugs of abuse.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (body weight 200–250 g) were obtained from the University of British Columbia (UBC) animal care unit. Protocols regarding animal care were followed in compliance with principles of the Canadian Council on Animal Care and were approved by the UBC Animal Care Committee (Protocol #A06-0419).

Materials

Somatostatin-14 was procured from Bachem (Torrance, CA, USA) and non-peptide SSTR5 agonist L-817818 was kindly provided by Dr. S. P. Rohrer from Merck & Co. WIN 55212-2 was purchased from the Tocris Cookson Inc., Ellisville, MO, USA (Authorization 31251.09.13). Normal goat serum (NGS) was purchased from Vector Laboratories, Burlingame, CA, USA. SSTR5 antibody was produced in our laboratory and has been well characterized as described previously (Kumar et al., 1999). CB1R anti-goat polyclonal antibody was purchased from Santa Cruz, CA, USA. Antibodies against hemagglutinin (HA) and cMyc were purchased from Sigma–Aldrich, Inc., St. Louis, MO, USA. Fluorescein isothiocyanate (FITC)- and cyanine 3 (Cy3)-conjugated secondary antibodies were obtained from Jackson ImmunoResearch, ON, USA. Rabbit polyclonal antibodies for p- and t-ERK1/2, and t-PI3K were purchased from Cell Signaling Technology, Danvers, MA. P-PKA, t-PKA, and p-PI3K antibodies were

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