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Research Report

Characterization of cannabinoid-1 receptors in the locus coeruleus: Relationship with mu-opioid receptors

Jillian L. Scavone[a,*], *Ken Mackie*[b], *Elisabeth J. Van Bockstaele*[a]

[a]*Department of Neurosurgery, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA, USA*
[b]*Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA*

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ABSTRACT

The locus coeruleus (LC)-norepinephrine system is a target of both cannabinoid and opioid actions. The present study investigated the anatomical distribution of cannabinoid-1 receptor (CB1r) in the LC and its association with mu-opioid receptor (MOR). Immunoreactivity for CB1r was localized to pre- and postsynaptic cellular profiles in the LC, 82% of which were dual-labeled for tyrosine hydroxylase (TH). Of the CB1r-immunoreactive structures, 66% were somatodendritic profiles, 22% were axon terminals, and the remaining 12% were associated with glial and small unmyelinated axon-like structures. CB1r immunoreactivity (-ir) in somatodendritic profiles was more often localized to the cytoplasm, whereas CB1r-ir located in axon terminals was more commonly localized on the plasma membrane. Somatodendritic profiles with CB1r-ir typically received input from axon terminals forming asymmetric-type synapses. In contrast, presynaptic profiles with CB1r-ir typically formed symmetric synaptic specializations. Anatomical studies confirmed the co-existence of MOR and CB1r-ir in common somatodendritic compartments of catecholaminergic neurons in the LC, and also revealed CB1r-positive axon terminals forming synaptic contact with MOR-containing dendrites. Our results provide evidence for a heterogeneous distribution of CB1r in the LC and demonstrate that CB1r and MOR co-exist in cellular profiles in this region. These data suggest important potential interactions between cannabinoid and opioid systems in LC neuronal profiles that may impact noradrenergic tone.

1. Introduction

G-protein coupled receptors are abundant transmembrane proteins that comprise approximately 50% of all pharmacological targets. Cannabinoid-1 receptors (CB1r) and mu-opioid receptors (MOR) are G-protein coupled receptors that are widely expressed throughout the brain. The opioid and cannabinoid receptors are major targets for many drugs of abuse and widely used analgesics (Demuth and Molleman, 2006; Manzanares et al., 1999; Pasternak, 2005). These

* *Corresponding author*. Farber Institute for Neuroscience, Jefferson Hospital for Neurosciences, 900 Walnut Street, Suite 417, Philadelphia, PA 19107, USA. Fax: +1 215 503 9238.
E-mail address: jillian.scavone@jefferson.edu (J.L. Scavone).

Abbreviations: CB1r, cannabinoid receptor type 1; CB1r–/–, CB1r knock out; EM, electron microscopy; FITC, fluorescein isothiocyanate; -ir, immunoreactivity; i.p., intraperitoneal; LC, locus coeruleus; MOR, mu-opioid receptor; NE, norepinephrine; PGi, nucleus paragigantocellularis; PrH, nucleus prepositus hypoglossi; Syn, synaptophysin; TRITC, tetramethyl isothiocyanate; TH, tyrosine hydroxylase

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receptors preferentially signal through the G_i/G_o alpha subunit of the heterotrimeric G-proteins, effectively decreasing cyclic adenosine monophosphate levels (Childers et al., 1992; Dhawan et al., 1996; Howlett et al., 2002; Massi et al., 2003).

A brainstem nucleus targeted by both opioids and cannabinoids is the locus coeruleus (LC), a norepinephrine (NE) enriched region that is involved in modulating arousal, stress, and anxiety (Espana and Berridge, 2006; Harburg et al., 2006; Rios et al., 2006; Van Bockstaele et al., 2000; Wang and Burns, 2006). The LC projects throughout the neuroaxis and serves as the primary source of NE to forebrain regions. This region regulates arousal, attention, pain and stress via projections to multiple limbic, cortical and autonomic-related brain areas (Aston-Jones et al., 1996, 2001; Carrasco and Van de Kar, 2003; Dunn et al., 2004; Nestler et al., 1999). The neuroanatomical substrates of the LC-NE axis and noradrenergic signaling in the brain implicate this system in the regulation of tolerance, addiction, sensitization and withdrawal from substances of abuse (Aston-Jones and Kalivas, 2008; Weinshenker and Schroeder, 2007).

The LC, a pivotal structure in opiate addiction and withdrawal, has been well-characterized in its sensitivity to opiate exposure and withdrawal (Koob et al., 1992; Nestler, 1993; Nestler et al., 1994, 1999; Rasmussen and Aghajanian, 1989; Rasmussen et al., 1990; Van Bockstaele et al., 2001). This nucleus is comprised of a fairly homogeneous cluster of noradrenergic neurons that possess a high density of postsynaptic MOR (Van Bockstaele and Commons, 2001). In addition to the LC's role in opioid signaling, evidence also supports modulation of noradrenergic activity and function by cannabinoids, suggesting a potential role for local cannabinoid signaling in the LC. Using a radiolabeled cannabinoid receptor agonist, regions with low to moderate cannabinoid receptor binding were noted in noradrenergic brainstem nuclei such as the LC and nucleus of the solitary tract (Herkenham et al., 1991). Electrophysiological evidence also suggests a role for cannabinoids in the LC. Studies have demonstrated that systemic administration of cannabinoid agonists increases spontaneous firing rates of neurons in the LC and blocks evoked inhibition (Mendiguren and Pineda, 2006a; Muntoni et al., 2006). From electrophysiology experiments using brain slices, there is evidence for cannabinoid receptor activity directly within the LC, where cannabinoid receptor activation was shown to suppress the glutamatergic component of potassium chloride-evoked excitation, and to enhance N-methyl-D-aspartic acid-induced excitation of these neurons (Mendiguren and Pineda, 2004, 2007).

Recent data from our laboratory and others provide additional evidence for cannabinoid modulation of the LC. Systemic and local administration of WIN55,212-2 has been shown to increase forebrain NE release as well as increase indices of noradrenergic activity (Corchero et al., 1997, 2002; Oropeza et al., 2005; Page et al., 2007, 2008; Paldy et al., 2008; Valverde et al., 2001). In further support of such modulatory actions, cannabinoid receptors have been localized to noradrenergic axon terminals in the frontal cortex (Oropeza et al., 2005; Oropeza et al., 2007; Page et al., 2007, 2008). Combined, these data have led us to further examine the anatomical substrates underlying cannabinoid effects on the LC by investigating the immunohistochemical localization of CB1r with respect to noradrenergic neurons in the brainstem. In addition, modulation of MOR and CB1r have both been found to alter indices of noradrenergic activity (Nestler, 1993; Nestler et al., 1999; Oropeza et al., 2005; Szabo and Schlicker, 2005) and may interact in the LC to regulate noradrenergic function. We investigated the dual localization of cannabinoid and opioid receptors in the LC to provide an anatomical substrate for potential interactions. Taken together, these studies contribute to advancing our understanding of the functional implications of cannabinoid signaling in the LC and reveal important cannabinoid–opioid receptor interactions that may impact noradrenergic activity.

2. Results

2.1. *Antisera specificity*

The specificity of the tyrosine hydroxylase (TH) and MOR antisera used in this study has been thoroughly established for detection of these antigens in the LC. Several control experiments were performed to verify the specificity of the CB1r antibody for detection of cannabinoid receptors in this region. Immunoperoxidase labeling for CB1r was carried out in $CB1r^{-/-}$ mice in multiple brain regions to verify the lack of immunoreactivity in knockout animals. In tissue sections from wild type CD1 mice, immunoperoxidase labeling for CB1r was visible in the cerebral cortex and hippocampus (Figs. 1A and B, respectively). CB1r immunohistochemistry performed simultaneously using tissue sections from the cerebral cortex and hippocampus of a $CB1r^{-/-}$ mouse revealed complete absence of CB1r immunoreactivity (Figs. 1E and F). Additional controls were performed in rat hippocampus sections processed for CB1r immunoperoxidase labeling (Fig. 1C), demonstrating similar immunoreactivity patterns to mouse CB1r. Preadsorption of the primary antisera with the corresponding blocking peptide abrogated CB1r immunoreactivity as compared to rat hippocampus sections in which standard CB1r immunohistochemistry was performed (Figs. 1G and C, respectively).

2.2. *Visualizing CB1r using light and fluorescence microscopy*

CB1r immunoreactivity was visualized using fluorescence and peroxidase labeling in rat brainstem tissue sections through the LC. In comparable rostrocaudal levels of the LC, single-labeling for CB1r using immunoperoxidase (Fig. 1D) displays a similar pattern to CB1r immunofluorescence labeling (Fig. 1H). Dual immunofluorescence studies were performed to examine the co-localization of CB1r immunoreactivity with TH, the rate limiting enzyme for norepinephrine synthesis. In LC sections labeled for CB1r and TH, TH immunoreactivity (green) reveals the localization of noradrenergic neurons in the LC (Fig. 2A). CB1r immunofluorescence (Fig. 2C, red) is scattered throughout the LC in what appears to be a mix of somatodendritic and presynaptic patterns of immunoreactivity. A merged image (Fig. 2E) reveals a considerable portion of CB1r immunoreactivity co-localized to TH-positive cells (arrows), but also CB1r labeling that is distinctly separate from TH immunoreactivity (arrowheads). To determine