available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Localization of CB1 cannabinoid receptor mRNA in the brain of the chick (*Gallus domesticus*)

*Todd L. Stincic, Richard L. Hyson**

Program in Neuroscience, Department of Psychology, Florida State University, Tallahassee, FL 32306-1270, USA

ARTICLE INFO

Article history:
Accepted 12 September 2008
Available online 20 September 2008

Keywords:
Avian
Comparative anatomy
Tectum
Striatum
Sensory system

ABSTRACT

The cannabinoid receptor one (CB1) is prevalent in the brains of many species. Receptor binding, in situ hybridization and immunohistochemical surveys have described the distribution of this receptor in a limited number of species. The current study used in situ hybridization to examine the expression of CB1 mRNA in the chick brain, a non-mammalian vertebrate. The results were compared to the observed patterns of expression for CB1 mRNA, protein, and agonist binding that have been reported for other avian species and mammals. Importantly, since CB1 receptors are typically located on neuronal terminals, comparison of the somatic mRNA expression with previously reported descriptions of the location of functional receptors, allows speculation about the circuits that make use of these receptors. The expression pattern for CB1 mRNA appears to be highly conserved across species in key areas such as the cerebellum and portions of the forebrain. For example, high levels of expression were observed in the avian amygdala and hippocampus, areas which express high levels of CB1 in mammals. The avian substantia nigra and ventral tegmental area, however, showed specific labeling. This finding is in stark contrast to the high levels of receptor binding or CB1 protein, but not CB1 mRNA in these areas of the mammalian brain. Moderate labeling was also seen throughout the hyperpallium and mesopallium. Throughout the brain, a number of regions that are known to be involved in visual processing displayed high levels of expression. For example, the tectum also had strong mRNA expression within layers 9–11 of the stratum griseum et fibrosum superficale and stratum album centrale.

1. Introduction

Cannabinoid (CB) signaling is rapidly gaining recognition as a widespread and influential form of signal modulation in the brain. At least two subtypes of receptors, CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993), have been identified. CB2 is predominately found in cells of the immune system, and it is only present in microglia within the brain (Nunez et al., 2004). CB1, however, is highly abundant in the brain and is involved in synaptic signaling. Analysis of sequence conservation has revealed high sequence identity of orthologs with human CB1 (97% rat, 91% zebra finch, and 83% newt salamander) (McPartland and Glass, 2003; Soderstrom and Johnson, 2000). The genome of the puffer fish, *Fugu rubripes*, also contains a CB1 ortholog (Yamaguchi et al., 1996), albeit with much lower sequence identity to human CB1, 59% (McPartland

* *Corresponding author*. Fax: +1 850 644 7739.
E-mail address: hyson@psy.fsu.edu (R.L. Hyson).
Abbreviations: CB, Cannabinoid; CB1, Cannabinoid Receptor 1; IHC, immunohistochemistry; ISH, in situ hybridization; ROI, region of interest

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.09.037

and Glass, 2003). These findings suggest that CB1 orthologs are present in a wide variety of vertebrate species, and that sequence conservation is high.

While the sequence of the CB1 receptor appears to be highly conserved across vertebrates, it is unclear if the localization of cells producing this receptor in the brain is likewise conserved. The pattern of mammalian CB1 localization has been well characterized utilizing receptor binding (Devane et al., 1988; Herkenham et al., 1991), in situ hybridization (ISH) (Mailleux et al., 1992; Matsuda et al., 1993), and immunohistochemistry (IHC) (Egertova and Elphick, 2000; Pettit et al., 1998; Tsou et al., 1998). However, relatively little is known of CB1 localization in non-mammalian species. The goal of this investigation was to determine the localization of CB1 mRNA in the chick brain, *Gallus domesticus*, a non-mammalian vertebrate. The publication of the chick genome (Consortium, 2004) makes this animal a potentially useful model for the study of gene expression in non-mammalian vertebrates. The localization of CB1 was examined throughout the chick brain using ISH and regions displaying distinct, above-background labeling were objectively analyzed by densitometry.

CB1 mRNA and protein have been observed in regions of the zebra finch telencephalon, and this receptor appears to be important for vocal production (Soderstrom and Johnson, 2000; Soderstrom et al., 2000; Soderstrom and Tian, 2006). Localization of CB1 receptor has also been described through binding assays in the budgerigar brain (Alonso-Ferrero et al., 2006). The present study of mRNA expression in the chick brain allows for two important comparisons with these previous studies. The first important comparison comes from the fact that the previous studies have focused on receptor localization in avian species capable of vocal learning. The present analyses of the chick, a non-vocal learning species, will aid in understanding which aspects of CB1 expression are general across avian species and which aspects may be specialized for vocal learning. The second important comparison comes from the fact that CB1 receptors are typically inserted at axon terminals, whereas mRNA is typically localized in cell bodies. Consequently, if CB1 is contained in projection neurons, then mRNA expression and protein expression patterns will not be identical. Thus, the comparison of the present mRNA results with prior results on the localization of functional receptors in birds (Alonso-Ferrero et al., 2006) not only provides information about which areas of the brain produce CB1 receptors, but also allows speculation into the functional circuitries that may utilize these receptors.

2. Results

2.1. *Probe specificity*

Probe specificity was confirmed in three ways. First, Northern analysis using our CB1 probe revealed a single band on lanes containing RNA from cerebellum and brain stem, but lanes containing heart and liver RNA had no labeling. Since CB1 is believed to be selectively expressed in the nervous system, the selective labeling of RNA from the nervous system tissue suggests that the probe was specifically labeling CB1. Hybridization of the 28S rRNA probe showed the presence of 28S rRNA in all lanes. This indicates that the lack of CB1 labeling in heart and liver was not because of insufficient RNA. Relative positions of the bands revealed that the CB1 mRNA was of greater length than 28S rRNA (4.7 kb). This is consistent with previous reports of CB1 mRNA in zebra finch (~5.5 kb) (Soderstrom and Johnson, 2000).

Quantitative RT-PCR was carried out to further establish the specificity and validity of our new chick CB1 mRNA probe. The CB1 primers with cerebellar cDNA showed detectable signal at 17.7 cycles. Both liver and cerebellum cDNA with RP27 primers (household gene) displayed a signal first at 20.6 and 20.8 cycles respectively. The CB1 primers with liver cDNA did not produce a signal until 26.4 cycles. Negative control wells showed no significant signal. PCR product was run on an agarose gel and a single band of the appropriate length was confirmed. Furthermore, the PCR product identity was confirmed through sequencing (FSU DNA Sequencing Laboratory).

Finally, the specificity of the ISH was confirmed using a riboprobe version of the CB1 probe. The anti-sense CB1 riboprobe produced a pattern of labeling that was identical

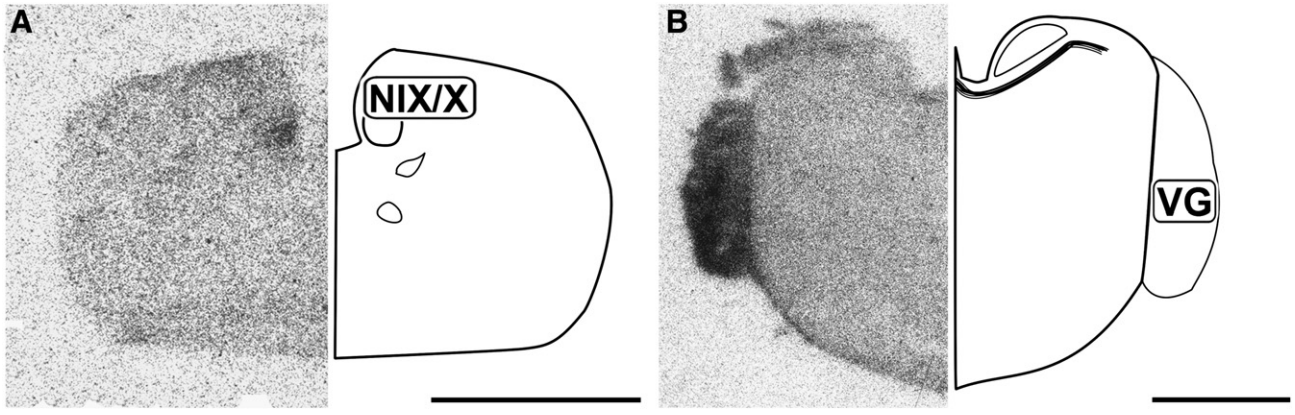

Fig. 1 – Coronal 20 μm autoradiographs showing CB1 mRNA expression (left) and corresponding anatomical schematic (right). Low levels of labeling are observed in the brain stem except for cranial nerve nuclei. NIX/X: Glossopharyngeal/vagal nucleus, VG: Vestibular ganglion. Scale bar=2 mm.