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Research Report

Cannabinoid system in the budgerigar brain

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ABSTRACT

Cannabinoid receptor density and cannabinoid receptor-mediated G protein stimulation were studied by autoradiographic techniques throughout the budgerigar (*Melopsittacus undulatus*) brain. The maximal CB_1 receptor density value (using [^{3}H]CP55,940 as radioligand) was found in the molecular layer of the cerebellum (Mol), and high binding values were observed in the nucleus taeniae amygdalae (TnA), nucleus preopticus medialis, and nucleus pretectalis. The highest net-stimulated [^{35}S]GTPγS binding values induced by the selective CB_1 receptor agonist WIN55,212-2 were observed in the nucleus paramedianus internus thalami, and high values of [^{35}S]GTPγS binding were observed in the TnA, Mol, arcopallium dorsale and arcopallium intermedium. The distribution data suggest that in the budgerigar, as previously indicated in mammals, cannabinoid receptors may be related to the control of several brain functions in the motor system, memory, visual system, and reproductive behavior. The discrepancies between the cannabinoid receptor densities and the cannabinoid receptor-mediated stimulation found in several budgerigar brain nuclei support the hypothesis, previously described for mammals, of the existence of different $G_{i/o}$ protein populations able to associate with the cannabinoid receptors, depending on the brain structure, and could reflect the relative importance that cannabinoid transmission could exerts in each cerebral area.

1. Introduction

In recent years, the study of the cannabinoid system in the brain has received much attention because of the widespread and complex effects on higher cognitive functions exerted by cannabis (*Cannabis sativa*). Two different cannabinoid receptor subtypes, CB_1 and CB_2, have been described (Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993; Piomelli et al., 2000). These receptors have been found to be coupled to $G_{i/o}$ proteins (Howlett et al., 1986, 1988).

Although the CB_1 receptor is the predominant subtype in the central nervous system (CNS) in mammals, the presence of the CB_2 subtype has also recently demonstrated (Van Sickle et al., 2005). CB_1 receptor is highly expressed in cerebral cortex, hippocampus, basal ganglia, and cerebellum (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992a; Glass and Felder, 1997). Interestingly, this subtype has been also found in striatal astrocytes (Rodriguez et al., 2001). Despite the conserved presence of this receptor in the CNS, different patterns of CB_1 receptor distribution have been found between

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humans and rodents. Thus, a significantly higher density of this receptor in the human amygdala and cingulate cortex as compared with those of rat and monkey has been described in the same brain areas (Herkenham et al., 1990). In addition to the reported presence in the brainstem, the distribution of the CB_2 receptor indicates that this subtype is primarily localized on cells related to the immune system, in particular, mature B cells and macrophages (Galiegue et al., 1995). Regarding studies on avian species, few authors have addressed cannabinoid receptors in birds, and no detailed distribution of these receptors has been provided (Soderstrom and Johnson, 2000). CB_1 receptors seem to be the only type of cannabinoid site in the CNS of birds (Soderstrom and Johnson, 2000), although a CB_2-like protein has been also described in the CNS in chick embryos but not in adult chickens (Fowler et al., 2001).

The type of neurons displaying the CB_1 receptor has been described to be efferent striatal GABAergic neurons and striatum–nigral and striatum–pallidal neurons, releasing substance P and enkephalins, respectively (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992b). Similarly, the existence of this subtype of receptor on hippocampal GABAergic interneurons (Tsou et al., 1998) and in cerebellar glutamatergic granular neurons has been reported (Levenes et al., 1998). Furthermore, the reported presynaptic localization of these receptors is consistent with the proposed role of endogenous cannabinoid compounds released by postsynaptic neurons as modulators of the release of excitatory and inhibitory neurotransmitters by presynaptic terminals (Hoffman and Lupica, 2000; Maejima et al., 2001; Diana et al., 2002). This proposed modulator role, together with the existence of very high densities of CB_1 receptors through the CNS, supports the relevance of the endocannabinoid system as a general mechanism of central regulation (Freund et al., 2003).

The budgerigar (*Melopsittacus undulatus*) belongs to the order Psittaciformes, which together with the Oscines and Trochiliformes are avian orders that contains vocal learning species (Ball and Hulse, 1998; Gahr, 2000; Jarvis et al., 2000; Janata, 2001; Roberts et al., 2001). Molecular and cladistic analyses provide evidences that vocal learning has evolved independently in these orders (Striedter, 1994). Species of the order Psittaciformes present a well-developed song control system which has recently been described (Durand et al., 1997) to show differences with that of the Oscines, as well as some differences in the functions of the song system, including the ability to imitate sounds (Hile et al., 2000; Plummer and Striedter, 2000).

The level of functionality of GPCRs can now be analyzed in deep by means of [^{35}S]GTPγS binding assays. In addition, a high density of membrane receptors does not always imply a high level of signal transduction. Because of that and taking into account the very limited information available on the presence and activity of cannabinoid receptors in the avian brain, a detailed study on the distribution of CB_1 receptor density and functionality (transductional properties) in the brain of budgerigar could provide important information about both the general role of the endocannabinoid system in birds nervous system and the specific involvement of these receptors in the regulation of song control systems.

Here, we present, for the first time, a complete and detailed distribution of the CB_1 receptor protein, together with the distribution of the degree of functionality mediated by this receptor, throughout the brain of this bird species.

2. Results

2.1. *Functional autoradiography*

Preliminary experiments were performed to determine the optimal concentrations of WIN55,212-2 able to stimulate [^{35}S]GTPγS binding mediated by cannabinoid receptors (unpublished results). In this sense, most reports have used 10 μM WIN55,212-2 for cannabinoid stimulation in functional autoradiographic assays using rat brain tissues (Sim et al., 1995; Berrendero et al., 1998; Breivogel et al., 1997). However, the need for higher concentrations (100 μM WIN55,212-2) for reaching maximal stimulation in the human brain has been reported (Rodriguez-Puertas et al., 2000). Our data using 10 μM and 100 μM WIN55,212-2 showed that both concentrations elicited maximal stimulation in budgerigar brain tissues. We used an agonist concentration of 10 μM for the distribution described here to maintain the standard protocols described for rat.

2.2. *Basal [^{35}S]GTPγS binding*

The highest basal [^{35}S]GTPγS binding values were found in the diencephalic nucleus preopticus medialis (POM). Very high levels of basal binding (>75% with respect to the structure of maximal basal binding value, POM) were found in some telencephalic structures such as nucleus striae terminalis lateralis (NSTL), and in the following diencephalic structures: nucleus dorsomedialis posterior thalami (DMP), nuclei habenularis medialis and lateralis (HM and HL). High levels of basal binding (50 to 75% with respect to POM) were found in nucleus paramedianus internus thalami (PMI) (diencephalon). The remaining structures showed moderate (25 to 50% with respect to POM) or low (0 to 25% with respect to POM) basal [^{35}S]GTPγS binding values. See Table 1 and Fig. 1.

2.3. *Total agonist-stimulated [^{35}S]GTPγS binding*

Total agonist-stimulated [^{35}S]GTPγS binding values obtained in the presence of the cannabinoid agonist WIN55,212-2 are shown in Table 1 and Fig. 1. The highest [^{35}S]GTPγS binding value in this condition was found in the diencephalic PMI nucleus. Very high [^{35}S]GTPγS binding values (>75% with respect to the structure of maximal density in the brain, the PMI) were found in the nucleus taeniae amygdalae (TnA) and NSTL (telencephalon) as well as in the POM, DMP, HM and HL (diencephalon). High binding levels (50 to 75% with respect to the PMI) were found in the hyperpallium apicale (HA), mesopallium (M), nucleus centralis nidopallii lateralis (NLC), nidopallium intermedium (NI), robust nucleus arcopallialis (RA), arcopallium dorsale (AD), arcopalium intemedium (AI), hippocampus (Hp) and area parahippocampalis (APH) (telencephalon), the nucleus superficialis parvocellularis (SPC) (diencephalon), the stratum griseum periventriculare (SGP) (mesencephalon), and