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EXPRESSION OF A CANNABINOID RECEPTOR IN BACULOVIRUS-INFECTED INSECT CELLS

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Abstract—A cannabinoid receptor recombinant baculovirus (AcNPV-THCR) has been constructed and employed to express rat neural cannabinoid receptors. Northern analysis of total RNA from *Spodoptera frugiperda* (Sf9) insect cells infected with AcNPV-THCR revealed novel hyper-production of a 3.3 kb transcript when probed with nick-translated rat cannabinoid receptor cDNA. Optimal viral protein expression was observed in ^{35}S-metabolically labeled AcNPV-THCR-infected Sf9 cells at a multiplicity of infection of 2.5. Transmission electron microscopy of AcNPV-THCR-infected Sf9 cells showed extensive membrane perturbation and electron-dense cytoplasmic perinuclear accumulation, indicative of receptor glycoprotein expression. Immunofluorescence staining using antiserum produced to a fusion protein consisting of the external domain of the cannabinoid receptor and hepatitis B core antigen revealed cannabinoid receptor expression in AcNPV-THCR-infected Sf9 cells. Scatchard–Rosenthal analysis of [^{3}H]CP55,940 receptor binding indicated a K_d of 3.4 nM and a B_{max} equal to 3.17 pmol/mg protein. Western immunoblotting performed on AcNPV-THCR-infected Sf9 cell lysates revealed immunoreactive bands with relative molecular weights ranging from 45 to 79 kDa. The predominant species (55 kDa) exhibited a relative molecular weight consistent with that predicted for the translational product obtained from the cannabinoid receptor cDNA coding sequence. *In vitro* translation using AcNPV-THCR mRNA also yielded a 55 kDa immunoreactive species. These data indicate that the baculovirus expression system is a viable means of expressing relatively large quantities of cannabinoid receptor recombinant protein.

Key words: radioligand binding; cannabinoid receptor; G protein-coupled receptor; baculovirus expression system; delta-9-tetrahydrocannabinol; anti-cannabinoid receptor antiserum

THC‡, the major psychoactive component in marijuana, can produce a multiplicity of effects in humans, including alterations in mood, perception, cognition, memory, psychomotor activity, as well as analgesia, antiemesis, and immunosuppression [1, 2]. It is unlikely that one mechanism is responsible for the multiplicity of effects observed with THC exposure. Indeed, substantial evidence exists for two possible mechanisms for THC-induced cellular effects. The highly lipophilic nature of THC has suggested that some of the cannabinoid-induced effects are due to membrane perturbation [3–5]. Such disruption of cellular membranes may alter signal transduction pathways, stimulate or inhibit membrane-associated enzymes, and alter membrane-associated ion channels. However, the stereospecificity and structural requirements for physiological activity suggest that the cannabinoids also can act via a specific receptor [6–10].

Cannabinoid-induced inhibition of cAMP accumulation in a mouse neuroblastoma cell line, N18TG2, was the first definitive evidence for the presence of cannabinoid receptors in neural tissue [11]. Radioligand binding studies that utilized a potent tritiated THC analog, CP55,940, further substantiated the presence of cannabinoid receptors in neural tissue [12]. Anatomical distribution of the cannabinoid receptor in rat brain was characterized by receptor autoradiography using [^{3}H]CP55,940, and dense binding was observed in cerebellum, hippocampus, and basal ganglia [13, 14]. The serendipitous discovery of a cDNA clone, isolated from a rat cerebral cortex cDNA library, shown to bind and functionally interact with cannabinoids, has allowed for the molecular characterization of this receptor [5, 15]. Sequence analysis of this clone has revealed a high level of homology with GPCR in that it contains an external domain that possesses glycosylation sites, seven conserved transmembrane domains, and an internal or cytoplasmic domain that is thought to couple to a G-inhibitory protein complex.

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‡ Abbreviations: THC, delta-9-tetrahydrocannabinol; AcNPV, *Autographa californica* multiply enveloped nuclear polyhedrosis virus; AcNPV-β-Gal, β-galactosidase recombinant baculovirus; BB, blocking buffer; THCR, cannabinoid receptor; AcNPV-THCR, cannabinoid receptor recombinant baculovirus; GPCR, G protein-coupled receptor; HBcAg, hepatitis B core antigen; HBcAg-THCR, hepatitis B core antigen cannabinoid receptor fusion protein; MOI, multiplicity of infection; PMSF, phenylmethylsulfonyl fluoride; pi, post-infection; Sf9, *Spodoptera frugiperda*; TCA, trichloroacetic acid; TBS, Tris-buffered saline; and PCR, polymerase chain reaction.