Endocannabinoid Signaling Pathways

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Endocannabinoids play an important role as powerful regulators of synaptic function in the central nervous system where they regulate neural functions and behaviors. As fundamental modulators of synaptic function, endocannabinoids can regulate neural functions such as cognition, motor control, pain, and feeding behavior. The interaction between endogenous or exogenous ligands and endocannabinoid receptors initiates signaling pathways.

endocannabinoid system receptor-mediated drug delivery endocannabinoid tone

1. Introduction

The discovery of the endocannabinoid system (ECS) began with the isolation of the active ingredient in an extract of *Cannabis sativa*, observation of endogenous ligand binding, the genetic and physical mapping of the human cannabinoid receptor gene, and identification of enzymes that synthesize and degrade endocannabinoids and has resulted in increased interest and global acceptance of the use of *Cannabis sativa* [1]. The growth in clinical evidence for the therapeutic efficacy of cannabinoid formulations can be attributed to knowledge of molecular genetics of cannabis, successful decoding of the genome, and crystal structure determination of cannabinoid receptor type 1 (CB1-R) and cannabinoid receptor type 2 (CB2-R) with their heterotrimeric complex formation [2] [NO_PRINTED_FORM].

The global trend of decriminalizing *Cannabis sativa* is not without controversy, and the legality and stigma associated with the plant slowed the transition of use into the pharmaceutical industry [3]. Challenges associated with pharmacokinetics, formulation of highly lipophilic cannabinoids, psychoactive and off-target effects, and high-dose dependency associated with delta-9-tetrahydrocannabinol (Δ9-THC) have further hindered the commercialization of cannabinoid-containing products [4]. Direct activation of CB1-R is responsible for most associated adverse effects, such as hypomotility, hypothermia, and catalepsy [5]. CB1-R activation can also induce sedation, hyperphagia, and physical or mental dependence [6]. While the desired anti-inflammatory and immunosuppressive properties of CB2-R activation in neurons are important and often sought, the peripheral CB2-R activation in T-cells which reduces immune response, may be unfavorable in pathogen-associated inflammation or cancer [7][8][9]. In an attempt to avoid direct activation of cannabinoid receptors, studies involving alteration in endocannabinoid tone by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibitors have yielded some promising preclinical results but have been unsuccessful thereafter [10].

The most promising receptors and molecular targets in the endocannabinoid system have been identified, which suggests strategic targeting approaches may improve drug delivery [11]. The literature is replete with target sites in the body and activates or inhibit signal transduction pathways with endocannabinoid (EC) degrading enzyme inhibitors, EC uptake inhibitors, upregulated cannabinoid receptors (CB-R) in pathophysiological states, cannabimimetic compounds, and other drugs which, when taken concurrently with cannabinoids, enhance the therapeutic properties of cannabinoids [12][13]. Following crystal structure elucidation CB-R was confirmed to be a seven-transmembrane G-protein coupled receptor (GPCR) [14]. The wealth of knowledge relating to GPCR-mediated drugs has been translated to commercial products and is important to consider when developing targeted delivery to CB-R [15]. GPCRs are the largest class of cell surface receptors which make up approximately one-third of currently marketed drugs [16]. GPCRs are involved in signaling pathways that control all essential processes, including vision and carcinogenesis, making them viable options for therapeutic intervention [17]. An understanding of signal transduction pathways following the orthosteric, biased, or allosteric binding of ligands and intracellular modulators to the CB-R-G-Protein complex has been identified as a viable strategy for specific targeting [18]. With the aid of homology and modeling drug concentration at the receptor site and the intensity of a drug effect can be elucidated [19]. However, receptor density on the cell surface may influence the drug-receptor response, signal transmission mechanism into the cell by secondary messengers, or regulatory factors that control gene translation and protein production [20][21][22]. While CB1-R and CB2-R mediate the function of multiple tissues, glands, and organs in the body, their expression varies throughout the body [23]. The location and distribution of CB receptors modulate their physiological effects therefore designing drug delivery systems for bio-specific targeting may be achieved when homology and modeling are used to precisely map the subcellular, cellular, tissue, and regional distribution of cannabinoid receptors. CB1-R is highly expressed in the central nervous system but is only present in the peripheral tissues in lower amounts, whereas CB2-R is expressed primarily in immune cells, with varying levels found throughout the body, including the brain, liver, myocardium, and coronary endothelial and smooth muscle cells [24]. CB2-R has been found in the adrenal glands, myocardium, endothelium, testis, uterus, bone, prostate, gut, the smooth muscle of the vasculature, and in different tumors [25][26][27]. The homology of the two receptors is structurally different, allowing the development of subtype-selective ligands for more specific targeting [28][29].

The strategies used to target sites where the endocannabinoid system is already active by enhancing endogenous cannabinoid tone appear to be more selective with minimal side effects and directly activate cannabinoid receptors [30][31]. However, the rationale for improving receptor-mediated drug delivery using nano precision tools which include nanocarriers properties consideration of factors such as size, shape, elasticity, surface charge, and interaction of materials with the immune system, blood, plasma, cell membranes, and biological barriers have been reported [32][33]. Surface modification by changing ligand surface density and surface patterning can affect cellular internalization and binding affinity [34][35].

2. Endocannabinoid Signaling Pathways

Endocannabinoids play an important role as powerful regulators of synaptic function in the central nervous system, where they regulate neural functions and behaviors [36]. As fundamental modulators of synaptic function, endocannabinoids can regulate neural functions such as cognition, motor control, pain, and feeding behavior [37]. The interaction between endogenous or exogenous ligands and endocannabinoid receptors initiates signaling pathways. Both CB1-R and CB2-R regulate the action of cAMP pathways and MAPK mechanisms [38]. Endocannabinoids are referred to as retrograde messengers because the principal signaling mechanism in the endocannabinoid system is retrograde signaling. It has been shown that the movement of endocannabinoids occurs backward across the synapse after production in the postsynaptic neuron and binding to the presynaptic CB1 receptor, which signals suppression of neurotransmitter release [39]. Evidence suggests endocannabinoid signaling also occurs through non-retrograde TRPV1 and postsynaptic CB1 receptors and/or an astrocytic manner [37]. The neurotransmitters controlled by CB1 receptors are the classic neurotransmitters glutamate, GABA, and glycine, and neuromodulators, including acetylcholine, norepinephrine, dopamine, serotonin, and cholecystokinin [40]. Endocannabinoids also activate the transient receptor potential vanilloid one receptor (TRPV1), which results in the release of a calcitonin-gene-related peptide from perivascular sensory fibers and a vasodilator response in isolated arteries.

2.1. CB1-R

Stimulation of the CB1 receptor transduces stimulation of mitogen-active phosphorylase kinase (MAP) and adenylyl cyclase inhibition, which decreases cyclic AMP production. CB1 receptors are coupled to ion channels through $G_{i/o}$ proteins [41]. They couple positively to inwardly rectify potassium channels and negatively to N-type and P/Q-type calcium channels. An enhanced outward potassium current is created when cAMP-dependent protein kinases are inhibited due to CB1-R activation. The inhibition of calcium channels is believed to decrease neurotransmitter release from CB1-R-expressing presynaptic terminals [42].

2.2. CB2-R

The CB2 receptor inhibits adenylyl cyclase in human lymphocytes and spleen cells in the mouse that expresses CB2 receptors [43]. The action of CB2 receptors in the immune system is to modulate cytokine release. Stimulation of CB2 receptors present on B- and T-cells lead to reduced responses when the immune system is challenged [44].

G-coupled proteins are secondary messengers that convey information to one or more effector proteins following receptor binding [45] and modulate the release of Ca^{2+} from intracellular stores, which then bind to and regulate ion channels. The heterotrimeric G-protein can be coupled to three subunits viz., α, β, and γ, which act independently in downstream signaling systems. When an agonist stimulates the receptor, a conformational change of the receptor allows for interaction with intracellular G-proteins, which results in the dissociation of the subunits from the G-protein. The dissociated dimer subunit $G_i\alpha$ proteins regulate adenylyl cyclase. The inhibition of cyclic AMP production was noted when cell lines expressing CB1 or CB2 receptors were stimulated [46]. The free dimer β_γ mediates the regulation of ion channels, mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-

kinase (PI3K). The two most studied endocannabinoid receptors, CB1-R and CB2-R are separated primarily by differences in their amino acid sequence signaling mechanisms and tissue distribution.

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