Cannabinoids and the Urinary Bladder

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Abstract

The presence of the Endocannabinoid System (ECS) in the urinary bladder has led to speculation that endocannabinoid-signalling is involved in the signal transduction pathways regulating bladder relaxation and may be involved in pathophysiological processes of the bladder. On the basis of this evidence, it was postulated that the binding of endocannabinoids to the cannabinoid receptors (CB1, CB2) may result in relaxation of the urinary bladder during the filling phase. Dysregulation of the ECS in human bladder may be responsible for the aetopathogenesis of Overactive Bladder Syndrome (OAB) and Detrusor Overactivity (DO).

Keywords: Cannabinoids; Endocannabinoids; Cannabinoid receptors; Endocannabinoid system

Introduction

Over the past decade, interest in the role of endocannabinoids in regulating many mammalian processes has increased and has been proposed to be involved in the signal transduction mechanism regulating micturition [1,2]. In a sub-analysis of a multicentre, randomized controlled trial of Cannabis in Multiple Sclerosis (CAMS) the effect of cannabinoids on reducing urge incontinence episodes without affecting voiding in patients with multiple sclerosis and Neurogenic Detrusor Overactivity (NDO) was tested [3]. 630 patients were randomized to receive an oral administration of the cannabis extract, Δ9-Tetrahydrocannabinol (THC) or matched placebo. Based on incontinence diaries there was a 25% reduction (p=0.005) in the cannabis extract group and THC showed a 19% reduction (p=0.039) in urinary incontinence episodes relative to placebo suggesting cannabis may modulate detrusor function [3]. This clinical effect of cannabis is supported by the localization and increased density of suburothelial CB2 receptors in patients with idiopathic detrusor overactivity and painful bladder syndrome compared with controls (p= 0.0123 and p= 0.0013 respectively) [2]. However, there are several possible CB receptor isomers and subtypes and their anatomical distribution, through which the Δ9-THC effect is mediated, remains unknown. Since Δ9-THC acts on the brain, improvement in urgency and urinary incontinence episodes observed in the CAMS study might be attributed to the effects of Δ9-THC at any point in the peripheral nervous system and/or in the micturition centres of the central nervous system.

Historical Review

Cannabis consists of the aerial, seeds and root parts of Cannabis sativa, which is an annual herb indigenous to central and western Asia and is cultivated in other tropical and temperate regions for the fibre used to produce ropes and carpets [4]. There have been more than 60 cannabinoids identified in Cannabis extracts of which the most abundant compound which induces the majority of the psychotropic effects of cannabis, is Δ9-THC [5]. Other constituents include cannabidiol, cannabigerol, cannabichromene and the relative acids [5]. Cannabis has been mentioned in early Hindu and Chinese medicine and its use spread through Persia to Arabia around at the time of the 10th century [6]. The therapeutic effects of cannabinoids were studied in the early 19th Century Irish physician Sir William B. O’Shaughnessy, who demonstrated the potential treatment in a range of disorders including cholera, rheumatic diseases, delirium and infantile convulsions [7]. Historically cannabis has been used in obstetrics and gynaecology for the treatment of menstrual irregularity, dysmenorrhoea, hyperemesis gravidarum, childbirth, postpartum haemorrhage, menopausal symptoms and urinary symptoms [8]. More common therapeutic applications of cannabis include analgesia, migraine, muscle spasms, seizures, attenuation of nausea and vomiting of cancer chemotherapy, anti-rheumatic and antipyretic actions [8,9].

The pharmacological effects of cannabinoids are mediated by two types of G Protein-Coupled Receptors (GPCR) called CB1 and CB2. CB1 was first identified in 1988 and subsequently cloned from rat cerebral cortex in 1990 [10,11]. It is most widely expressed in central nervous system regions involved with pain transmission and is the most abundant GPCR in the brain [12]. The CB1 receptor was cloned from human promyeloicytic leukaemia cells (HL-60 cells) in 1993 and is mainly expressed in immune tissues but is also expressed in low levels in the CNS in both microglia and some neurons [13,14]. The localization of CB1 receptors in immune tissues implies that some cannabinoid-induced immunosupression involves a receptor-mediated process. The cannabinoid receptors are activated by natural ligands with arachidonyl ethanolamine (anandamide) being the first endogenous ligand to be isolated. Anandamide mimics the effects of Δ9-THC by binding to CB receptors, but lacks the psychocactive effects probably because it is highly susceptible to enzymatic hydrolysis [10,15].

The Endocannabinoid System

The Endocannabinoid System (ECS) consists of the cannabinoid receptors, the endogenous ligands for the cannabinoid receptors, the enzymes involved in the synthesis and degradation of these ligands and the transport systems involved in the transfer of these ligands across the cell membrane.

Cannabinoid Receptors

There are currently three known cannabinoid receptors; CB1, CB2, and G protein-coupled receptor 55 (GPR55), which are GPCRs activated by cannabinoid ligands. CB1 is a G protein-coupled receptor expressed mainly in the central nervous system, and plays a major role in pain modulation. CB2 is expressed mainly in immune cells and is involved in immune response regulation. GPR55 is a novel cannabinoid receptor that is expressed in a wide range of tissues, including the brain, gut, and immune system.
by endocannabinoid ligands that are arachidonic acid-derived lipid mediators [16]. There are two principal signal transduction pathways involving the cannabinoid receptors; the Cyclic-adenosine monophosphate (cAMP) signal pathway and the phosphatidylinositol signal pathway, which are mediated by the various subunits of G-proteins [16]. Most GPCRs are capable of activating more than one G-α subtype, but they show a preference for one subtype over another [16]. The effector of both the G_{αs} and G_{αi/o} pathways is the enzyme Adenylyl Cyclase (AC), which catalyzes the conversion of Cytosolic Adenosine triphosphate (ATP) to cAMP [17]. This mechanism is stimulated by G-proteins of the G_{αs} class and conversely, interaction Adenosine triphosphate (ATP) to cAMP [17]. This mechanism is stimulated by G-proteins of the G_{αs} class and conversely, interaction Adenosine triphosphate (ATP) to cAMP [17]. This mechanism is stimulated by G-proteins of the G_{αs} class and conversely, interaction Adenosine triphosphate (ATP) to cAMP [17].

The effector of the G_{αq/11} pathway is phospholipase C-β (PLCβ), to bind and activate enzymes such as the Ca2+/calmodulin-dependant 4,5-biphosphate (PIP2) into the second messengers inositol (1,4,5) trisphosphate (IP3) and Diacylglycerol (DAG)[17]. IP3 acts on IP3 receptors found in the membrane of the Endoplasmic Reticulum (ER) to elicit Ca2+ release from the ER, while DAG diffuses along the plasma membrane where it may activate any membrane localized forms of a second ser/thr kinase called Protein Kinase C (PKC) [17]. Since many isoforms of PKC are also activated by increases in intracellular Ca2+, both these pathways can also converge on each other to signal through the same secondary effector [18]. Elevated [Ca2+], also binds and allosterically activates proteins called calmodulins, which in turn go on to bind and activate enzymes such as the Ca2+/calmodulin-dependant kinases (CAMKs) [19]. Finally, the effectors of the G_{α12/13} pathway are three RhoGEFs (p115-RhoGEF, PDZ-RhoGEF, and LARG), which, when bound to G_{α12/13} allosterically activate the cytosolic small GTPase, Rho [19]. Once bound to GTP, Rho can then go on to activate various proteins responsible for cytoskeleton regulation such as Rho-kinase (ROCK) [19]. Most GPCRs that couple to G_{α12/13} also couple to other sub-classes, often G_{αq/11}.

Endocannabinoids

After the cannabinoid receptors were identified as the molecular targets for Δ9-THC, natural compounds, which bind to these receptors, were discovered. This group of bioactive lipid signalling molecules was collectively referred to as endogenous cannabinoids or endocannabinoids. N-arachidonoyl ethanolamide (anandamide, AEA) was the first endogenous ligand identified for the cannabinoid receptors in 1992, following its isolation from porcine brain [20]. Since then, a number of bioactive lipid signalling molecules with differing affinities for the cannabinoid receptors have been identified. Additional endocannabinoids include, N-docosatetra-7,10,13,16-ethylethanolamine, 2-arachidonoylglycerol (2-AG), 2-arachidonoylglycerol ether (noladin ether), O-arachidonoyl ethanolamine (virodhamine), N-dihomo-γ-linoenoyl ethanolamine, N-docosatetraenoyl ethanolamine, oleamide, N-Arachidonoyl Dopamine (NADA) and N-Oleoyl Dopamine (OLDA) (Figure 1). Potency determinations are complicated by the possibility of differential susceptibility of endogenous ligands to enzymatic conversion.

Biosynthesis and degradation of N-acylthanolamides

AEA synthesis involves a series of enzymatic reactions, the final stage of which involves the enzyme N-arachidonoylphosphatidylethanolamine specific phospholipase D (NAPE-PLD). NAPE-PLD can be stimulated by Ca2+, Mg2+, Cd2+, Mn2+, Ba2+ and Sr2+ and other organic cations [21]. Whilst spermine, spermidine, and putrescine are also stimulatory [21]. Initial characterization of NAPE-PLD revealed the enzyme to be membrane associated and it lacks the ability to catalyze a transphosphatidylation reaction, which is a common feature of known PLDs [22]. NAPE-PLD is the first PLD-type phosphodiesterase which belongs to the metallo-β-lactamase family [23]. Unlike classical neurotransmitters and neuropeptides, its primary product, AEA is not stored in vesicles but synthesized and released “on demand” in response to physiological and pathological stimuli, hormones neurotransmitters and depolarizing agents from its direct biosynthetic precursor N-arachidonoylphosphatidylethanolamine (NAPE) a phospholipid commonly found in biological membranes [24,25]. Figure 2 shows an outline of the major pathways through which anandamide and 2-AG are produced and degraded.

Fatty Acid Amide Hydrolase (FAAH) is the enzyme primarily involved in the hydrolysis of AEA, but can also degrade other endocannabinoids. FAAH was first cloned and purified from rat liver.
The CB₁ receptor is a member of the rhodopsin subfamily of GPCRs [32]. There are three cytosolic loops and a putative fourth loop formed by palmitoylation at the juxtamembrane C-terminal region, which contribute to the activation of the G-proteins [33]. The proximal CB₁ receptor intracellular C-terminal domain is critical for G-protein coupling and the distal C-terminal tail domain modulates signal transduction [33]. Most cannabinoid effects are sensitive to Pertussis Toxin (PTX) implicating a CB₁ and CB₂ receptor coupling to a G protein [34]. The binding of endocannabinoids and cannabinoids to CB₁ and CB₂ results in a decrease of intracellular cAMP levels and activation of mitogen-activated protein kinase through the coupled Gαs proteins [34-36]. Cannabinoid-mediated inhibition of cAMP has been demonstrated in slices of rat hippocampus, striatum, cerebral cortex and cerebellum [36]. CB₂ can also stimulate the formation of cAMP through G, under certain conditions [37]. It may also be that CB₁ receptors can exist as two distinct subpopulations, one coupled to Gαs proteins and the other to Gs [38,39]. The level of cytosolic cAMP may then determine the activity of various ion channels as well as members of the ser/thr specific protein kinase A (PKA) family [32,40,41]. Thus cAMP is considered a second messenger and PKA a secondary effector.

In addition, activation of CB₂ receptor modulates ion channels through Gαs proteins leading to the activation of A-type and inwardly rectifying potassium channels [42-45]. This is due to decreased phosphorylation of the channels, as protein kinase A activity is decreased due to cannabinoid induced inhibition of AC [45]. Thus cannabinoids increase the efflux of potassium. In addition, activation of CB₂ causes a cAMP-independent, but Gαs-dependent inhibition of N-type and P/Q-type calcium channels and activation of inwardly rectifying potassium channel proteins (e.g. GIRK1, GIRK2), leading to a decrease calcium influx and increase in potassium efflux [42-44].

Similarly, CB₁, CB₂ receptors can modulate AC and MAP kinase activity, through their ability to couple to Gαs proteins [46]. The MAP kinase pathway is a key signalling mechanism that regulates many cellular functions such as cell growth, transformation, differentiation, gene expression and apoptosis [47]. Activation of the MAP kinase pathway is associated with the activation of a tyrosine kinase-linked receptor which activates the intracellular G protein Ras and sets up a signaling cascade beginning with the activation of the serine/threonine kinase Raf (MAP kinase kinase kinase) [32]. Raf activates MAP kinase kinase (MEK) leading to phosphorylation and activation of MAP kinase, which can phosphorylate various cytoplasmic and nuclear proteins [32]. CB₂ receptors have been shown to link positively to MAP kinase [48]. However, in contrast to CB₁, CB₂ receptor stimulation is believed not to modulate ion channel function as seen in AoT-20 cells transfected with CB₂ receptors and Xenopus oocytes transfected with CB₁ [49,50]. In addition, unlike CB₁ receptors, CB₂ receptors do not appear to couple to Gαs, suggesting there is a difference between CB₁ and CB₂ receptor signalling [51].

There is evidence that GPR55 is a novel cannabinoid receptor that has a different signalling pathway to that of CB₁ and CB₂ [52,53]. GPR55 is also a rhodopsin-like GPCR, which has been implicated in diverse physiological and pathological processes such as inflammatory and neuropathic pain, bone development and cancer. However, GPR55 shares only low amino acid sequence identity with CB₁ (13.5%) and CB₂ (14.4%) and lacks the typical functional response elicited by these receptors [54]. Activation of the GPR55 receptor coupled to the Gαs, RhoA, actin, phospholipase C pathway triggers the release of Ca²⁺ from IP₃-R-gated stores, which leads to increased intracellular Ca²⁺ [53] (Figure 4). GPR55 can be activated by Lysophosphatidylinositol (LPI), which is an agonist, which can be antagonized by CP55940 and cannabidiol.

**Figure 3:** Chemical structure of cannabinoid ligands.
Cannabinoid Receptor in the Urinary Bladder

Cannabinoid Receptor distribution in the urinary bladder

The effect of cannabis on DO symptoms is probably mediated through a mechanism that depends on endocannabinoids [3]. The mechanism of this effect is far from clear and published data on the expression and functional sites of cannabinoid receptors in the bladder are contradictory. It is thought that endocannabinoids bind to CB₁ and CB₂ receptors, resulting in a relaxation of the detrusor muscle during the filling phase [55,56]. CB₁ receptors are mainly found at the central and peripheral neuron terminals of the bladder, inhibiting neurotransmitter release [55]. Several studies have localized both cannabinoid receptors in the urinary bladder of humans, rats, mice, and monkeys [2,55-60]. In rat detrusor muscle, cannabinor (a CB₂ selective agonist) did not attenuate carbachol-induced contractions in isolated detrusor preparations, suggesting that the action of the CB₂ receptor is not directly involved in post-junctional regulation of smooth muscle contractility [1]. A recent study showed that both pure Cannabidiol (CBD) and Cannabis Sativa extract enriched with CBD also termed as “CBD Botanic Drug Substance” (CBD BDS), which are devoid of psychotropic activity, inhibited human and rat bladder contractility via a postsynaptic site of action [63].

The differences seen between the results of these studies may be due to inter-species differences in cannabinoid receptor expression and distribution. The effect of these receptors on the release of contractile transmitters and anatomical variations in bladder innervation. Inter-species differences in the neuroanatomy of the mammalian bladders are known to exist [62]. For example there are several parasympathetic ganglia in isolated bladder tissue from guinea pigs and humans while there are none in the urinary bladders of mice and rats [64,65].

Cannabinoid Receptor function in the urinary bladder

Studies have demonstrated that the activation of presynaptic CB₁ and CB₂ receptors inhibit electrically evoked contractions in isolated mammalian tissue when using THC and the non-selective CB receptor agonists CP55940, CP55244, JWH015, which corresponds to the localization of CB₁ receptors in nerve fibres of the suburothelium and in human and rat detrusor muscle [2,58,60]. However, another study did not detect the CB₂ receptor in rat urothelium or nerve fibres but reported immunoreactivity for CB₁, in these structures and in ganglion cells of the outflow region [1,55]. In addition, human bladder studies identifying the presence of gene transcripts by quantitative Polymerase Chain Reaction (qPCR) and tissue expression and localization by Immunohistochemistry (IHC), revealed a higher abundance of the CB₁ receptor in the urothelium compared to the detrusor [57]. Similar results were found for CB₂, but overall, receptor protein expression was much lower when compared to CB₁ receptor protein expression [57].

In contrast, another study found an attenuation of EFS evoked human detrusor contraction in the presence of both CB₁ (ACEA) and CB₂ (GP1a) agonists [57]. These findings suggest cannabinoids act on pre-junctional nerve endings attenuating contractile responses. These data, however, must be interpreted with caution because quantification of the effect by GP1a or vehicle (dimethyl-sulfoxide) control experiments were not presented [57]. Supporting that cannabinoids act on pre-junctional nerve endings to attenuate a contractile response, Gratze et al. demonstrated co-localization of vesicular acetylcholine transporter protein (VACHT) nerve structures and CB2 immunoreactive terminal varicosities. They also showed inhibitory effects of CP55, 940 on nerve mediated contractions but not on carbachol induced contractions in detrusor preparations, suggesting a modulatory function of CB₂ on cholinergic neurotransmission [55]. Similarly, cannabinor (a CB₂ agonist) did not attenuate carbachol-induced contractions in isolated rat detrusor tissue, suggesting that the action of the CB₂ receptor is not directly involved in post-junctional regulation of smooth muscle contractility [1]. A recent study showed that both pure Cannabidiol (CBD) and Cannabis Sativa extract enriched with CBD also termed as “CBD Botanic Drug Substance” (CBD BDS), which are devoid of psychotropic activity, inhibited human and rat bladder contractility via a postsynaptic site of action [63].

Cannabinoid receptors as therapeutic targets

The most studied cannabinoid compound is Cannabidiol (CBD)
which exerts a number of pharmacologic effects such as analgesic, anti-inflammatory, antioxidant, and anti-tumoral [70]. It has been clinically evaluated for the treatment of anxiety, psychosis, and movement disorders and has been found to have a safe clinical profile [70]. CBD is the main component of Sativex, which also contains Δ9-THC, a cannabis-derived drug used for the treatment of pain and spasticity associated with multiple sclerosis. Sativex is licensed for this indication in patients with multiple sclerosis. In a clinical survey, administration of Δ9-THC improved nocturia and detrusor overactivity in patients with multiple sclerosis [71]. To date, a small number of open-label and placebo-controlled studies have demonstrated that oral administration of cannabinoids may alleviate OAB/DO symptoms as first line. Most of these studies have been carried out on patients with advanced multiple sclerosis using preparations containing Δ9-THC and/or CBD. One such example is a recent study involving a group of severe incontinent patients with multiple sclerosis [72,73]. Other cannabinoid receptor agonists are already used clinically to suppress nausea and vomiting provoked by anticancer drugs (nabilone) or to boost the appetite of AIDS patients but these have not been studied for their effects upon urinary symptoms [9].

However, the oral use of cannabinoids may induce undesirable CNS effects including hypoactivity, hypothermia and catalepsy, but may in turn improve OAB symptoms, which are known to be afferently mediated [3,74]. What remains unclear is whether the latter beneficial effects are centrally mediated or whether a local bladder component acting on the afferent bladder pathway, plays a significant role. There are no human data that exists which can answer this question. Data from animal studies support a local effect on bladder afferents where cannabinoid administration systemically and intravesically, improved parameters associated with OAB and DO [55,75].

In addition to using an intravesical route of administration for cannabinoid drugs in order to bypass the CNS effects associated with activation of CB1, the use of CB2 agonists and FAAH inhibitors is being explored and appears promising [76,77]. There is emerging evidence that activation of CB2 inhibits tissue inflammation and has analgesic properties [78-80]. The CB2 subtype is mainly expressed outside the CNS, as described earlier, so it can act as a potential endocannabinoid target where analgesic effects may be separated from psychotropic effects by activating the peripheral receptors. In addition, pharmacological targeting of the homeostasis of endogenous cannabinoids by manipulating the degradation enzymes, may also offer the possibility of avoiding the CNS side effects of exogenous cannabinoids. FAAH, an enzyme that specifically degrades anandamide has been localised in the urinary bladder [56,77,81]. Inhibition of FAAH activity with FAAH inhibitor OLEAyl Ethyl Amide (OEtA), significantly increased inter-contraction intervals, micturition volume, bladder capacity and threshold pressure urodynamic parameters in rats which reflect sensory functions of micturition. These effects were prevented by a selective CB2 antagonist. Similarly, another FAAH inhibitor, URB597 has been found to have a functional role in the colon, where FAAH has been localized by reducing inflammation [77,82,83]. The use of a FAAH inhibitor needs to be explored further in the urinary bladder because it may be the way forward in treating OAB symptoms. However, the complexity of the endocannabinoid system at the tissue level may mean that we are still a long way from obtaining a clinically useful compound for treatment.

The Future

Modulation of the endocannabinoid system is currently being investigated for a wide range of potential therapeutic applications including smoking cessation, treatment of obesity, epilepsy and other CNS related conditions. Similarly, the presence of the endocannabinoid system in the urinary bladder has led to speculation that endocannabinoid-signalling is involved in the signal transduction pathways regulating bladder relaxation and may be involved in pathophysiological processes of the bladder. This role of the endocannabinoids in the lower urinary tract supports their therapeutic potential in conditions of OAB and DO, whereas evidence already exists for their role in bladder inflammation [2,59,75,84]. There are still a number of unanswered questions in the understanding of cannabinoid pharmacology in the urinary bladder. Clearly, further research is required to investigate the role of cannabinoid receptors and their exogenous modulators on bladder control prior to embarking on a clinical trial involving cannabinoids and healthy volunteers with OAB. The inhibitory effects of CB2 and the effect of FAAH inhibitors on lower urinary tract control should be the focus of future studies.


