Functional role of cannabinoid receptors in urinary bladder

Pradeep Tyagi, Vikas Tyagi, Naoki Yoshimura¹, Michael Chancellor
Departments of Urology, William Beaumont Hospital, MI 48073 and ¹University of Pittsburgh, PA 15213, USA

ABSTRACT
Cannabinoids, the active components of Cannabis sativa (marijuana), and their derivatives produce a wide spectrum of central and peripheral effects, some of which may have clinical applications. The discovery of specific cannabinoid receptors and a family of endogenous ligands of those receptors has attracted much attention to the general cannabinoid pharmacology. In recent years, studies on the functional role of cannabinoid receptors in bladder have been motivated by the therapeutic effects of cannabinoids on voiding dysfunction in multiple sclerosis patients. In this review, we shall summarize the literature on the expression of cannabinoid receptors in urinary bladder and the peripheral influence of locally and systemically administered cannabinoids in the bladder. The ongoing search for cannabinoid-based therapeutic strategies devoid of psychotropic effects can be complemented with local delivery into bladder by the intravesical route. A greater understanding of the role of the peripheral CB₁ and CB₂ receptor system in lower urinary tract is necessary to allow the development of new treatment for pelvic disorders.

Key words: Bladder, cannabinoids, irritation, protein-coupled receptor, receptor expression

DOI: 10.4103/0970-1591.60440

PHARMACOLOGY OF PHYTOCANNABINOIDS GUIDING RECEPTOR DISCOVERY
For many centuries, phytocannabinoids obtained from cannabis plant (marijuana) have been consumed for their analgesic, anxiolytic, antiemetic and antispasmodic properties especially in the oriental culture.[1] However, therapeutic utility of cannabis plant or its products in the evidence based medicine continues to remain a lightning rod for controversy on social, legal and medical fronts. Pharmacological and chemical investigation on cannabis plant found more than 50 compounds, of which the main psychoactive principal was identified as ∆²-tetrahydrocannabinol, (∆²-THC) [Figure 1] apart from two other bioactive cannabinoids, cannabidiol (CBD) and cannabiol (CBN).[2] Further studies dealing with the search of biological targets for the (-) ∆⁹-THC led to the cloning and identification of two CB₁ and CB₂ receptors belonging to the heptahelical G protein-coupled receptor (GPCR) superfamily.[3] The receptor CB₁ is the most abundant of all receptor types in the brain and other CNS regions involved with pain transmission and modulation, specifically in the spinal dorsal horn and periaqueductal gray.[4,5] CB₂ receptors are also located peripherally in both neuron and non-neuronal tissue, while CB₂ receptors are mainly found in immune cells and brain glial cells.[4,6] These receptors have been found to have many physiological and patho-physiological functions, including mood alteration, control of feeding and appetite, motor and co-ordination activities, analgesia, immune modulation and gut motility.[1]

Given the ubiquitous expression of CB₁ and CB₂ receptors, cannabinoids have been shown to produce wide spectrum of effects including induction of proliferation, growth
arrest, or apoptosis in a number of cells, including neurons, lymphocytes, and various neural and non neural cells. Alterations in the reproductive system produced by cannabis motivated the studies leading up to the discovery of CB₁ receptor expression in reproductive tract. CB₂ receptors have been detected in the testis, prostate and vas deferens. In addition, expression of functional CB₂ receptors on sperm and presence of the archetypal endocannabinoid anandamide in reproductive secretions have also been detected. It can be said therefore that discovery of cannabinoid receptors in bladder lagged behind the discovery of these metabotropic receptors in other organs lining the genitourinary tract.

CB₁ and CB₂ receptors and signaling
Cannabinoids elicit their well known diverse effects by activating numerous signaling pathways. CB₁ and CB₂ receptors exhibit 48% amino acid sequence identity and both of them are negatively coupled to adenylyl cyclase to inhibit cyclic AMP and mitogen-activated protein kinase [Figure 2]. In addition, CB₂ receptors couple via pertussis toxin-sensitive G₉/o proteins to inhibit L-, N-, and P/Q-type calcium channels and activate potassium channels. The endogenous ligands for these receptors are called as endocannabinoids (ECB), which are namely anandamide, 2-arachidonoylglycerol, virodhamine, and noladin ether (2-arachidonoylglycerol ether).

These ECBs structurally resemble eicosanoids as they are derived from arachidonic acid; a polyunsaturated fatty acid that serves as precursor for a plethora of other bioactive metabolites such as prostaglandins, thromboxanes, leukotrienes etc. In animal studies, the pharmacological action of Δ⁸-THC was mimicked by endocannabinoids. Most of the ECBs derived from arachidonic acid act in a receptor-dependent manner and have the ability to act as retrograde inhibitors of synaptic neuromodulation in GABAergic and glutamatergic synapses, as well as modulators of post-synaptic transmission, involving norepinephrine and dopamine. These ECBs are transported into cells by a specific uptake system and degraded by two well-characterized enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase. The ECBs are synthesized on demand and have short-lived effects due to effective metabolic pathways.

Expression of cannabinoid receptors in bladder
The results of a recently completed large randomized, controlled, multicenter clinical trial known as the cannabinoids in multiple sclerosis study (CAMS) sparked the interest in studying the expression of cannabinoid receptors in resident bladder tissue of urothelium and detrusor. The multi-center CAMS study randomized 630 patients to receive either oral administration of cannabis extract, Δ⁸-THC or a matching placebo. Patients completed incontinence diaries throughout the study. Significant reduction in urge incontinence episodes and improvement in bladder control from baseline were noted at the end of the study with the use of cannabis extract (38%) or Δ⁹-THC (33% reduction). The small increase in efficacy of cannabis extract over pure Δ⁸-THC seems to suggest that ingredients other than Δ⁸-THC in cannabis extract such as CBD and CBN may antagonize some of the undesirable effects of Δ⁸-THC and contribute positively to bladder symptoms. The placebo arm of the trial only showed 18% decrease in incontinent episodes relative to baseline to further suggest a distinct clinical effect of cannabinoids on bladder symptoms.

Cannabinoid receptors in rodent bladder
In earlier studies, the presence of CB₂ receptors has been indirectly demonstrated in the rodent bladder using specific CB₂ agonist and antagonists. Results from an isolated bladder strip study suggested that these receptors are located in the prejunctional neuron. Further, systemic administration of CB₂ agonist and antagonists in spinal cord...
injured rats with detrusor overactivity demonstrated the role of a tonically active ECB system in pathological voiding.\cite{24}

Previously, the expression of muscarinic, neurokinin and beta 3 adrenoceptors in bladder have been successfully demonstrated using molecular and pharmacological techniques.\cite{25} Literature accounts on the expression of CB1 and CB2 receptors in different organs have relied on different approaches, such as autoradiography, in situ hybridization of receptor messenger RNA\cite{26} or functional assays.\cite{27} A recent study determined the localization of CB1 and CB2 receptors in rat bladder by immunohistochemistry and a functional assay.\cite{28}

Hayn et al., positively identified the expression of CB1 receptors in rat bladder by the immunoreactivity of CB1 bladder comparable to that in cerebellum. Similarly, the presence of immunoreactivity for CB2 in spleen was used as positive control for the positive localization of CB2 receptors in bladder by the same antibody.\cite{28} The known ability of peripheral cannabinoid receptors to modulate afferent transmission by modulating the stimulus-evoked neuropeptide release was used to design a functional assay.\cite{29} Studies show that quantification of released calcitonin gene related peptide (CGRP) can be a suitable marker for measuring afferent neuronal activity in rat bladder.\cite{30} The bladder of adult female rats receives approximately 16,000 axons (i.e., is the target of that many ganglion neurons) of which at least half are sensory.\cite{31} Virtually all bladder sensory fibers that originate from dorsal root ganglia are immunoreactive for capsaicin receptor transient receptor potential vanilloid (TRPV1) and CGRP.\cite{32} The role of TRPV1 in voiding has been well established\cite{33} but the potential role of CB1 and CB2 receptors in micturition and pain originating from bladder is yet to be completely investigated.

The presumed expression of CB receptors on capsaicin-sensitive sensory nerves,\cite{34} being coupled to inhibition of neurotransmitter release, was demonstrated in an isolated rat bladder model. Application of the mixed CB1/CB2 receptor agonist, ajulemic acid (AJA) inhibited the evoked release of CGRP from afferent nerve terminals in isolated rat bladder. Sensory afferent axons in the bladder are the only structures in the bladder that contain high levels of CGRP released upon nerve depolarization or chemical stimulation by capsaicin [Figure 3].\cite{35} Pharmacological specificity of the inhibitory effect of AJA on sensory neuronal activity originating from the bladder through CB1 and CB2 receptors was demonstrated using selective receptor antagonists.

### Cannabinoid receptors in human and primate bladder

Encouraged by expression in rat bladder, the expression of CB1/CB2 receptors in bladder obtained from human cadavers was recently investigated using different techniques.\cite{36} Expression of functional CB1 and CB2 receptors in human detrusor and urothelium was demonstrated using real-time quantitative polymerase chain reaction QPCR and protein expression using immunohistochemistry and Western blot. QPCR was done using customized CB1 and CB2 primers which amplified gene products from the open reading frame of single exon human CB1 and CB2 genes [Figure 4]. Expression of CB1 and CB2 receptors was demonstrated in the detrusor and urothelium, with the expression for both receptors approximately two fold higher in the urothelium than in the detrusor (P < 0.05). The mRNA expression of the CB1 receptor was significantly higher than that of the CB2 receptor in both tissue types (P < 0.05). Immunofluorescence results show that expression of CB1 and CB2 is specific to bladder and not contributed by infiltrating cells.

**Figure 3:** Proposed mechanism of locally administered cannabinoid agonist in irritated bladder. Bladder irritated by acetic acid, in the animal model, activates TRPV1 on urothelium and adjoining nerves to release CGRP. Irritation evoked release of CGRP is blocked by mixed CB1/CB2 agonist ajulemic acid entrapped into liposome that activates CB1 and CB2 receptors on bladder surface and nerves.

**Figure 4:** Amplification plot from a typical real-time PCR experiment to detect expression of CB receptors in human urothelium relative to housekeeping gene \(\beta\) actin. The urothelium specimens were obtained from organ donors. M = 9000P instrument measures the fluorescence of dsDNA intercalating dye SYBR Green twice in each PCR cycle. This inflection point is called the cycle number (Ct) at which fluorescent signal generated passes over threshold baseline. The Ct value was determined for each specimen to measure receptor expression.
Expression of CB1 and CB2 receptors detected at the mRNA level by QPCR was confirmed at the protein level by immunoreactivity and Western blot analysis. Activation of CB1 and CB2 receptors attenuated the electrically evoked contraction of detrusor strips. These inhibitory effects of cannabinoid receptor agonists were suggested to be attributable to prejunctional CB receptors that decrease contractile transmitter release. It is well accepted that endogenous cannabinoids and CB2 receptors are involved in the regulation of smooth muscle contractility, through a mechanism mainly related to reduction of acetylcholine release from cholinergic nerve endings. The modulatory action of the cannabinoid agents on the non-adrenergic non-cholinergic neurotransmission operating in the bladder is yet to be studied.

Using similar techniques of Western blot and immunohistochemistry, similar results on expression of CB1/CB2 receptors in human bladder were also reported by Gratzke et al., 2009. Apart from humans, this group also investigated the distribution of CB1 and CB2 receptors in the rat and monkey species. Higher expression of CB2 receptor, but not CB1, was noted in the urothelium relative to detrusor. Expression of CB2 receptors in urothelium was localized to the sensory and cholinergic nerves in the bladder obtained from humans as well as other species of rats and rhesus monkeys. Co-localization of CB2 receptor antibody stain with the stain for CGRP, TRPV1, and vesicular acetylcholine transporter (VACHT) protein specific for cholinergic nerves further confirmed the expression of CB2 receptor by bladder afferents. Nerve fibers containing CB2 and VACHT were also located in the detrusor. The co-expression of VACHT and CB2 receptor, and effects by CP55940 on nerve mediated contractions suggest a CB2 receptor mediated modulatory effect on cholinergic nerve activity in bladder.

The localization of CB2 receptor with nerves argues for a role of CB2 in bladder afferent signals which can be best demonstrated by cystometric studies. These in vivo effects of CP55940 (CB1/CB2 receptor agonist) on urodynamic parameters. MI and TP may be considered parameters that indirectly represent sensory functions during cystometry. Lack of a direct effect of CB1/CB2 agonist CP55,940 and the nonselective agonist anandamide on carbachol induced contractions indicated the absence of direct CB mediated functions of isolated detrusor smooth muscle.

Recent studies demonstrated the modulatory effects of CB1/CB2 agonists on nerve induced contractions in detrusor preparations obtained from most mammalian species. In contrast, according to a previous report CB1/CB2 agonists fail to show any effect on the electrically evoked contractions of bladder preparations isolated from dogs, pigs, cynomolgus monkeys and humans. However, the same report was able to reproduce the inhibition of neuronally evoked contractions in isolated rat and mouse bladders by CB1/CB2 agonists. Definitive characterization of CB receptor in tissues depends on the availability of selective agonists and antagonists and difference in the selectivity of agents used in different studies may explain the different results. In addition, there may be differences in the age and associated pathology of organ donors who donated the bladder for the muscle strip studies. Previously published studies in the spinal cord injured rat revealed that peripheral CB receptors are involved in detrusor overactivity. The inhibitory effect of CB agonists on detrusor pressure observed by Blyweert et al., further corroborates the inhibitory effects of CB agonists on isolated human detrusor strips in organ bath.

**Unique pharmacology of anandamide in bladder**

One of the interesting aspects of study reported by Gratzke et al., 2009, is the difference in the pharmacology of a synthetic CB1/CB2 receptor agonist and ECBs such as anandamide. The difference was best illustrated by anandamide led 26% increase in the TP and 19% decrease of MI in cystometric studies relative to increase of 124% (TP) and 46% (MI) by CP55940 ($P < 0.05$ and $< 0.01$, respectively). Furthermore, nerve mediated contractions were enhanced by anandamide and decreased by CP55940 ($P < 0.05$).

The unique pharmacology of anandamide have been explained in the past by its ability to elicit effects by acting on both via G protein-coupled CB1 receptors and transient receptor potential (TRP) channels (chiefly TRPV1 receptors). The pre-synaptic inhibitory effect of anandamide is evident at low concentration and at higher concentrations the activation of TRPV1 counters the effect on CB1/CB2 receptors. The activation of TRPV1 channels can lead to desensitization and loss of pre-synaptic inhibition observed in various studies.

Anandamide has also been noted to aggravate cyclophosphamide induced cystitis in rodents through its activation of TRPV1 ion channels and thereby causing detrusor overactivity and hyperalgesia. In contrast, anandamide have been also shown to mediate attenuation of detrusor overactivity induced by nerve growth factor instillation in bladder. The attenuation of detrusor overactivity by anandamide unmasked the role of CB receptors expressed in bladder in controlling the pain initiated locally in bladder. Concentration and time of exposure may be critical in determining which of these opposite effects of anandamide ultimately prevails.

Most TRPV1 receptor-expressing cells are also known to co-express the CB1 receptors as well. The close proximity of CB1 and TRPV1 may facilitate the dual, concentration-dependent effect of anandamide observed in different studies. Dual dose dependent effect of cannabinoids have also been previously noted in relation to immune system where...
low doses of cannabinoids may enhance cell proliferation and high doses of cannabinoids may induce growth arrest or apoptosis. The ability of same ligands to activate both metabotropic CB₁/CB₂ receptors and ionotropic TRPV1 receptors suggest possible interactions between the two signaling systems. Stimulation of CB₁ and desensitization of TRPV1 could be a strategy to protect against inflammation in bladder.

To further confound the pharmacology of anandamide in bladder, the responses to anandamide as reported by Gratzke et al., (2009) were attenuated but not abolished after desensitization by capsaicin. Further, anandamide response were partially attenuated by an prostaglandin receptor EP1 antagonist and almost abolished by indomethacin, a cyclooxygenase inhibitor. Neither the CB₁ antagonist AM251 nor the CB₂ antagonist AM630 had any effect on the response to anandamide, to suggest possible role of EP1 receptor.

Cannabinoid receptors fueling drug discovery

The distribution of these CB₁/CB₂ receptors at key sites involved in nociceptive processing is instrumental in the analgesic effects of phytocannabinoids (plant source) or synthetic cannabinoids developed in last 30 years. Synthetic cannabinoids are chemicals having action similar to cannabis on their cognate receptors. Studies have shown that synthetic and semi-synthetic cannabinoids that lack psychotropic effects are effective against severe pain states refractory to even opioids. The activation of nociceptive sensory neurons leads to nociception. However, CB₁/CB₂ agonists are capable of altering nociceptor activity without producing nociceptive behavior. CB₁/CB₂ agonists have been able to suppress the nociceptive transmission and inhibit pain-related behavior in animal models of acute and persistent nociception by their activity at spinal, supraspinal and peripheral sites.

The new drugs based on pharmacology of cannabinoids can be classified into two categories: Direct and indirect agonists. Direct agonists selectively activate either CB₁ or CB₂ receptor. CB₁ receptor agonists are not associated with the adverse side-effects of CB₁-selective agonists and therefore may provide an alternative analgesic target. Indirect agonists work on the principle that metabolic degradation is the rate-limiting step in the therapeutic effects of ECB and the efficacy can be magnified by blocking the ECB metabolism either through cellular reuptake or enzymatic hydrolysis. Such compounds can theoretically act selectively on tissues with ongoing synthesis and degradation of ECB, thus producing fewer unwanted effects than direct agonists. By acting through up-regulation of ECB, another advantage of indirect agonists is that they may produce beneficial actions through actions on other receptors as well such as CB₁, CB₂ or TRPV1 receptors.

The drug discovery of synthetic cannabinoids is also fuelled by the notorious toxicity of cannabis or phytocannabinoids. Some synthetic cannabinoids with limited side effects and abuse liability have already been approved for clinical use in Canada. Nabilone and dronabinol are classical synthetic cannabinoids, with chemical structure based on Δ⁹-THC, approved for treating severe nausea and vomiting associated with cancer chemotherapy has motivated the off-label use of nabilone and dronabinol in chronic pain management in a few clinical trials, case reports or case series. To avoid the possible risk of abuse of these drugs by patients, experts in the field have put guidelines for clinical use of these agents.

These peripherally acting agents could evoke profound pain relief in animal models as well as in a few small clinical studies, but the underlying mechanism and signaling pathways mediating these effects are yet to be completely understood. A major challenge facing the biomedical research community is the identification of compounds that are safe and effective in treating pain, particularly chronic pain such as painful bladder syndrome (PBS) or interstitial cystitis (IC). Various methods, medicines, and devices are available to IC/PBS patients to reduce their pain and symptoms but many of these conventional therapies have significant limitations. Based on the known effects of cannabinoids, in preclinical and clinical studies, it can expected that peripherally acting cannabinoid receptor agonists can modulate bladder sensory pathways by acting on nociceptive pathways originating from bladder.

Route of administration affects bioavailability and toxicity of cannabinoids

As described in earlier sections here, experimental factors such as drug concentration, timing of drug delivery and location of drug administration can influence the therapeutic and adverse response of cannabinoids. The acute adverse effects reported with the consumption of cannabis by smoking or by oral route includes increased food intake, tachycardia, orthostatic hypotension, pulmonary irritation, impaired motor coordination, cognitive impairment, anxiety, paranoia, and psychosis. In addition, the bioavailability of CBs from the oral route is uncertain as illustrated by unpredictable pharmacokinetics of (Δ⁹-THC) after oral administration. This has generated a lot of interest in alternative routes for delivering cannabinoids. It was recently shown that topical application of 30 μg of Δ⁹-THC reduced allergic inflammation in mouse ear model of allergic dermatitis. The activation of cutaneous CBs lead to attenuation of nociceptor excitation, pain and itch perception, and decreased the release of neuropeptides, particularly CGRP, from terminal afferents.

It is definitely more logical to develop local CB delivery
with predictable bioavailability that rules out central side effects. Route of inhalation is also a distinct possibility for the therapeutic delivery of cannabinoids, as shown by the recent approval of an oromucosal (sublingual) spray standardized for the Δ⁶-THC and CBD (1:1 ratio) in Canada, as adjunctive treatment for neuropathic pain of multiple sclerosis patients. The rationale for the combination of CBD and treatment for neuropathic pain of multiple sclerosis bladder, selective CB₁/CB₂ agonist AJA was loaded inside Guided by the results of CGRP experiments in isolated bladder, selective CB₁/CB₂ agonist AJA was loaded inside the liposomes and instilled into bladder to unmask the role of peripheral cannabinoid receptors in bladder. AJA is a synthetic derivative of nabilone that is currently approved in Canada for chemotherapy induced emesis. AJA has been efficacious in animal models of chronic pain by activation of the CB₂ receptor with a superior therapeutic index compared to other CB compounds. AJA binds to human CB₁/CB₂ receptors in vitro, with high affinity at human CB₁ (Ki 6nM) as well as h CB₂ (Ki 56 nM) receptors. In a previous study, by our group, on systemic administration of AJA, the role of CB₁/CB₂ receptors in micturition was more evident in the irritated condition of bladder than in normal condition. Female rats were pretreated with AJA (0.5 ml of liposomal for 30 min) prior to irritation with acetic acid [Figure 5]. First, baseline cystometric parameters were derived through transurethral open cystometry (CMG) under urethane anesthesia (dose 1.0 g/kg body weight), with saline infusion at the rate of 0.04 ml/min. Bladder irritation was induced by infusing acetic acid (0.125% v/v) in saline into the bladder. As shown in the CMG tracing of Figure 5, infusion of acetic acid in bladder reduces the micturition interval MI of rats instilled with saline previously, because acetic acid irritates the afferents in the bladder to induce hyperexcitability. As revealed by cystometric parameters, local activation of cannabinoid system in bladder by intravesical administration can blunt the pain initiated in the bladder by acid infusion. The therapeutic effect of instilled drugs was assessed by the per cent reduction in MI after infusions of acetic acid. Cystometric data supported the hypothesis that instilled CB₁/CB₂ agonist can buffer the pain signals emerging from bladder following acetic acid infusion. Local action of CB₁ agonist in the bladder may involve action at CB₁ receptors.

**Local administration of cannabinoids inside bladder**

Studies done on other tissues such as ear and paw have already shown that locally administered CB agonists act on peripheral receptors and attenuate the pain behavior induced by localized tissue damage or irritation. Local administration of anandamide via intraplantar injection suppressed neuropathic pain in rats. These observations lend support to the concept that CB receptors in the periphery participate in the intrinsic control of pain initiation and locally generated endocannabinoid such as anandamide and may mediate this effect. Recent studies indicate a possible antinociceptive synergy from cannabinoid action on peripheral receptors with that on spinal sites.

Intravesical drug delivery offers an attractive opportunity to focus the potency of potentially toxic drugs only on site of action as demonstrated by clinical management of PBS/IC with intravesical DMSO. In recent years, considerable effort has been expended into developing formulation suitable for intravesical administration of antimuscarinics, capsaicin, resiniferatoxin and local anesthetics. The success of delivering antimuscarinics, capsaicin and botulinum toxin into the bladder encouraged intravesical delivery of cannabinoids using liposomes. Phyto cannabinoids and synthetic cannabinoids are generally not soluble in water and formulation of these agents into liposomes can overcome the aqueous solubility of these agents. Guided by the results of CGRP experiments in isolated bladder, selective CB₁/CB₂ agonist AJA was loaded inside the liposomes and instilled into bladder to unmask the role of peripheral cannabinoid receptors in bladder. AJA is a synthetic derivative of nabilone that is currently approved...
The diverse effects of CB₁ and CB₂ receptor system in lower urinary tract may be novel targets for therapies designed to treat diseases afflicting lower urinary tract. The growth-inhibiting action of cannabinoids acting on these receptors expressed on transformed cells might be useful for managing pain associated with IC/PBS.

**Knockout mice of cannabinoids receptors**

It can be a challenge to determine the *in vivo* mechanism of CB agonists administered systemically or intravesically, given the ability of CB ligands to activate other receptors, namely TRPV1, at varying concentrations. This can also explain the difficulty faced by researchers engaged in investigating the role of CB₁ and CB₂ receptors using pharmacological antagonists and agonists. For example, SR-141716, a CB₁ receptor antagonist, can also show agonist property because of its other effects. Therefore, the use of a genetic approach has gained favor among scientists to complement the pharmacological analysis of the cannabinoid system, and mice with targeted deletions in the cannabinoid receptor genes have been generated to study role of of the endocannabinoid system in addiction research. The pharmacological specificity of CB₁/CB₂ agents administered systemically or intravesically can be easily determined by comparing results in knockout mice with wild type littermates. The development of transgenic CB₁⁻/⁻ and CB₂⁻/⁻ receptor knockout mice using homologous recombination has opened up the opportunity to study the role of the CB₁ and CB₂ receptor system in lower urinary tract. The concerns of global deficit of CB₁ and CB₂ receptors, in the survival of these mice, can be ameliorated using time-dependent and bladder specific deletion of CB₁ and CB₂ receptors.

**Take home message and important points**

1. Expression of CB₁ and CB₂ receptor in lower urinary tract is relevant to effects of cannabinoids on voiding dysfunction.
2. Expression of CB₁ and CB₂ receptor in bladder demonstrated by molecular, immunofluorescence, detrusor strip contraction and cystometric studies.
3. Pharmacology of ECBs is complex due to their ability to act on multiple receptors.
4. Route of administration have a drastic influence on therapeutic index of cannabinoids.

**CONCLUSIONS**

The diverse effects of CB₁ and CB₂ receptor system in lower urinary tract may be novel targets for therapies designed to treat diseases afflicting lower urinary tract. The growth-inhibiting action of cannabinoids acting on these receptors expressed on transformed cells might be useful for managing pain associated with IC/PBS.
for the management of malignancy in bladder. Recently published pre-clinical studies have demonstrated that cannabinoids appear to act principally as prejunctional modulators of neurotransmission to affect the micturition process indirectly by affecting the nociceptive responses pathways. It is likely that CB1 and CB2 receptors located in periphery such as in bladder participate in the intrinsic control of initiation of afferent stimulus. Emerging studies show that ECBs are mediators of spinal activity-dependent pain sensitization to create a future role for pharmacological antagonists CB1 and CB2 receptors in control of neuropathic pain.

Historically, products from cannabis or its synthetic analogues have faced many obstacles in getting investment from the pharmaceutical industry and acceptance from regulatory agencies. Currently, opioids are the most effective prescription-based analgesics for the painful symptom emanating from the lower urinary tract with limited efficacy and serious toxicity, such as tolerance development, physical dependence, sedation, respiratory depression and gastrointestinal symptoms. The assurance of safety, from ongoing search, of cannabinoid-based therapeutic strategies devoid of psychotropic effects can be complemented with the development of new treatments.

REFERENCES

1. Zuardi AW. History of cannabis as a medicine: A review. Rev Bras Psiquiatr 2006;28:153-7.
2. Khiabani HZ, Bramness JG, Bjorneboe A, Morland J. Relationship between THC concentration in blood and impairment in apprehended drivers. Traffic Inj Prev 2006;7:111-6.
3. Felder CC, Dickson-Chesterfield AK, Moore SA. Cannabinoids biology: The search for new therapeutic targets. Mol Interv 2006;6:149-61.
4. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors 10.1124/pr.54.2.161. Pharmacol Rev 2002;54:161-202.
5. Avelino A, Cruz F. TRPV1 (vanilloid receptor) in the urinary tract: Functional and immunohistochemical characterization of cannabinoid receptors CB1 and CB2 in the urinary bladder. Eur J Pharmacol 2002;456:99-106.
6. Tyagi, et al.: Cannabinoid receptors in urinary bladder
Tyagi, et al.: Cannabinoid receptors in urinary bladder

Expression, function and clinical applications. Naunyn Schmiedebergs Arch Pharmacol 2006;373:287-99.

Kalsi V, Fowler CJ. Therapy Insight: Bladder dysfunction associated with multiple sclerosis. Nat Clin Pract Urol 2005;2:492-501.

Ghasemi M, Sadeghipour H, Shafaroodi H, Nezami BG, Gholipour T, et al. The anti-hyperalgesic actions of cannabinoids in the rat, monkey and human bladder. J Urol 2009;181:1939-48.

Su HC, Wharton J, Polak JM, Muldery PK, Ghati MA, Gibson SJ, et al. Calcitonin gene-related peptide immunoreactivity inafferent neurones supplying the urinary tract: Combined retrograde tracing and immunohistochemistry. Neuroscience 1986;18:1727-47.

Tyagi V, Philips BJ, Su R, Smaldone MC, Erickson VL, Chancellor MB, et al. Differential expression of functional cannabinoid receptors in human bladder detrusor and urothelium. J Urol 2009;181:1932-8.

Ghasemi M, Sadeghipour H, Shafraroodi H, Nezami BG, Gholipour T, Hajarsooliha AR, et al. Role of the nitric oxide pathway and the endocannabinoid system in neurogenic relaxation of corpus cavernosum in the eel. J Urol 2007;178:2153-7.

Calignano A, Grimaldi A, Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277-81.

Morisset V, Ahluwalia J, Urban L. Possible mechanisms of cannabinoid-induced antinociception in the spinal cord. Eur J Pharmacol 2001;429:93-100.

Costa B, Siniscalco D, Trovato AE, Comelli F, Sotgiu ML, Colleoni M, et al. Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurones in cystitis. J Neurosci 2004;24:11253-63.

Jaggar SL, Hassie F3, Sellaruty S, Rice AS. The antihyperalgesic actions of the cannabinoid anandamide and the putative CB receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. Pain 1998;76:189-99.

Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: An overview. Int J Obes (Lond) 2006;30:513-8.

Yoshihara D, Ograzul. Lack of cross-tolerance to the antinoceptive effects of systemic and topical cannabinoids in morphine-tolerant mice. Neurosci Lett 2004;371:122-7.

Mahan J, Philip T, Ibrahim MM, Lai J, Vanderah TW, Makriyannis A, Porreca F, CB2 cannabinoid receptor agonists: Pain relief without psychoactive effects? Current Opinion in Pharmacology 2003;3:62-7.

Beaulieu P. Effects of nabilone, a synthetic cannabinoid, on postoperative pain: [Les effets de la nabilone, un cannabinoide synthetique, sur la douleur postoperative]. Can J Anaesth 2006;53:769-75.

Berlach DM, Shir Y, Ware MA. Experience with the synthetic cannabinoid nabilone in chronic nocancer pain. Pain Med 2006;7:25-9.

Clarke AJ, Lynch ME, Ware M, Beaulieu P, McGilveray IJ, Gourlay D. G guidelines for the use of cannabinoid compounds in chronic pain. Pain Res Manag 2005;10:44A-5A.

Fisher BA, Ghurau A, Vadmalavi V, Antonios TF. Cardiovascular complications induced by cannabis smoking: A case report and review of the literature. Emerg Med J 2005;22:679-80.

Grottenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 2003;42:327-60.

Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S, et al. Attenuation of allergic contact dermatitis through the endocannabinoid system. Science 2007;316:1494-7.

Maekawa T, Nojima H, Kuraishi Y, Aisaka K. The cannabinoid CB receptor inverse agonist JTE-907 suppresses spontaneous itch-associated responses of NC mice, a model of atopic dermatitis. Eur J Pharmacol 2006;542:179-83.

Burns TL, Ineck JR. Cannabinoid analgesia as a potential new therapeutic option in the treatment of chronic pain. Ann Pharmacother 2006;40:251-60.

Brady CM, DasGupta R, Dalton C, Wiseman OJ, Berkley KJ, Fowler CJ. An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis. Mult Scler 2004;10:425-33.

Barnes MP. Sativex: Clinical efficacy and tolerability in the treatment of symptoms of multiple sclerosis and neuropathic pain. Expert Opin Pharmacother 2006;7:607-15.

Dongrul A, GuL H, Akar A, Yildiz O, Bilgin F, Guzeldemir E. Topical cannabinoi antinociception: Synergy with spinal sites. Pain 2003;103:11-6.

Sokal DM, Elmes SJ, Kendall DA, Chapman V. Intraplantar injection of anandamide inhibits mechanically-evoked responses of spinal neurones via activation of CB receptors in anaesthetised rats. Neuropsychopharmacology 2003;45:404-11.

Tyagi P, Tyagi S, Kaufman J, Huang L, de Miguel F. Local drug delivery to bladder using technology innovations. Urol Clin North Am 2006;33:519-30.

Tyagi P, Chancellor MB, Li Z, De Groot WC, Yoshimura N, Fraser MO, et al. Urodynamic and immunohistochemical evaluation of intravesical capsicin delivery using thermosensitive hydrogel and liposomes. J Urol 2004;171:483-9.

Chuang YC, Thomas CA, Tyagi S, Yoshimura N, Tyagi P, Chancellor MB. Human urine with solifenacin intake but not tolterodine or darifenacin intake blocks detrusor overactivity. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:1353-7.

Chuang YC, Tyagi P, Huang CC, Yoshimura N, Wu M, Kaufman J, et al. Urodynamic and Immunohistochemical Evaluation of Intravesical Botulinum Toxin A Delivery Using Liposomes. J Urol 2009182:786-92.

Tyagi V, Yoshimura N, Chancellor MB, Tyagi P. Local Activation Of Cannabinoid CB1 Receptors Bladder Suppresses Acetic Acid Induced Bladder Irritation. J Urol 2008;179:539-54.

Tyagi V, Yoshimura N, Chancellor MB, Tyagi P. Inhibition of Inflammatory Hyperalgesia by Down-modulating Mast Cell Activation. J Urol 2009;181:129-37.

Dionne KA, Lepinski AM, Gordon SM, Jabbe L, Bramh JS, Hargreaves KM. Adverse effects of periurethral administered opioids in clinical models of Bladder Irritation. J Urol 2007;179:202-8.

Izzo AA, Courtts AA. Cannabinoids and the digestive tract. Handb Exp Pharmacol 2005;168:573-98.

Guitierrez T, Farthing JN, Zvonok AM, Makriyannis A, Hohmann AG. Activation of peripheral cannabinoid CB and CB receptor suppresses the maintenance of inflammatory nociception: A comparative analysis. Br J Pharmacol 2007;150:153-63.

Mazzari S, Canella R, Petrelli L, Marcolongo G, Leon A. N-(2-hydroxyethyl) hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. Eur J Pharmacol 1996;308:227-36.

Rahn EJ, Makriyannis A, Hohmann AG. Activation of cannabinoid CB(1) and CB(2) receptors suppresses neuropathic nociception evoked by the chemotherapeutic agent vincristine in rats. Br J Pharmacol 2007;152:765-77.

Ossipov MH, Porreca F. Challenges in the development of novel treatment strategies for neuropathic pain. NeuroRx 2005;2:650-61.

Indian J Urol, Jan-Mar 2010, Vol 26, Issue 1
of acute and chronic inflammation. Clin Pharmacol Ther 2001;70:66-73.
73. Di Marzo V, Griffin G, De Petrocellis L, Brandi I, Bisogno T, Williams W, et al. A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. J Pharmacol Exp Ther 2002;300:984-91.
74. Shire D, Calandra B, Bouaboula M, Barth F, Rinaldi-Carmona M, Casellas P, et al. Cannabinoid receptor interactions with the antagonists SR 141716A and SR 144528. Life Sci 1999;65:627-35.
75. Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 1999;283:401-4.

How to cite this article: Tyagi P, Tyagi V, Yoshimura N, Chancellor M. Functional role of cannabinoid receptors in urinary bladder. Indian J Urol 2010;26:26-35.

Source of Support: Nil, Conflict of Interest: None declared.