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Voiding Dysfunction

Effects of Cannabinor, a Novel Selective Cannabinoid 2 Receptor Agonist, on Bladder Function in Normal Rats

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Abstract

Background: Cannabinoid (CB) receptors may be involved in the control of bladder function; the role of CB receptor subtypes in micturition has not been established.
Objectives: Our aim was to evaluate the effects of cannabinor, a novel CB2 receptor agonist, on rat bladder function.
Design, setting, and participants: Sprague Dawley rats were used. Distribution of CB2 receptors in sensory and cholinergic nerves of the detrusor was studied. Selectivity of cannabinor for human and rat CB receptors was evaluated. Effects of cannabinor on rat detrusor and micturition were investigated.
Measurements: Immunohistochemistry, radioligand binding, tritium outflow assays, organ bath studies of isolated bladder tissue, and cystometry in awake rats were used.
Results and limitations: CB2 receptor immunoreactivity was expressed in the urothelium and in sensory and cholinergic bladder nerves. Cannabinor exhibited similar binding at human and rat CB2 receptors and a 321-fold functional selectivity for the CB2 receptor versus the CB1 receptor. Cannabinor had no effect on isolated detrusor muscle function. In vivo, cannabinor 3.0 mg/kg increased micturition intervals and volumes by 52% ($p < 0.05$) and 96% ($p < 0.01$), respectively, and increased threshold and flow pressures by 73% ($p < 0.01$) and 49% ($p < 0.001$), respectively. Cannabinor 0.3 or 1.0 mg/kg or vehicle did not affect urodynamic parameters.
Conclusions: Considering that CB2 receptors are localized on sensory nerves and on the urothelium and that cannabinor had effects on "afferent" urodynamic parameters, peripheral CB2 receptors may be involved in sensory functions of rat micturition. Effects of cannabinor on cholinergic nerve activity in normal bladder tissue appear to be limited.

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1. Introduction

Confirming previous observations [1], a randomized placebo-controlled study (Cannabinoids in Multiple Sclerosis–Lower Urinary Tract Symptoms [CAMS-LUTS]) reported reduced urgency incontinence episodes in patients with multiple sclerosis by cannabis extract and Δ9-tetrahydrocannabinol (THC) [2], and focused interest on cannabinoid (CB) receptors as pharmacologic targets in lower urinary tract (LUT) disorders.

In accordance, nonselective CB receptor agonists (WIN55212 and CP55940) have been demonstrated to increase the threshold for micturition and to increase micturition intervals in preclinical urodynamic models [3–5]. It has not been clarified if these actions are related to CB receptors in the central nervous system (CNS), at peripheral sites in the LUT, or both. Furthermore, it is not known which of the two CB receptor subtypes, CB1 or CB2, is of primary importance for regulation of micturition.

High levels of CB1 receptors are expressed in the CNS, whereas CB2 receptors are found predominantly outside the CNS [6,7]. The CB1 receptor has also been demonstrated in the urinary bladder of humans and mammals, but diverging results on expression and functional activity of the receptor in different species have been reported [5,8–10]. However, CB1 receptor–related CNS effects on cognition, memory, mental state, and consciousness [6] may raise questions about this receptor as a suitable target for drugs aimed at the treatment of bladder overactivity.

Recently, the CB2 receptor was demonstrated on the urothelium and nerves of the urinary bladder from humans, monkeys, and rodents [5,10,11]. In human bladders, expressions of CB2 receptors were reported to be higher in the mucosa than in the detrusor [5,10], and based on experimental data, a role for CB2 receptors in sensory signals from the bladder was suggested [5,10].

The objective of the current study was to evaluate the effects of cannabinor (Procter & Gamble, Cincinnati, OH, USA), a novel selective CB2 receptor agonist, on isolated detrusor muscle and on urodynamic parameters of conscious rats during cystometry.

2. Materials and methods

2.1. Animals and ethical permission

The protocol was approved by the Animal Ethics Committee, County Court of Lund, Sweden. Thirty-eight female Sprague Dawley rats (200–250 g), maintained at a 12:12 light/dark cycle with free access to food and water, were used. Xylazine (Rompun; 50 mg/kg^{-1}) and ketamine (Ketalar; 10 mg/kg^{-1}) were used as anesthetics. Rats were killed by carbon dioxide asphyxia.

2.2. Immunohistochemistry

Bladder specimens were processed for immunohistochemistry [12]. Antibodies for CB2 (rabbit; 1:500; Alomone Labs, Jerusalem, Israel), calcitonin gene-related peptide (CGRP) (guinea pig; 1:1000; Euro-Diagnostica, Malmö, Sweden), goat antiserum to vesicular acetylcholine transporter (VAChT, 1:1600; Chemicon, Malmö, Sweden), and Alexa fluorescence antibodies (1:600; Molecular Probes Inc, Leiden, The Netherlands) were used. Sections were analyzed using a laser microscope (Olympus Corp, Osaka, Japan). Control staining without primary antibodies did not yield immunoreactive signals.

2.3. Radioligand binding assays

Membranes of HEK-293 cells expressing human or rat CB2 receptors were incubated with 1–1.5 nM tritiated CP55940 (PerkinElmer, Boston, MA, USA) in the presence or absence of increasing concentrations of cannabinor. The effect of cannabinor on stimulation of binding of sulfur 35–GTPgS in HEK-293 cell membranes expressing human CB1 receptor and Sf9 membranes expressing human CB2 (hCB2) receptor (PerkinElmer) was compared with CP55940 (full CB receptor agonist). Reactions were terminated by filtering onto GF/C filter plates (PerkinElmer). The plates were counted in a TopCount (PerkinElmer). Efficacy (E_{max}), mean inhibition constant (Ki), and median effective concentration (EC_{50}) values were calculated with GraphPad Prism (GraphPad, San Diego, CA, USA).

2.4. Functional in vitro studies

Detrusor preparations (2 × 2 × 5 mm) were dissected. Experiments were performed in aerated organ baths (37 °C, pH 7.4) containing Krebs solution, as previously described [5]. Electrical field stimulation (EFS) was performed with a Grass S48 stimulator (Grass Instruments, Grass Instrument Co, Quincy, MA, USA) [5]. The effects of cannabinor (0.1, 1, and 10 μM) on contractions to carbachol (0.1–100 μM) and EFS were studied.

2.5. Tritium outflow experiments

Detrusor specimens were incubated with tritiated choline (2.7 Ci/mmol; New England Nuclear, Boston, MA, USA) containing aerated Krebs solution. Preparations were mounted in perfusion chambers, and superfusates were collected as previously described in detail [13].

2.6. Cystometry

As previously described [5,12], polyethylene (PE-50; Clay-Adams, Parsippany, NJ, USA) catheters were positioned in the bladder and in the femoral vein. Three days later, intravesical pressure and micturition volumes were recorded during cystometries of conscious rats [5,12]. After baseline registration, vehicle or cannabinor (0.1, 0.3, or 3.0 mg/kg) was given intravenously. Recorded parameters included (1) basal pressure (BP), (2) threshold pressure (TP), (3) flow pressure (FP; pressure at start of flow [14]), (4) maximal pressure (MP), (4) micturition volume (MV), (5) residual volume (RV), (6) bladder capacity (BC; equals MV plus RV), and (6) micturition interval (MI) [5,12].

2.7. Drugs and solutions

Cannabinor, CP55940, and carbachol (Sigma, St. Louis, MO, USA) were used. Cannabinor was dissolved in phosphate-buffered saline; carbachol was dissolved in saline. The Krebs solution contained NaCl, 119 mM; KCl, 4.6 mM; $CaCl_2$, 1.5 mM; MgCl, 1.2 mM; $NaHCO_3$, 15 mM; NaH_2PO_4, 1.2 mM; and glucose, 5.5 mM.

2.8. Calculations

Values are given as mean plus or minus standard error of mean. The two-tailed student t test was used for paired or unpaired observations. A p value <0.05 was regarded as significant.

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3. Results

3.1. Immunohistochemistry

Immunoreactivity (IR) for the CB2 receptor was expressed in the urothelium (Fig. 1A). Suburothelial and mural varicosities coexpressed CGRP-IR and CB2-IR (Fig. 1B and C). Single ganglion cells of the outflow region expressed CB2-IR and VAChT-IR (Fig. 1D and E). In the detrusor wall, VAChT-positive nerve fibers also expressed CB2-IR (Fig. 1F and G).

3.2. Radioligand binding assay

Cannabinor exhibited similar potency at human and rat CB2 receptors with Ki values ($n = 10$) of 16 and 13.5 nM.

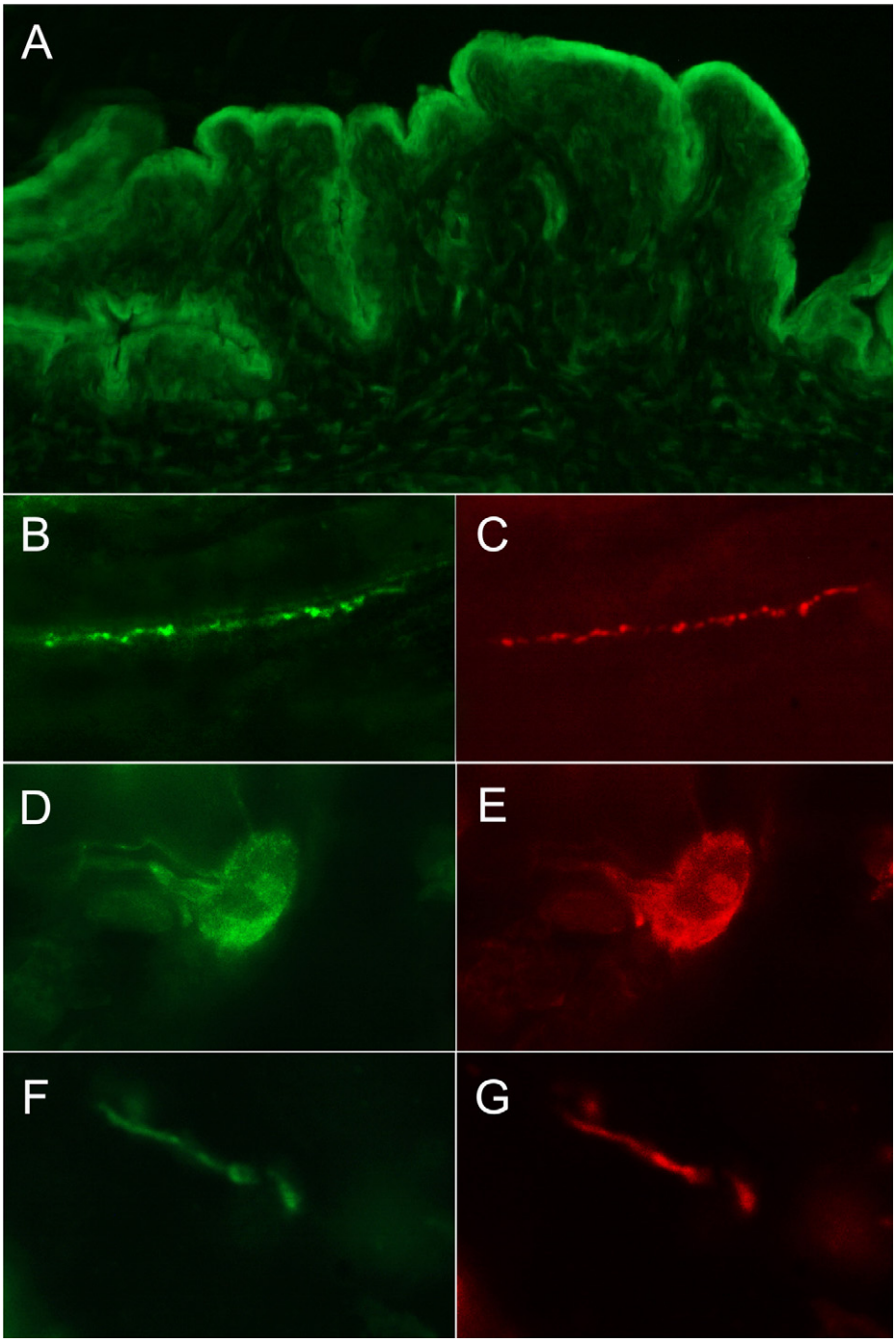

Fig. 1 – Immunohistochemistry. (A) Cannabinoid 2 (CB2) receptor immunoreactivity (IR) in the urothelium (Alexa Green, ×200 magnification). (B) CB2-IR in the suburothelial varicose nerve terminal (Alexa Green, ×1000 magnification). (C) Same section as in (B) showing calcitonin gene-related peptide IR (Alexa Red). (d) CB2-IR in the ganglion cell of the outflow region of the rat urinary bladder (Alexa Green, ×400 magnification). (E) Same section as in (D) showing vesicular acetylcholine transporter (VAChT) IR (Alexa Red). (F) CB2-IR in the mural nerve fiber (Alexa Green, ×1000 magnification). (G) Same section as in (F) showing VAChT-IR (Alexa Red).

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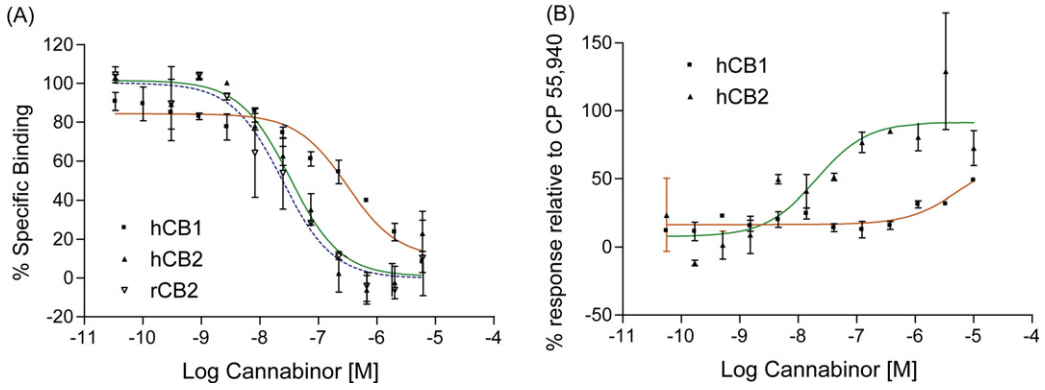

Fig. 2 – Functional GTPgS assay system for human cannabinoid 1 (solid squares) and 2 (solid triangles). Level of activation is expressed relative to the response of 370 nM CP55940 (full agonism). Mean median effective concentration (EC_{50}) at hCB1 receptor is 5595 nM, and 95% confidence interval (CI) of EC_{50} values is between 1697 and 18 400 nM ($n = 4$). Mean EC_{50} at the hCB2 receptor is 17.4 nM, and 95% CI of mean inhibition constant (Ki) is between 6.18 and 48.8 nM ($n = 4$).
hCB1 = human cannabinoid 1 receptor, hCB2 = human cannabinoid 2 receptor; rCB2 = rat cannabinoid 2 receptor.

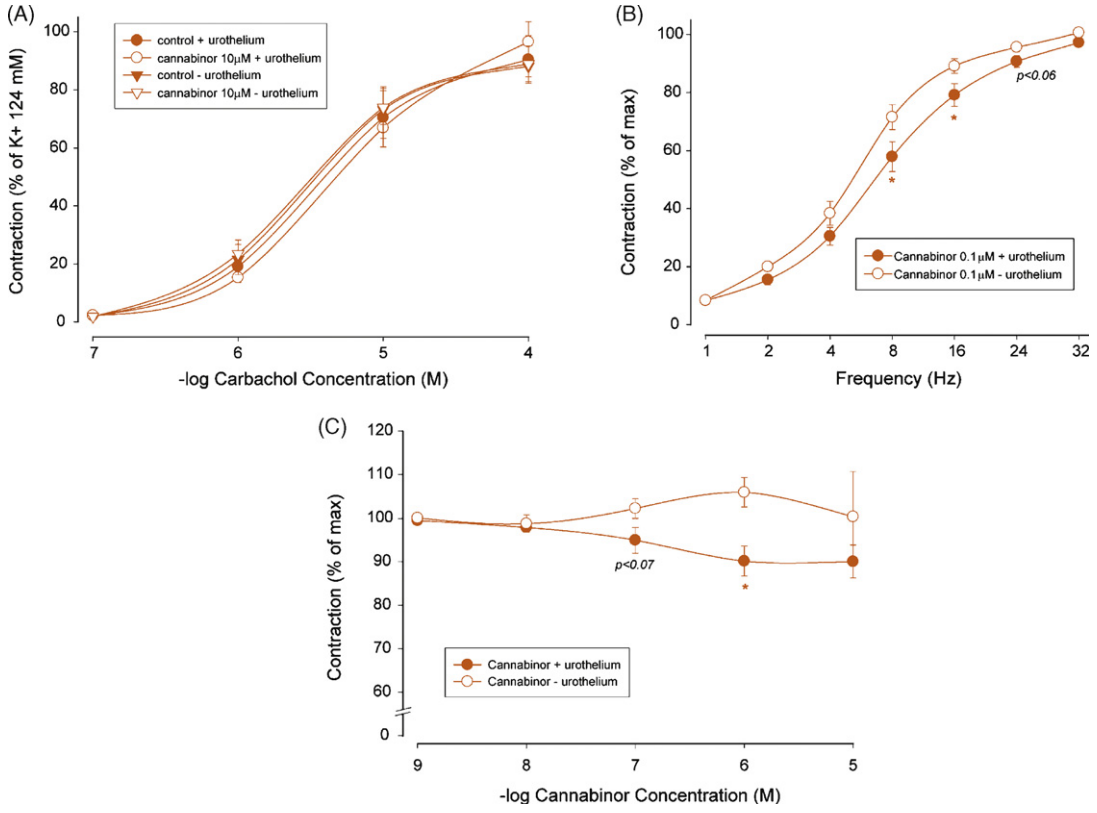

Fig. 3 – Functional in vitro studies. (A) Concentration-response curves to carbachol (1 nM to 10 μM) before and after administration of cannabinor, 10 μM, in preparations with ($n = 5$) or without ($n = 5$) urothelium; (B) frequency-response curves to electrical field stimulation in preparations with ($n = 5$) or without ($n = 5$) urothelium before and after administration of cannabinor, 0.1 μM; (C) effect by cannabinor (1 nM to 0 μM) on continuous activation of nerves (16 Hz) in preparations with ($n = 5$) or without ($n = 5$) urothelium. Values are given as mean plus or minus standard error of the mean.
*** $p < 0.05$ (t test).**

Cannabinor was a potent and efficacious agonist at hCB2 receptors with EC_{50} values of 17.4 nM (E_{max} of 98% ± 13.5% relative to CP55940) (Fig. 2B). Cannabinor exhibited 321-fold functional selectivity at hCB2 versus CB1 receptors (EC_{50} of 5595 nM; E_{max} of 67.64% + 27.7% relative to CP55940).

3.3. *Functional in vitro studies*

Cannabinor ($n = 5$) did not affect carbachol-induced (Fig. 3A) or EFS-induced contractions. In preparations ($n = 5$) with or without urothelium, the responses to EFS

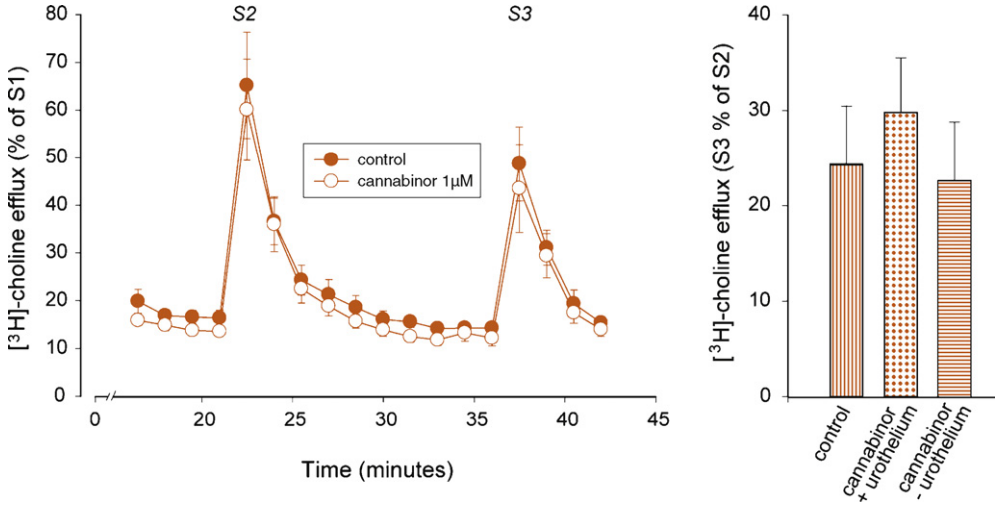

Fig. 4 – Release experiments. (A) Effect of vehicle (control) or cannabinor (1 μM) on electrically induced tritium efflux in urothelium-intact preparations. Data for S2 and S3 are expressed as percentage of S1; (B) relative tritium efflux between S3 and S2 in control preparations with urothelium and in preparations with or without urothelium after exposure to cannabinor (1 μM). Values are given as mean plus or minus standard error of the mean.

at midrange frequencies were different, however (Fig. 3B). At 16 Hz, contractions amounted to 78% ± 3%, 79% ± 5%, and 79% ± 4% versus 96% ± 1% ($p < 0.05$), 91% ± 1% ($p < 0.05$), and 89% ± 2% ($p < 0.05$) for preparations with or without urothelium at cannabinor concentrations 0.1, 1, and 10 μM. Similarly, at 8 Hz, contractions amounted to 55% ± 3%, 58% ± 5%, and 58% ± 5% (urothelium) versus 68% ± 3% ($p < 0.05$), 70% ± 4% ($p = 0.07$), and 72% ± 4% ($p < 0.05$) for urothelium-denuded preparations. During continuous stimulation (16 Hz) of preparations with urothelium ($n = 5$; Fig. 3C), 0.1 μM and 1 μM of cannabinor *reduced* contractions by 5% ± 2% and 10% ± 3%, respectively. At the same concentrations in urothelium-denuded preparations (Fig. 3C), cannabinor *increased* contractions by 2% ± 2% ($p = 0.07$) and 6% ± 3% ($p < 0.05$), respectively.

3.4. *Tritium outflow experiments*

For urothelium-intact preparations exposed to cannabinor (1 μM; $n = 5$), no differences were recorded for the efflux of tritium induced by EFS (Fig. 4A). The relative fractional efflux (S3 vs S2) was 24% ± 6% and 29% ± 6% for controls and cannabinor (Fig. 4B). Also, no difference was noted for urothelium-denuded preparations exposed to cannabinor (1 μM; $n = 5$), which exhibited a relative efflux of 23% ± 6% (Fig. 4B).

3.5. *Cystometry*

Cannabinor 0.3 or 1.0 mg/kg had no effect on MI, MV, TP, FP, RV, and BC ($n = 8$; Table 1). Cannabinor 3.0 mg/kg ($n = 8$)

Table 1 – Urodynamic parameters

Urodynamic parameters	Intervals and volumes				Pressures			
	MI, min	MV, ml	RV, ml	BC, ml	BP, cm H_2O	TP, cm H_2O	FP, cm H_2O	MP, cm H_2O
Cannabinor 0.1 mg/kg								
Baseline ($n = 7$)	4.7 ± 0.7	0.99 ± 0.11	0.01 ± 0.01	1.00 ± 0.11	5.3 ± 1.6	14.6 ± 1.9	27.9 ± 3.3	70.2 ± 8.7
Vehicle ($n = 7$)	5.6 ± 1.1	1.08 ± 0.11	0.01 ± 0.01	1.10 ± 0.12	6.1 ± 1.9	13.7 ± 1.5	28.1 ± 4.3	68.7 ± 13.3
Cannabinor 0.1 mg/kg ($n = 7$)	5.4 ± 1.0	1.08 ± 0.18	0.02 ± 0.01	1.10 ± 0.18	5.3 ± 1.7	14.7 ± 1.8	29.3 ± 4.9	63.3 ± 10.9
Cannabinor 1.0 mg/kg								
Baseline ($n = 8$)	4.0 ± 0.5	0.92 ± 0.13	0.00 ± 0.00	0.92 ± 0.13	5.2 ± 1.6	13.6 ± 2.3	24.4 ± 2.3	68.6 ± 8.1
Vehicle ($n = 8$)	4.4 ± 0.7	0.89 ± 0.12	0.00 ± 0.00	0.89 ± 0.12	4.5 ± 1.3	13.0 ± 2.3	24.4 ± 2.6	63.2 ± 6.2
Cannabinor 1.0 mg/kg ($n = 8$)	5.8 ± 0.9	1.02 ± 0.13	0.01 ± 0.01	1.03 ± 0.13	4.8 ± 1.4	14.4 ± 2.4	27.5 ± 2.7	65.6 ± 8.5
Cannabinor 3.0 mg/kg								
Baseline ($n = 8$)	5.4 ± 1.0	0.82 ± 0.14	0.01 ± 0.01	0.83 ± 0.14	6.5 ± 1.8	12.7 ± 2.0	24.7 ± 3.2	74.7 ± 9.0
Vehicle ($n = 8$)	5.0 ± 0.9	0.96 ± 0.16	0.01 ± 0.01	0.97 ± 0.12	5.9 ± 1.9	13.4 ± 2.4	25.8 ± 4.3	71.4 ± 12.5
Cannabinor 3.0 mg/kg ($n = 8$)	7.0 ± 1.0*	1.40 ± 0.17**§	0.02 ± 0.02	1.42 ± 0.18**§	7.9 ± 3.0	23.8 ± 7.7**§	36.8 ± 5.3**§§	78.0 ± 16.2

BC = bladder capacity; BP = basal pressure; FP = flow pressure; MI = micturition interval; MP = maximal pressure; MV = micturition volume; RV = residual volume; TP = threshold pressure.
* $p < 0.05$ versus baseline.
** $p < 0.01$ versus baseline.
§ $p < 0.05$ versus vehicle.
§§ $p < 0.01$ versus vehicle.

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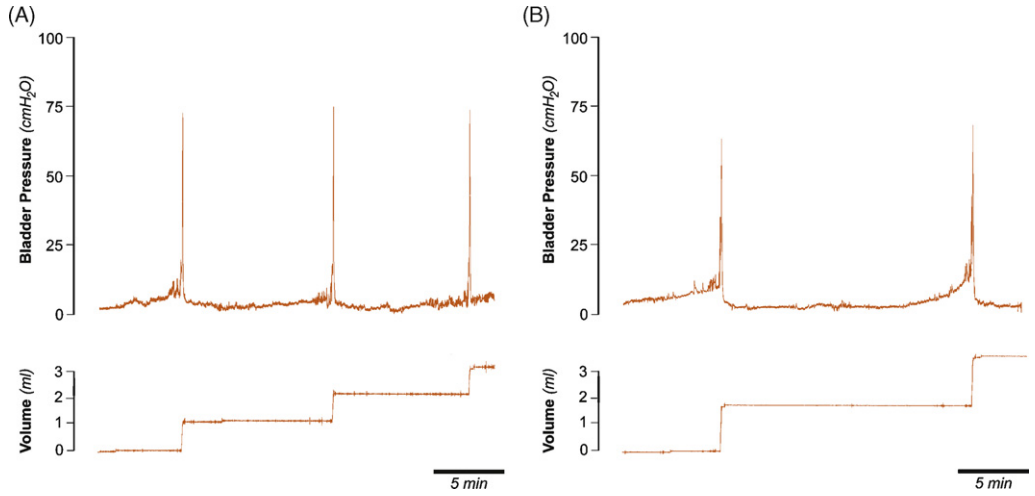

Fig. 5 – Cystometry. Original pressure and volume recordings from a rat (A) before and (B) after intravenous administration of cannabinor (3.0 mg/kg). Micturition interval, threshold pressure, and micturition volume are increased after administration of cannabinor.

increased MI from 4.9 ± 0.7 min to 7.0 ± 1.0 min ($p < 0.05$) and MV from 0.82 ± 0.14 ml to 1.40 ± 0.17 ml ($p < 0.01$) (Fig. 5). At this dose, RV was unaffected, whereas BC increased from 0.83 ± 0.14 ml to 1.42 ± 0.18 ml ($p < 0.01$). Cannabinor never affected BP or MP. After cannabinor 3.0 mg/kg, TP was increased from 12.7 ± 2.0 cm H_2O to 23.8 ± 7.7 cm H_2O ($p < 0.01$). Similarly, FP was increased from 24.7 ± 3.2 cm H_2O to 36.8 ± 5.3 cm H_2O ($p < 0.01$).

Vehicle had no effect on urodynamic parameters. All rats exhibited normal behavior and normal motor functions after administration of cannabinor.

4. Discussion

The results of the current study show that CB2 receptor–mediated signals can influence bladder function in awake rats during cystometry. Cannabinor was characterized as a high-affinity CB2 receptor selective full agonist with a 321-fold functional selectivity for the CB2 receptor versus the CB1 receptor. At the highest investigated dose (3.0 mg/kg), MI, TP, and FP increased by 44%, 87%, and 49%, respectively. Compared with previous in vivo results from rats using the same experimental setup, the CB receptor agonist CP55940 increased the MI by 38% and TP by 116% [5]. Based on morphologic data and functional in vitro experiments, it was proposed that part of the effects by CP55940 on rat micturition was attributed to CB2 receptor–mediated modification of afferent signals from the bladder [5]. Considering that in the present study, similar effects were obtained with cannabinor, a highly selective CB2 receptor agonist, as previously with CP55940, it seems reasonable to assume that the main action of cannabinor was exerted at CB2 receptors. The nonsubtype selective CB receptor agonist WIN55212 increased the threshold for micturition in anesthetized rats, and this effect was counteracted by the CB1 receptor antagonist SR141716A [4]. In this study, CB1 receptor–mediated CNS effects of the drug were not excluded [4]. In anesthetized rats, IP-751 (10 mg/kg), a synthetic analog of THC, increased the micturition intervals by 63% and threshold pressures by 53%, and these effects were counteracted by AM251 (CB1 receptor antagonist) but not by AM630 (CB2 receptor antagonist) [15]. At doses >10 mg/kg, IP-751 exhibited overt central effects, affecting motor performance and resulting in catalepsy in the rats [15]. Even if the main effects of cannabinor seem to be exerted peripherally, we cannot completely exclude a CNS site of action.

In isolated detrusor tissue, cannabinor did not affect baseline tension or carbachol-induced contractions, suggesting that CB2 receptor signals are not directly involved in postjunctional regulation of smooth muscle contractility. Similarly, cannabinor did not have any effect on basal intravesical pressures. These findings are consonant with results obtained with CP55940 on contractions by carbachol in isolated detrusor and basal pressures in vivo [5]. Cannabinor did not have any effects on nerve-induced contractions of isolated detrusor. In contrast, the CB2 receptor agonist GP1a was reported to decrease EFS-induced contractions of the human bladder [10]. However, quantification of the effect by GP1a or vehicle (dimethylsulfoxide) control experiments were not presented [10]. Interestingly, even if cannabinor did not affect EFS-induced control responses, a difference in the effect of the drug was noted between preparations with or without urothelium, which together with urothelial CB2 expression suggests a role for CB2 receptors in mucosal regulation of nerve activities.

The nonselective CB receptor agonists CP55940, CP55244, JWH015, and THC have been shown to produce inhibitory effects on nerve-induced contractions of mouse, rat, monkey, or human detrusor preparations [5,8]. In the mouse detrusor, effects of CB receptor agonists could be attributed to CB1 receptor activation, whereas the effects of JWH015 on the rat detrusor were consistent with possible actions at both CB1 and CB2 receptors [8,9,16]. Varying effects by WIN55212 on nerve-induced contraction of isolated detrusor from dogs, pigs, monkeys, or humans have been reported [9]. However, similar to anandamide,

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WIN55212 has been reported to interact with the function of the TRPV1 and TRPA1 ion channels, which are co-expressed with CB receptors on nerves and urothelium in rat and human LUT tissues [5,17].

We verified the localization of CB2 receptors to cholinergic bladder nerves and also, for the first time, demonstrated that CB2 receptors are expressed in ganglion cells of the outflow region. Even if cannabinor did not exert any effects on EFS-induced contractions, CB2 receptors may mediate effects on cholinergic nerve activity. This assumption is based on morphologic data and previous functional results with CB receptor agonists [5,9,10]. However, tritium outflow experiments showed that cannabinor did not affect the release of tritiated choline at baseline or during activation of detrusor nerves. Because EFS activates all nerves in the preparations (not only cholinergic nerves), it is likely that the urothelium-dependent CB2 receptor–mediated effects observed are related to other transmitter systems.

Recently, CB2 receptors in sensory pathways were suggested to be involved in modulation of afferent signals from peripheral tissues [7]. Inhibition of sensory functions by CB2 receptor activation, but not CB1 receptor activation, has been demonstrated in models of pain [7]. These effects are present in CB1 receptor–negative mice but are absent in CB2 receptor–negative mice, which also exhibit low thresholds for pain [7,18]. A main finding of this study was that cannabinor exhibited significant effects on MI, TP, and FP, which are the urodynamic parameters considered to reflect afferent signals during micturition. In accordance with findings in patients with advanced multiple sclerosis for whom maximum cystometric capacity was increased by THC [2], cannabinor also increased MI and BC of rats during cystometry. In other models, activation of the peripheral CB2 receptors has been found to suppress C-fiber activation and mechanical or inflammation-evoked neuronal activity at the level of the CNS [19–21]. The mixed CB1/CB2 receptor agonist IP-751 was recently reported to reduce chemically evoked local release of CGRP in the rat detrusor, and these effects were reported to be mediated by both CB1 and CB2 receptors [11]. The CB2 receptor active agonists JWH133 and GW 833972A were both found to suppress sensory nerve fiber activation of human and guinea pig airways [22,23]. In the rat mesenteric arterial bed, THC was reported to inhibit CGRP release and neurosensory vasorelaxation [24]. Calcium-imaging studies reported CB2 receptor–mediated inhibitory effect on rat dorsal root ganglion (DRG) neurons [25]. In cultures of human DRG neurons, selective CB2 receptor agonists blocked capsaicin-induced cation currents and Ca^{2+} influx, effects that were counteracted by GW818646X, a CB2 receptor antagonist [26].

The current results cannot establish at which level cannabinor acts to modify afferent signals of micturition. Control stainings verified that rats used in the present study expressed CB2 immunoreactivities on sensory nerves and urothelium, structures of the bladder that form the basis for mechanoafferent regulation of micturition. Because preparations with and without urothelium revealed different effects of cannabinor on nerve-induced contractions, CB2 receptor–mediated effects of the drug on sensory nerve activity in the bladder may be considered. However, we cannot exclude the possibility that cannabinor may also act at the level of the DRG.

5. Conclusions

Cannabinor, a selective CB2 receptor agonist, had significant effects on "afferent" urodynamic parameters, and it increased bladder capacity in normal awake rats during cystometry. Considering that CB2 receptors are localized on sensory nerves and on the urothelium, peripheral CB2 receptors may be involved in the regulation of mechanoafferent functions of the rat bladder.

Author contributions: Karl-Erik Andersson had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Andersson, Hedlund, Gratzke.
Acquisition of data: Gratzke, Streng, Hedlund.
Analysis and interpretation of data: Gratzke, Hedlund, Andersson.
Drafting of the manuscript: Hedlund, Andersson.
Critical revision of the manuscript for important intellectual content: Andersson, Stief, Rosenbaum.
Statistical analysis: Gratzke, Hedlund.
Obtaining funding: Andersson, Hedlund.
Administrative, technical, or material support: Downs, Alroy.
Supervision: Andersson, Stief.
Other (specify): None.

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