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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.