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Sexual Medicine

Localization and Function of Cannabinoid Receptors in the Corpus Cavernosum: Basis for Modulation of Nitric Oxide Synthase Nerve Activity

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Abstract

Background: Anandamide, a proposed endogenous cannabinoid (CB) agonist, has been shown to enhance neurogenic responses *in vitro* of the rat corpus cavernosal tissue (CC). However, no information is available on the distribution of CB-receptors or effects by anandamide in CC from primates or humans.

Objective: To characterize the distribution of CB-receptor isoforms in the human and primate CC and to investigate the effects of anandamide on isolated CC preparations.

Design, setting, and participants: CC tissue was excised from the crura penis of six rhesus monkeys and five patients. Expression and distribution of CB1 and CB2 receptors were characterized with Western blot analyses and immunohistochemical investigations. The effects of anandamide on isolated CC preparations were analyzed during pharmacologic and nerve-mediated activation of primate tissue in aerated organ baths.

Measurements: The expression and localization of CB1 and CB2 receptors in the primate CC and effects of anandamide on nerve-mediated relaxations and pharmacologically evoked contractions.

Results and limitations: Western blot experiments revealed CB1 and CB2 receptors at expected band weights. Within and between strands of CC smooth muscle, CB1 and CB2 immunoreactivity (IR) was found in nerve fibers that also expressed IR for nitric oxide synthase (NOS) or transient receptor potential V1 (TRPV1). Neither CB1-IR nor CB2-IR nerves were colocalized with calcitonin-gene–related peptide (CGRP)-containing or tyrosine hydroxylase-containing nerves. No differences were observed between primate and human CC sections.

Anandamide (10^{-9} to 10^{-4} M) had no contractile effects on CC smooth muscle, no relaxant effects on precontracted preparations, and no effect on phenylephrine-induced contractions. However, anandamide (10 μM) inhibited electrically evoked smooth-muscle relaxations (34–48%; $p \leq 0.05$).

Conclusions: CB1 and CB2 receptors are located on NOS-containing nerves in primate and human CC tissue. In contrast to findings in rats, anandamide antagonized nerve-mediated relaxations of the primate CC, suggesting important species differences for CB-mediated functions. The results also suggest a peripheral mechanism for cannabis-related sexual dysfunction.

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1. Introduction

The corpus cavernosal (CC) smooth-muscle tone determines the degree of penile flaccidity, tumescence, erection, or detumescence [1,2]. The balance between contracting and relaxing factors is maintained by central and peripheral mechanisms involving several transmitters and vasoactive substances [3,4]. Penile erection is thus a complex neuromuscular event, and even if the *major players* have been identified, it is clear that a full understanding of the complex interrelationships remains elusive.

In this regard, experimental evidence has suggested a potential role for the endocannabinoid system in the mechanism of erectile and sexual functions in rats. Administration of endogenous and exogenous cannabinoids was associated with changes in penile erection and modulation of male sexual behavior [5,6]. One potential explanation is that cannabinoid 1 (CB1) receptors, present in the paraventricular nucleus, influence erectile function and sexual activity possibly by modulating the activity of oxytocinergic neurons that are involved in regulation of sexual responses [7]. In addition, peripherally, it has also been proposed that anandamide, an endogenous CB-receptor agonist, potentiates the nonadrenergic noncholinergic-mediated relaxations through CB1 receptors, transient receptor potential V1 (TRPV1) ion channels, and nitric oxide (NO), in CC smooth muscle of rats [8–11].

Cannabis, used as a recreational drug, is known to cause sexual side effects and infertility [12]. Cannabinoid agonists, used in experimental studies as stimulatory agents for the treatment of anorexia, have also been reported to cause sexual side effects in humans [13]. To what extent these effects are centrally mediated or are caused by direct effects on peripheral organs such as CC tissue, is currently unclear.

To the best of our knowledge, no information is available on the effects of the endogenous cannabinoid agonist anandamide on CC smooth muscle of primates. The distribution of CB1 and CB2 receptors in primate and human CC tissue is also not known. We therefore aimed to study the effects of anandamide on pharmacologically and nerve-evoked responses in isolated CC smooth-muscle strips from rhesus monkeys. Furthermore, we aimed to assess the distribution of CB1 and CB2 receptors in relation to markers for autonomic cholinergic and adrenergic nerves as well as markers for sensory nerves in CC from primates and humans.

2. Material and methods

2.1. Tissue

CC tissue samples from six male rhesus monkeys, and from five male patients who had undergone gender reassignment, were used in this study. Tissue from rhesus monkeys was used for Western blots, immunohistochemistry, and functional studies, while tissue from male patients was used for immunohistochemistry only. Experiments were conducted in accordance with the regulations of the Ethics Committee of the Animal Care and Use Committee at Wake Forest University (United States) and the Ethics Committee of Lund University (Sweden).

2.2. Western blotting

CC tissue from rhesus monkeys was snap frozen and homogenized, followed by lysis in radio immunoprecipitation assay (RIPA) buffer (1X phosphate-buffered saline [PBS], 1% Triton X-100, 0.5% sodium deoxycholate [DOC], 0.1% sodium dodecyl sulfate [SDS]) supplemented with 1:10 protease-inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Protein concentration was determined using DC protein assay (Bio-Rad Laboratories). Some 1–3 μg/ml of total protein for all samples was separated on the 12% SDS-polyacrylamide gel by electrophoresis, transferred to a polyvinylidene difluoride membrane (Millipore Corporation, Bedford, MA, USA), and probed with antibodies against CB1 and CB2 receptors (Sigma-Aldrich, St Louis, MO, USA) and TRPV1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight, followed by secondary horseradish peroxidase-labeled immunoglobulin G (IgG). Images were obtained using an LAS3000 Imager (Fujifilm Life Science USA, Stamford CT, USA). β-actin was used to ensure equal loading of total protein. Anti-CB1, anti-CB2, and anti-TRPV1 were diluted in an appropriate range in 5% w/v skim milk/1X phosphate-buffered saline Tween-20 (PBST; 0.5% v/v Tween-20). A broad-range molecular marker (Bio-Rad Laboratories, Hercules, CA, USA) and a biotynylated protein ladder (Cell Signaling Technology, Danvers, MA, USA) were used to determine molecular masses. Negative controls without primary antibodies were performed for all samples.

2.3. Immunohistochemistry

CC tissue from all primates and male patients was processed for immunohistochemistry as previously described [14]. Cryostat sections were preincubated in PBS with 0.2% Triton X-100 for 2 h and then incubated overnight at 4 °C in the presence of goat antisera against CB1 (1:500), CB2 (1:1000; Sigma-Aldrich), and antiserum for calcitonin-gene–related peptide (guinea-pig; 1:2000; Euro-Diagnostica, Malmö, Sweden), tyrosine hydroxylase (TH) (mouse; 1:2000, Diasorin AB, Bromma, Sweden) nitric oxide synthase (NOS) (sheep; 1:2000, P.C. Emson, Babraham Institute, Cambridge, UK), and TRPV1 (goat; 1:1000, Santa Cruz Biotechnology). After rinsing in PBS (three rinses for 10 min), the sections were incubated for 90 min with appropriate Alexa Red- or Green-conjugated affinity purified IgG. In control experiments, no IR could be detected in sections incubated in the absence of the primary antisera. Because cross-reactions to antigens sharing similar amino-acid sequences cannot be completely excluded, the structures shown are referred to as CB1-IR, CB2-IR, CGRP-IR, TH-IR, NOS-IR, TRPV1-IR and α-actin–IR. The sections were examined under a fluorescence microscope with epi-illumination and filter settings for Alexa Red and Green immunofluorescence.

2.4. Functional investigations

The primate penises were placed in chilled Krebs solutions. The tunica albuginea was carefully opened, and the CC tissue was microsurgically dissected. Silk ligatures were applied at both ends of the strip preparations (4 mm × 0.5 mm × 0.5 mm), which were then suspended between two metal prongs in thermostatically controlled organ baths (5 ml, 37 °C) containing Krebs solution, routinely changed every 20 min and bubbled with a mixture of 95% oxygen (O_2) and 5% carbon dioxide (CO_2) (pH 7.4). Isometric tension was recorded by means of DMT Myograph system 800MS force transducers (Danish Myo Technology A/S, Aarhus, Denmark). A pre-tension of 2.5 mN (250 mg) was applied, and the tissue was allowed to equilibrate for 60 min without further mechanical manipulation. 13 CC tissue strips from six monkeys were precontracted with phenylephrine, and when the contractions were stabilized, frequency–response curves for electrical field stimulation (EFS) were obtained, using consecutive 8-s stimulations (150V, 3-ms duration every 120 s) at the frequencies of 2 Hz, 4 Hz, 8 Hz, 16 Hz, and 32 Hz before and after administration of anandamide 10 μM.

2.5. Drugs and solutions

Stock solutions of phenylephrine (10 mM; Sigma-Aldrich) were made in deionized water and were stored at −20 °C. Anandamide (arachidonoylethanolamide or AEA, Sigma-Aldrich) solutions were made in ethanol. Subsequent dilutions of the drug were made on the day of the experiment using deionized water. Reported concentrations are the final concentrations in the organ-bath solution. Krebs solution was composed of 119 mM sodium chloride (NaCl), 4.6 mM potassium chloride (KCl), 1.5 mM calcium chloride ($CaCl_2$), 1.2 mM magnesium chloride ($MgCl_2$), 15 mM sodium bicarbonate ($NaHCO_3$), 1.2 mM sodium phosphate (NaH_2PO_4) and 11 mM glucose. High K^+ Krebs solution (60 mM) was prepared by replacing NaCl with equimolar amounts of KCl.

2.6. Calculations and statistical analysis

Values are given as mean plus or minus standard error of the mean (SEM). Student *t* test (two-tailed) was used for paired or unpaired observations (Statview software). A probability of $p < 0.05$ was regarded as significant. The number of animals or patients used is denoted by *n*.

3. Results

3.1. Western blot analysis of primate corpus cavernosal tissue

Western blot analysis was conducted using CB1-, CB2-, and TRPV1-selective antibodies. Characteristic bands at the expected molecular weights of 60 kDa, 60 kDa, and 100 kDa, respectively, were observed (Fig. 1). Bands for β-actin were characteristically visualized at 42 kDa (not shown).

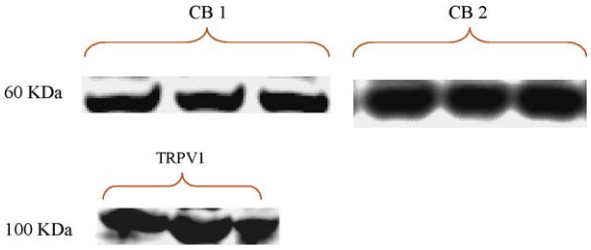

Fig. 1 – Western blot analysis: The cannabinoid agonist 1 (CB1), CB2, and transient receptor potential V1 (TRPV1) antibody detected clear bands of the expected molecular weights (60 kDa, 60 kDa, and 100 kDa) in tissue from the nonhuman primate corpus cavernosal smooth muscle.

3.2. Immunohistochemistry of primate and human corpus cavernosal tissue

CB1-IR or CB2-IR was found in nerve fibers that were similarly distributed within and between strands of CC smooth-muscle tissue of human and primate CC sections (Fig. 2). NOS-IR nerve fibers were also located heterogeneously between smooth-muscle bundles of CC from both species (Figs. 2 and 3). As assessed in double-stained

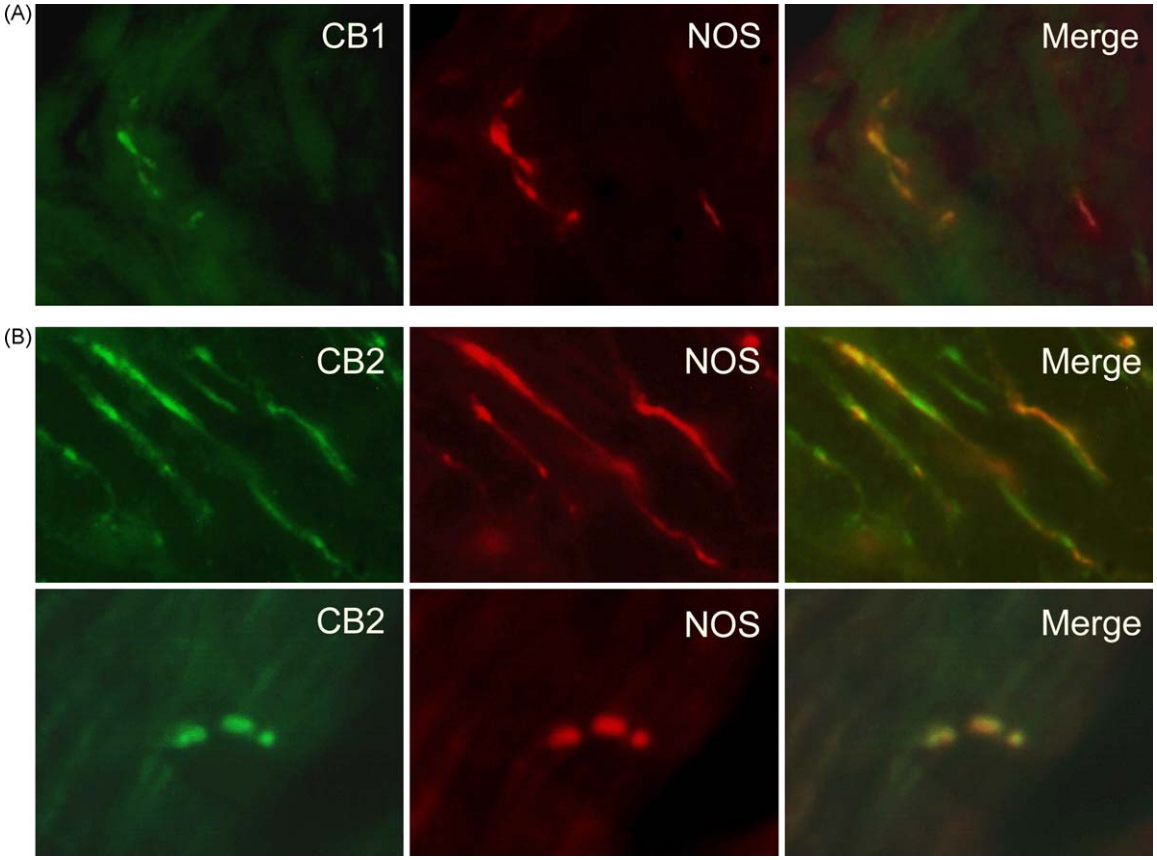

Fig. 2 – Cannabinoid agonist 1 (CB1) and CB2 immunoreactivity (IR) was found in nerve fibers within and between strands of corpus cavernosal (CC) smooth muscle. CB1-IR (A: human CC, ×1000 magnification) and CB2- IR (B: upper panel: primate CC, ×600 magnification; lower panel: human CC, ×1000 magnification) nerves also expressed immunoreactivity for nitric oxide synthase (NOS).

sections of human or primate CC tissue, CB1-IR or CB2-IR nerves also expressed IR for NOS and TRPV1. In nerve terminals, IR for NOS and CB1 or CB2 was colocalized in terminal varicosities of human or primate CC tissue (Fig. 2). Also, slender NOS-IR nerve fibers exhibited coinciding profiles with nerves expressing IR for CB1 or CB2 (Fig. 2). IR for TRPV1, a marker mainly for C-fiber afferents, was generally expressed in slender, varicose nerve terminals of CC sections. In double-stained sections, CB1-IR or CB2-IR was colocalized in terminal TRPV1-IR varicosities (Fig. 3).

IR for TH, a marker for adrenergic nerves, and for CGRP, a marker for sensory nerves, was observed interspersed between bundles of smooth-muscle cells. However, neither

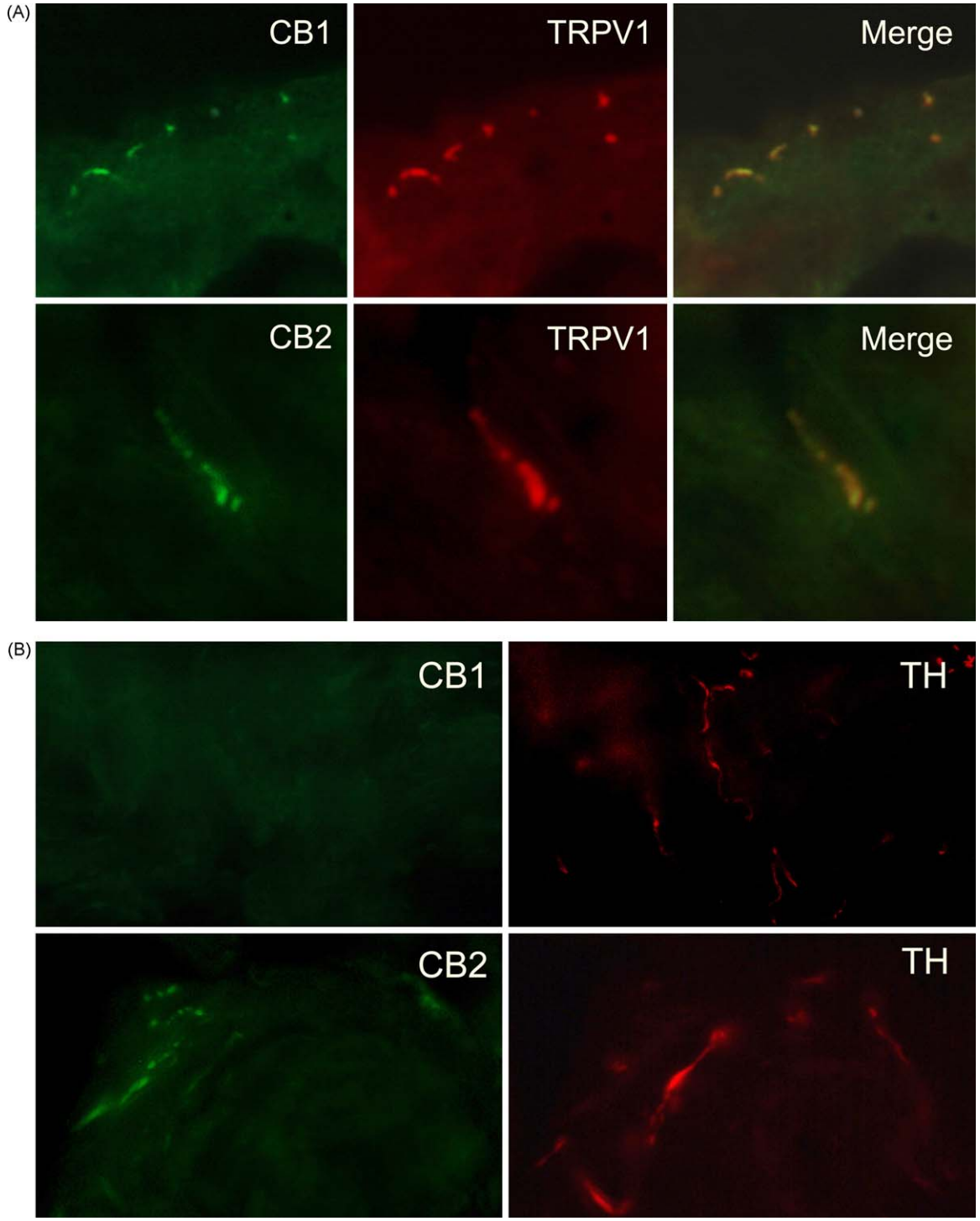

Fig. 3 – A: Cannabinoid agonist 1 (CB1) immunoreactivity (IR) (upper panel: human corpus cavernosal [CC] tissue) or CB2-IR (lower panel: human CC) was coexpressed with transient receptor potential V1 (TRPV1)-IR in varicose nerve terminals (×1000 magnification). B: Neither CB1-IR (upper panel: human CC, ×200 magnification) nor CB2-IR (lower panel: human CC) was located for nerve fibers expressing tyrosine hydroxylase (TH)-IR (×400 magnification).

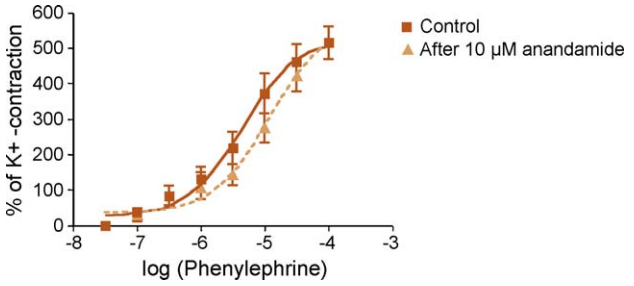

Fig. 4 – Phenylephrine concentration–response curves (CRCs) before and after administration of anandamide (10 μM) on isolated primate corpus cavernosal smooth-muscle preparations ($n = 6$). Values represent mean plus or minus standard error of the mean.

CB1 nor CB2 nerves were colocalized with CGRP- or TH-containing nerves in primate or human CC (Fig. 3).

3.3. *Functional investigations of isolated primate corpus cavernosal tissue*

At baseline, anandamide did not have any effect on level of tension of the preparations per se.

In phenylephrine (PE)-contracted preparations (10 μM), anandamide (100 nM to 10 μM) did not show any detectable effect on steady-state contraction. There was also no significant change in the magnitude of the time-dependent PE-induced contractions after pretreatment of anandamide (10 μM) compared to controls. Consistent with this observation, the reverse logarithm of the half-maximal effective concentration (pEC50) values for PE-induced contractions did not differ significantly (−5.342 vs −4.934; Fig. 4).

In PE-contracted preparations, the EFS generated frequency-dependent and tetrodotoxin-sensitive relaxant responses. The magnitude of these responses was attenuated by anandamide at any investigated frequency. Specifically, anandamide (10 μM) inhibited nerve-induced smooth-muscle relaxations by 48% (2 Hz; $p \leq 0.01$), 41% (4 Hz; $p \leq 0.01$), 39% (8 Hz; $p \leq 0.01$), 34% (16 Hz; $p \leq 0.05$), and 34% (32 Hz; $p \leq 0.01$), respectively (Fig. 5).

4. Discussion

The present study reports for the first time that CB1 and CB2 receptors are expressed in human and primate CC. In correspondence to these findings, the CB1-receptor protein has previously been demonstrated in rat CC using Western blot analyses. However, in contrast to the human and primate CC, CB2 receptors were not located in the rat CC [9]. These results suggest that there are differences between rats and humans as well as nonhuman primates in the expression of CB-receptor subtypes in the erectile tissue.

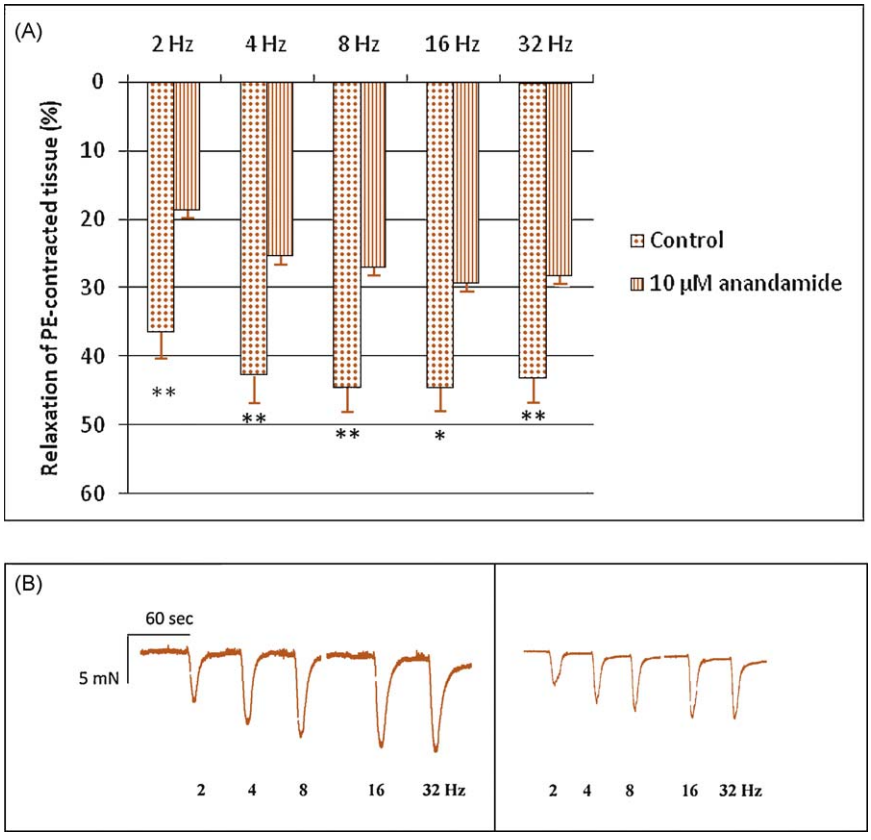

Fig. 5 – A: Effects of electrical field stimulation (EFS) before and after administration of anandamide (10 μM, left) on isolated corporal smooth muscle preparations ($n = 6$). Values represent mean plus or minus standard error of the mean. B: Original tracings show the effects of anandamide on nerve-induced relaxations.

Such differences may be of importance when investigating the role of cannabinoid-mediated effects on penile erection. Until now, no information on which cellular structures of the CC contain CB receptors has been available to further interpret a relationship between cannabinoid activity on erectile function and a site of action within the CC. The present results show that IRs for CB1 and CB2 are expressed on NOS-containing nerves in human and primate CC. A similar arrangement of CB1-receptors, but not CB2-receptors, on autonomic motor neurons has been found in the gastrointestinal (GI) tract, the cardiovascular system, and the vas deferens, suggesting presynaptic modulator functions for CB1-active cannabinoids in these tissues [15,16].

In functional experiments, the endogenous cannabinoid anandamide had no effect on baseline tension, did not produce relaxations of α_1-adrenoceptor precontracted preparations, and did not affect concentration–response curves to phenylephrine in preparations of the primate CC. These findings are consistent with a lack of a postjunctional site of action for anandamide in CC tissue.

It is well established that nerve-mediated and NO-dependent relaxations of rodent, rabbit, dog, primate, and human CC are crucial for normal erectile responses [3,17]. In the present study, transmural activation of nerves produced frequency-dependent relaxation responses ranging from approximately 30% to 50% of the α_1-adrenoceptor precontracted preparations. Consistently, at any investigated frequency, anandamide significantly attenuated these responses. These findings contrast with previous results using isolated rat CC smooth muscle to study effects of anandamide on nerve-induced relaxation. In those studies, Ghasemi et al reported that the relaxation responses to electrical stimulation in CC tissue from normal rats, rats with diabetes, or rats with biliary cirrhosis, were enhanced in the presence of 1 μM and 3 μM of anandamide [8–10]. No potentiating effects were noted at 2 Hz, but increases up to roughly 20% in the middle-range frequencies (5 Hz, 10 Hz and 15 Hz) were observed. The effect of anandamide on relaxant responses was attenuated by either a CB1-receptor antagonist (AM251) or by the vanilloid-receptor (VR) antagonist capsazepine, but not by a CB2-receptor antagonist (AM630). The authors concluded that the effects of anandamide on nerve-induced relaxations of the rat CC tissue probably involved CB1- and VR1-receptor–mediated mechanisms [9].

Based on our present findings, the discrepancy in functional effects of anandamide on nerve-mediated relaxations in rat and primate erectile tissue may be attributed to differences in the expression and distribution of CB-receptor subtypes. The CB2 receptor is present in primate CC, but not in rat CC. That differences in the distribution of CB-receptor subtypes may have an impact on functional CB responses in related tissues has been reported for the GI tract. Thus, CB1 receptors, but not CB2 receptors, were expressed throughout the GI-tract (eg, stomach, ileum, colon) and were related to inhibition of autonomic nerve activity, as well as inhibition of nonadrenergic, noncholinergic, neuromuscular responses [15]. In the gastric fundus, expression of both CB1 and CB2 receptors was reported, and anandamide at 1 μM and 10 μM was shown to inhibit relaxant responses to activation of nerves via a CB2-sensitive mechanism [18]. Interestingly, in the gastric fundus, nerve-mediated relaxant functions have been shown to depend upon NO and to have an important role for relaxation of this tissue [19].

Neuronal relaxation responses of the rat CC were shown to differ from other mammals, including humans, and probably involve NO and other transmitters [20], which also suggests that species differences in the peripheral regulation of penile erection may contribute to discrepancies in functional results obtained in rat CC compared to CC from other mammals. In previous investigations [8,9], studying the effect of anandamide on nerve-mediated relaxations of the rat erectile tissue, preparations were pretreated with atropine (ie, blockade of muscarinic activity) and guanethidine (ie, inhibition of adrenergic nerve activity), and it cannot be excluded that differences in the experimental approach may contribute to the differences in results obtained compared to the current study.

The role of CBs in modulation of neurotransmission is complex and may involve both inhibitory actions via CB receptors and excitatory actions via TRPV1 receptors. In addition, CB and TRPV1 receptors may also have different sensitivities to certain cannabinoids or vanilloids, so there may be preferential activation of one or the other receptor [16]. Knowledge of the expression of TRPV1 in the CC and effects of TRPV1-active agents on functional responses of CC tissue is limited. One study reported expression of TRPV1 in human and rat CC, as revealed by Western blot analysis, but did not give any information about the structural location of the receptor [21]. In a small clinical study of 20 men, intraurethral application of capsaicin was reported to induce erectile responses [22]. Even if it was proposed that the erectile responses induced involved local reflexes within the corpus spongiosum and CC (ie, axonal reflexes), the precise mechanism behind this effect remains unclear. Based on the current morphological findings, a close relation between TRPV1-mediated, NOS-mediated, and CB-mediated functions can be assumed.

A role of CB receptors in pathophysiologic mechanisms for erectile dysfunction may also be expected. For example, administration of CB agonists in vivo may lead to attenuation of the release of NO from nerves, thereby decreasing CC smooth-muscle relaxation and reducing erectile function. Effects of CB agonists on erectile mechanisms or other sexual responses may also be related to findings demonstrating a modulatory role of CB1-receptors in the central nervous system. Thus, it was shown that CB1 receptors present in the paraventricular nucleus (PVN) may influence erectile function and sexual activity [7].

A study of 3004 men and women showed that use of marijuana was associated with inhibited orgasm, dyspareunia, or any sexual dysfunction [23]. Furthermore, erectile dysfunction was found to be a side effect in a randomized, controlled study investigating whether dronabinol (delta-9-tetrahydrocannabinol), the major active component of cannabis, had stimulatory effects on appetite in patients

with anorexia [13]. These observations suggest that both central and peripheral effects on sexual function may be related to cannabinoid-mediated signals.

5. Conclusions

CB1 and CB2 receptors are located on NOS-containing nerves in human and primate CC tissue. Taken together with functional results of the present study, it is suggested that the endogenous cannabinoid, anandamide, depresses nerve-mediated relaxant responses, probably by presynaptic modulation of the activity of NOS-containing nerves. These findings point to the possibility of a peripheral mechanism for cannabis-related sexual dysfunctions.

Author contributions: Petter Hedlund 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: Hedlund, Andersson, Gratzke.
Acquisition of data: Gratzke, Hedlund.
Analysis and interpretation of data: Hedlund, Gratzke.
Drafting of the manuscript: Hedlund, Andersson, Stief, Gratzke.
Critical revision of the manuscript for important intellectual content: Hedlund, Andersson, Stief, Christ.
Statistical analysis: Gratzke, Hedlund.
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Supervision: Hedlund, Andersson, Stief, Christ.
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References

[1] Andersson KE, Wagner G. Physiology of penile erection. Physiol Rev 1995;75:191–236.
[2] Melman A, Christ GJ. Integrative erectile biology. The effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. Urol Clin North Am 2001;28:217–31, vii.
[3] Andersson KE. Pharmacology of penile erection. Pharmacol Rev 2001;53:417–50.
[4] Moreland RB, Hsieh G, Nakane M, Brioni JD. The biochemical and neurologic basis for the treatment of male erectile dysfunction. J Pharmacol Exp Ther 2001;296:225–34.
[5] Shrenker P, Bartke A. Suppression of male copulatory behavior by delta 9-THC is not dependent on changes in plasma testosterone or hypothalamic dopamine or serotonin content. Pharmacol Biochem Behav 1985;22:415–20.
[6] Ferrari F, Ottani A, Giuliani D. Inhibitory effects of the cannabinoid agonist HU 210 on rat sexual behaviour. Physiol Behav 2000;69:547–54.
[7] Melis MR, Succu S, Mascia MS, Argiolas A. Antagonism of cannabinoid CB1 receptors in the paraventricular nucleus of male rats induces penile erection. Neurosci Lett 2004;359:17–20.
[8] Ghasemi M, Sadeghipour H, Dehpour AR. Anandamide improves the impaired nitric oxide-mediated neurogenic relaxation of the corpus cavernosum in diabetic rats: involvement of cannabinoid CB1 and vanilloid VR1 receptors. BJU Int 2007;100:1385–90.
[9] Ghasemi M, Sadeghipour H, Mani AR, et al. Effect of anandamide on nonadrenergic noncholinergic-mediated relaxation of rat corpus cavernosum. Eur J Pharmacol 2006;544:138–45.
[10] Ghasemi M, Sadeghipour H, Shafaroodi H, et al. Role of the nitric oxide pathway and the endocannabinoid system in neurogenic relaxation of corpus cavernosum from biliary cirrhotic rats. Br J Pharmacol 2007;151:591–601.
[11] Van Diepen H, Schlicker E, Michel MC. Prejunctional and peripheral effects of the cannabinoid CB(1) receptor inverse agonist rimonabant (SR 141716). Naunyn Schmiedebergs Arch Pharmacol 2008;378:345–69.
[12] Saso L. Effects of drug abuse on sexual response [in Italian]. Ann Ist Super Sanita 2002;38:289–96.
[13] Jatoi A, Windschitl HE, Loprinzi CL, et al. Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. J Clin Oncol 2002;20:567–73.
[14] Hedlund P, Ny L, Alm P, Andersson KE. Cholinergic nerves in human corpus cavernosum and spongiosum contain nitric oxide synthase and heme oxygenase. J Urol 2000;164:868–75.
[15] Pertwee RG. Cannabinoids and the gastrointestinal tract. Gut 2001;48:859–67.
[16] Ralevic V. Cannabinoid modulation of peripheral autonomic and sensory neurotransmission. Eur J Pharmacol 2003;472:1–21.
[17] Okamura T, Ayajiki K, Toda N. Monkey corpus cavernosum relaxation mediated by NO and other relaxing factor derived from nerves. Am J Physiol 1998;274:H1075–81.
[18] Storr M, Gaffal E, Saur D, Schusdziarra V, Allescher HD. Effect of cannabinoids on neural transmission in rat gastric fundus. Can J Physiol Pharmacol 2002;80:67–76.
[19] Ny L, Pfeifer A, Aszodi A, et al. Impaired relaxation of stomach smooth muscle in mice lacking cyclic GMP-dependent protein kinase I. Br J Pharmacol 2000;129:395–401.
[20] Hedlund P, Alm P, Andersson KE. NO synthase in cholinergic nerves and NO-induced relaxation in the rat isolated corpus cavernosum. Br J Pharmacol 1999;127:349–60.
[21] Stein RJ, Santos S, Nagatomi J, et al. Cool (TRPM8) and hot (TRPV1) receptors in the bladder and male genital tract. J Urol 2004;172:1175–8.
[22] Lazzeri M, Barbanti G, Beneforti P, Turini D. Intraurethrally infused capsaicin induces penile erection in humans. Scand J Urol Nephrol 1994;28:409–12.
[23] Johnson SD, Phelps DL, Cottler LB. The association of sexual dysfunction and substance use among a community epidemiological sample. Arch Sex Behav 2004;33:55–63.

Editorial Comment on: Localization and Function of Cannabinoid Receptors in the Corpus Cavernosum: Basis for Modulation of Nitric Oxide Synthase Nerve Activity

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Penile erection is a complex neurovascular event that relies on vasodilatation of erectile tissues due to neural- and endothelial-derived nitric oxide (NO) released by activation of parasympathetic nerves on sexual stimulation and by further shear-stress–dependent stimulation of the cavernous endothelial lining [1]. Cannabinoids are signaling molecules derived from the cell membranes of nerves, blood cells, and endothelial cells that have earlier been related to erectile function and male sexual behavior [2,3]. Endogenous and exogenous cannabinoids were initially thought to regulate erectile function and sexual activity through the modulation of neural transmission of brain oxytocynergic neurons from paraventricular nucleus [2]. Endocannabinoid CB1 receptors, however, have recently also been found to regulate peripheral nitrergic neurotransmission in the rat corpus cavernosum (CC) [4].

The work by Gratzke et al [5] provides novel information on the role of cannabinoids in the peripheral neurotransmission controlling penile erection and CB2 receptors, usually found in association with immune tissues, identified for the first time in the CC in association with nitrergic nerves. Both CB1 and CB2 receptors were co-localized with NO synthase and with the vanilloid transient receptor potential 1 (TRPV1), mainly expressed in sensory nerves. Of special interest is the fact that the study was carried out in humans and in nonhuman primates; therefore, the results are potentially transferable to humans. In this regard, the functional experiments of the study show that the endogenous CB1 and CB2 agonist anandamide depresses the nonadrenergic noncholinergic (NANC) relaxations of the primate CC; these observations would be consistent with epidemiologic data associating the use of cannabis with sexual dysfunction in men [3]. The functional results with anandamide and the fact that both CB1 and CB2 are found in the primate CC conflict with the results obtained in the rat, which suggests that caution must be taken when translating results obtained in animal models to humans and points out the need to carry out control experiments in animal species closer to man, such as nonhuman primates.

References

[1] Andersson KE, Wagner G. Physiology of penile erection. Physiol Rev 1995;75:191–236.
[2] Castelli MP, Piras AP, Melis T, et al. Cannabinoid CB1 receptors in the paraventricular nucleus and central control of penile erection: immunocytochemistry, autoradiography and behavioral studies. Neuroscience 2007;147:197–206.
[3] Johnson SD, Phelps DL, Cottler LB. The association of sexual dysfunction and substance use among a community epidemiological sample. Arch Sex Behav 2004;33:55–63.
[4] Ghasemi M, Sadeghipour H, Mani AR, et al. Effect of anandamide on nonadrenergic noncholinergic-mediated relaxation of rat corpus cavernosum. Eur J Pharmacol 2006;544:138–45.
[5] Gratzke C, Christ GJ, Stief CG, Andersson K-E, Hedlund P. Localization and function of cannabinoid receptors in the corpus cavernosum: basis for modulation of nitric oxide synthase nerve activity. Eur Urol 2010;57:342–9.

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