181

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CB1 AND CB2 RECEPTORS IN THE URINARY BLADDER OF DIFFERENT SPECIES: MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION

Hypothesis / aims of study

Recent studies have shown beneficial effects of cannabis-based extracts on symptoms in patients with urgency incontinence due to multiple sclerosis (1, 2). However, the underlying mechanism is not known. The aim of the present study was to characterize the distribution of CB1 and CB2 receptors in the urinary bladder of different species and to analyze the possible involvement of CB1 / CB2 receptors in contractile mechanisms of bladder smooth muscle in vitro and in vivo.

Study design, materials and methods

The expression of the CB1- and CB2 receptor was studied with Western blot analysis and fluorescence immunohistochemistry in rat, monkey and human bladder. Urothelium and detrusor were analyzed separately. Double-staining was performed for CB1/CB2 receptors, vesicular acetylcholine transporter (VAChT), CGRP, and transient receptor potential V1 ion channel (TRPV1). The effects of anandamide, an endogenous CB1/CB2 receptor agonist which also activates TRPV1, and CP 55,940, a potent mixed CB1/CB2 receptor agonist on contractions induced by transmural activation of nerves or by pharmacological activation of isolated bladder smooth muscle were recorded in tissue baths. In addition, the effects of anandamide (intravesical administration) and CP 55,940 (i.v.-injection) on bladder function were assessed with cystometric investigations in conscious rats.

Results

Western Blot analysis for CB1 and CB2 displayed clear bands in the rat, monkey and human bladder, respectively. When normalized to ß-actin, the density of CB1 receptor bands was similar in the human urothelium and detrusor, whereas the density of CB2- receptor bands in the urothelium was 72.8 % higher than in the detrusor ($p<0.05$). Using immunohistochemistry, CB2 immunoreactivity was localized urothelial cells, but CB1- immunoreactive (-IR) structures could not be detected in the urothelium. CB2-IR nerve fibres were observed between strands of smooth muscle cells of the detrusor. In double stained sections, CB2-IR terminal varicosities exhibited coinciding profiles with VAChT-IR nerve structures. In the suburothelial region, larger amounts of CB-2-immunoreactive nerve fibres were observed in comparison to the muscular wall. Slender CB2-IR nerve fibres that extended into the urothelium also expressed immunoreactivity for TRPV1. A majority of CB2- IR nerve fibres and varicosities expressed immunoreactivity for CGRP. Immunoreactivity for CB1 was not detected in nerve structures of the detrusor. In all species (n= 6-8), transmural activation of nerves produced frequency-dependent contractions, which were enhanced by 10 µM anandamide (16 and 32 Hz, $p<0.05$) (Fig 1). Administration of 10 µM CP 55,940 decreased nerve-induced contractions of the detrusor (16 and 32 Hz, $p<0.05$) (Fig. 1). Neither anandamide nor CP 55,940 had any effects on carbachol-induced contractions. In vivo, CP 55,940 (n=6) increased micturition interval (MI) and threshold pressure (TP) by 51.8 ± 5.9 % ($p<0.05$) and 51.1 ± 13.4 % ($p<0.05$), respectively. In contrast, anandamide (n=6) reduced MI and basal pressure by 18.8 ± 3.4 % ($p<0.05$) and 61.5 ± 4.9 % ($p<0.005$) after pretreatment with protamine sulphate, whereas no effect was observed on TP (Fig. 2).

Interpretation of results

The distribution of CB2- immunoreactivity on primary afferents and effects by CP55940 on “afferent” urodynamic parameters (i.e. MI, TP) suggest a role for CB2 receptors in sensory signals of the detrusor. A role in mechanoafferent functions may be proposed for urothelial CB2 receptors. The co-expression of immunoreactivities for VAChT and CB2, and inhibitory effects by CP55940 on nerve-mediated, but not carbachol-induced contractions suggest a possible modulatory effect on cholinergic nerves by CB2 receptor activities. Anandamide may not be a good tool for studies of CB receptor functions and we speculate that the current functional findings are related to the compounds activity at TRPV1.

Concluding message

CB2-receptors appear to be involved in sensory functions of micturition and a basis for CB2 receptor-mediated modulation of cholinergic nerve activity may be considered. Further studies aimed to investigate the role for the CB2 receptors in urgency disorders would be of interest.

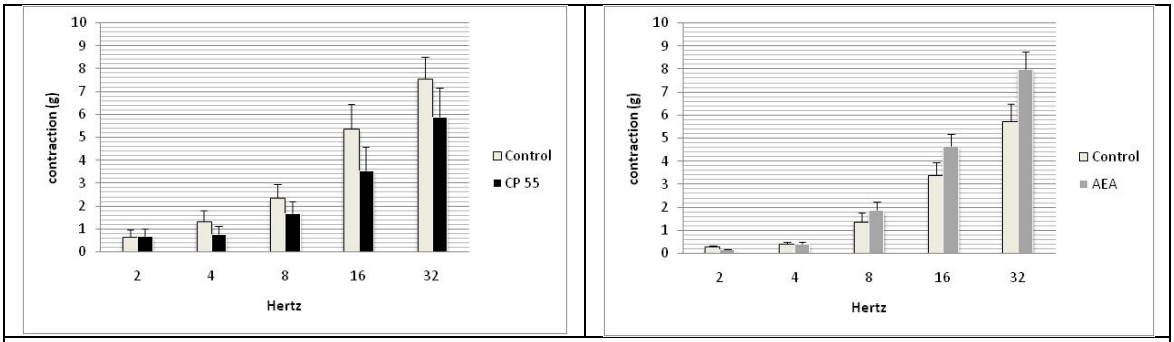

Figure 1: Responses of bladder smooth muscle of rhesus monkeys to electrical field stimulation (EFS, 2,4,8,16 and 32 Hertz). While administration of anandamide (AEA, 10 µM) increased contractile responses, CP 55,940 (10 µM) decreased responses significantly at 16 and 32 Hz(*p<0,05).

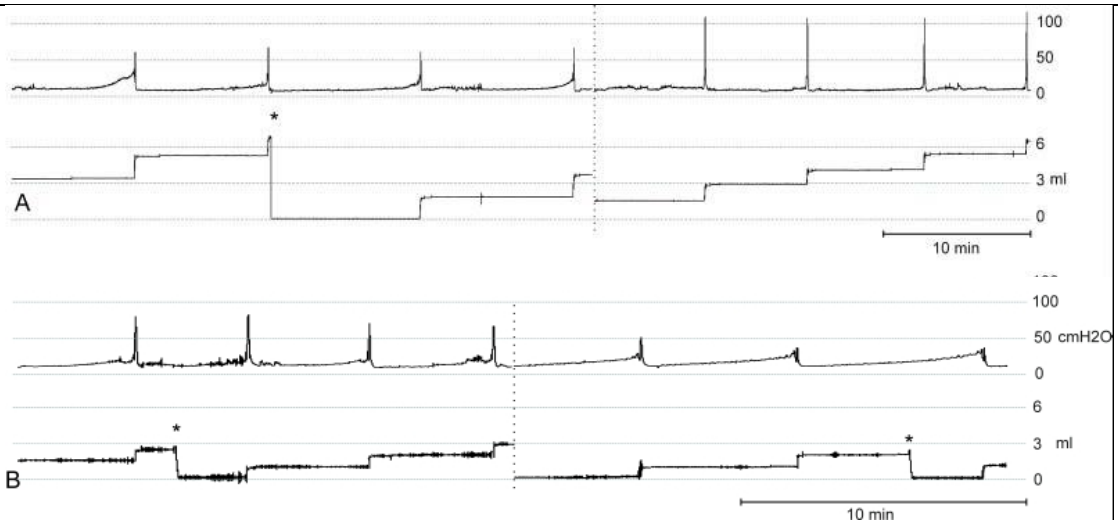

Figure 2: Cystometrogram from one rat showing bladder pressure (upper line) and voided volume (lower line) before and after intravesical infusion of anandamide after pretreatment with protamine sulphate (A) and intravenous injection of CP 55,940 (B).

References

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3.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**All procedures were conducted in compliance with guidelines established by the Wake Forest University Animal Care and Use Committee and the University of Lund Ethics Committee.**