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DISTRIBUTION OF THE ENDOCANNABINOID SYSTEM IN THE RAT AND HUMAN BLADDER

Hypothesis / aims of study

Cannabis has an effect on urge incontinence(1) probably mediated through endocannabinoid system-dependent mechanisms. Although cannabinoid expression has been identified in the human and rat bladder, the results are inconsistent and the main components of the endocannabinoid system have yet to be reported. Consequently we investigated the expression and distribution of the cannabinoid receptors (CB1 & CB2), transient receptor potential vanilloid type 1 (TRPV1) receptor, and cannabinoid-modulating enzymes, fatty acid amide hydrolase (FAAH) and N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD) in the rat and human bladder.

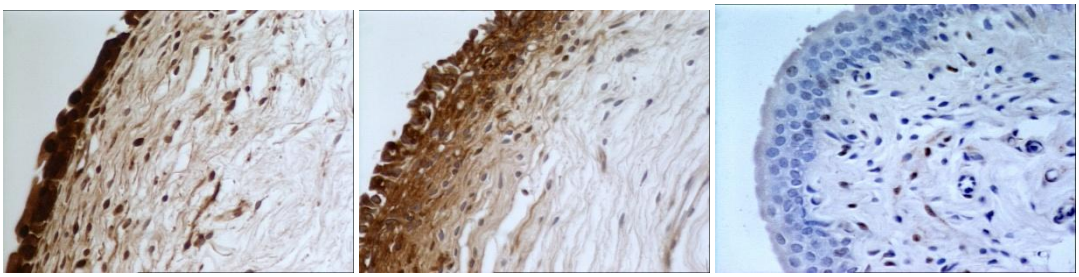
Study design, materials and methods

Immunohistochemistry (IHC) was performed on 4% paraformaldehyde fixed, paraffin-embedded 5μm sections of rat and human bladder using polyclonal antibodies to CB1, CB2, NAPE-PLD, FAAH & TRPV1. Rat brain was used as a postive control for CB1, NAPE-PLD, FAAH. Rat spleen was used as a positive control for CB2 and human skin was a positive control for TPVR1. CB1 and CB2 peptides were used on the rat and human bladder sections to confirm specificity of staining and rat tongue was used as a negative control. Sections were stained with 3,3'-diaminobenzidine and counterstained using haematoxylin. Western Blots were carried out on rat and human bladder, brain, spleen, and tongue samples which were homogenised, centrifuged and subjected to SDS-PAGE. Samples were probed with the same antibodies as for IHC overnight. Sigma anti-rabbit horseradish peroxidase was used as the secondary antibody. CHO cells were cultured and transfected with human CB1 DNA using Fugene 6. These served as positive and negative controls for the western blots.

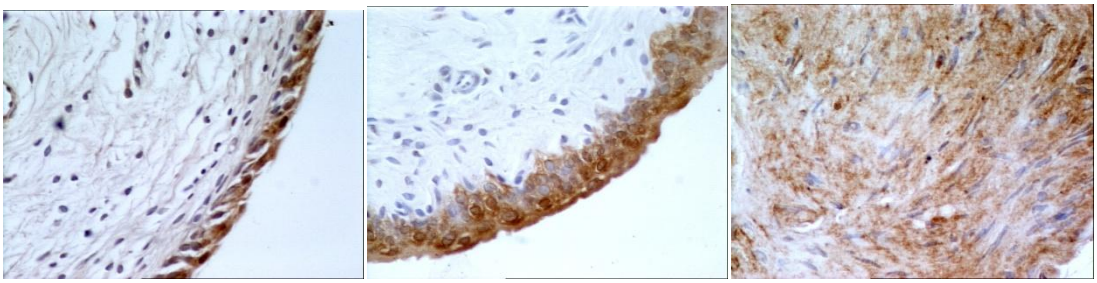
Results

Immunoreactivity for CB1, FAAH, NAPE-PLD & TPVR1 was observed in rat and human urothelium and detrusor (brown staining). CB2 immunostaining was observed only in detrusor muscle of the rat and only in the human urothelium. See figures below (all x400).

A: Human Urothelium CB1 B: Human Urothelium CB2 C: Rat Urothelium CB2



D: Human Urothelium NAPE E: Rat Bladder FAAH F: Human Detrusor TPVR1



Immunoblots indicated an identical molecular mass of 45kDa for CB2 in rat bladder and spleen and 43kDa in the human bladder. CB1 produced specific bands of 45kDa in the rat bladder and brain and 40kDa in the human bladder. Immunoblots for FAAH showed specific bands at 42kDa in human bladder,46kDa in rat bladder and 45kDa in brain. Specific bands using the NAPE-PLD antibody were seen at 53 kDa in the brain and the rat bladder and 45 kDa in the human bladder. TPVR1 showed a specific band at 104 kDa in the human and rat bladder and rat brain.

Interpretation of results

The current study provides qualitative immunohistochemical and quantitative molecular data for the expression of the endocannabinoid system (receptors and modulating enzymes). Our data confirm the presence of the endocannabinoid system in the human bladder. The recent completion of Cannabinoids in Multiple Sclerosis study(1) showed a significant reduction in urinary incontienence episodes in the cannabis group when compared to placebo. Our results showing the localization of the

endocannabinoid system in the human bladder provide evidence of the pathway through which the clinical effects of cannabis may be mediated, and are the first step towards identifying a new target for bladder control which may become amendable to pharmacological manipulation in the future.

Concluding message

For the first time, all the main components of the endocannabinoid system (receptors and enzymes) have been localised in the rat and human bladder. Cannabinoid and TRPV1 receptors and the modulating enzymes were expressed. CB2 expression in the Wistar rat bladder was different to that previously described in other rat species (2).

References

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2. Hayn MH, Ballesteros I, Miguel F et al. Functional and immunohistochemical characterisation of CB1 and CB2 receptors in rat bladder. Urol 2008;72 Suppl 5:1074-8.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Leicestershire, Northamptonshire & Rutland Research Ethics Commitee One**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**