selective 5-HT_{1A} receptor antagonist is endowed with favourable effects on the bladder, inducing increase of bladder capacity without derangement of bladder contractility.

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70

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Title (type in CAPITAL LETTERS, leave one blank line before the text):
CHARACTERISATION OF MUSCARINIC RECEPTORS IN THE PIG BLADDER DOME, BLADDER BASE AND PROXIMAL URETHRA

Aims of study: It has been reported that contraction of urinary bladder body is mediated via the smaller population of M_3-receptors, but M_2-mediated contraction has been demonstrated following M_3-receptor inactivation and elevation of cAMP levels. The present study investigates the characterisation of muscarinic receptor subtypes in the bladder dome, bladder base and proximal urethra of the female pig.

Methods: In receptor binding studies, displacement experiments using [^{3}H]QNB with 4-DAMP (M_3-selective antagonist) and methoctramine (M_2-selective antagonist) determined the M_2:M_3 receptor ratio in membranes of pig bladder and urethra. In the functional studies *in vitro,* the affinity of these antagonists against carbachol induced contractions of tissue strips were also calculated in normal tissues and following selective M_3-inactivation (incubation with 40μM 4-DAMP mustard in the presence of 1μM methoctramine to "protect" M_2-receptors), precontraction with 50mM KCl and relaxation with isoprenaline (30μM).

Results: In saturation binding studies, receptor density was significantly ($p<0.05$) more in bladder dome and base than in urethra, being 137.5±56.4, 130.5±25.7 and 44.1±13.2 fmol/mg protein, respectively. Dissociation constant (Kd) for [^{3}H] QNB in bladder dome, base and urethra was similar, being 0.27±0.04, 0.27±0.11and 0.26±0.07 nM, respectively. In competition binding studies, displacement of [^{3}H] QNB by 4-DAMP and methoctramine best fitted a 2-site model with Hill's slopes<1.0 in bladder dome and base, the high and low affinity site indicating M3 and M_2 receptor, respectively, and an M_2: M_3 ratio of 3:1. In urethra, displacement of [^{3}H] QNB by 4-DAMP and methoctramine best fitted 1-site model with Hill's slopes close to unity, the affinity indicating M_2 receptor.
On normal detrusor muscle strips in vitro, 4-DAMP had a high affinity in both bladder dome (n=12) and base (n=18), with Schild slopes close to unity, pKB value of 9.4±0.07 and 9.5±0.07,respecively. Methoctramine had a relatively low affinity in bladder dome (pKB=6.1±0.05, n=18). These results indicated that the M_3-subtype mediates contraction of the bladder dome and base. 4-DAMP also had a high affinity in proximal urethra (pKB=9.46±0.15, n=9), however the Schild slope was less than unity (0.56±0.08). Methoctramine demonstrated pKB values of 6.90±0.14 with Schild slopes close to unity in urethra (n=12). These results suggested that the contraction of urethra was mediated by M_3 and M_2 receptors. In tissues where the M_3-receptors had been inactivated and cAMP levels elevated, the affinity of 4-DAMP was significantly reduced in bladder dome (8.7±0.1, n=27,$P<0.001$) and base (8.5±0.08, n=12,$P<0.0001$) compared with normal tissues.

Conclusions: Bladder dome and base have similar distribution of muscarinic receptor subtypes, the M_3: M_2 ratio being 3:1. Urethra appears to have predominantly M_2 receptor. *In vitro*, the M_3-subtype appears to mediate contraction of the normal pig bladder dome and base, and an involvement of M2-receptors in contraction was noted following selective M_2-inactivation and cAMP elevation. Contraction of the pig urethra appears to be mediated by M_2 and M_3 receptors.

71

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Title (type in CAPITAL LETTERS, leave one blank line before the text):
IMMUNOHISTOCHEMICAL TOPOGRAPHY OF THE VANILLOID RECEPTOR IN THE NORMAL HUMAN BLADDER: PRELIMINARY DATA.

Aims of the study: Sofar the topography of the vanilloid receptor in the bladder has only been studied with direct autoradiographical methods (1) or with indirect immunofluorescence studies of afferent nerves. These studies located the receptor on the afferent nerves. Moreover a higher concentration of the receptor was noted at the bladder neck in relation to the body of the bladder. Recently a rabbit anti-capsaicin receptor polyclonal antibody has

become commercially available. The aim of the study was to confirm or to reject the current knowledge on the topography of the vanilloid receptor in the normal human bladder.

Methods: Bladder tissue was taken from cystectomy specimens or transurethrally.

In all cases a biopsy of the bladder neck or trigone was taken as well as a biopsy of the fundus of the bladder, including the muscularis.

Immunohistochemstry was performed on frozen bladder specimens with a polyclonal anti-capsaicin-antibody (Chemicon Int.®), using a three-step unlabeled peroxidase-anti-peroxidase (PAP) method, as described previously (2). Colocalisation studies were performed for CGRP and Substance P using laser confocal microscopy.

Results:

1) The presence of the vanilloid receptor on unmyelinated nerves is confirmed.

2) Immunoreactivity was also noted on the Schwann cells (perineurium) of myelinated nerves.

3) Strong immunoreactivity was noted on the smooth muscle cells of the muscularis layer and of the lamina propria. The heterogeneity of the immunoreactivity in the muscular layer pleads against eventual background colouring.

4) The reported higher concentration at the level of the bladder neck is caused by the presence of more muscle fibers in the region and by the compactness and orientation of these fibers and not by a higher individual immunoreactivity.

Discussion: The presence of the receptor on the unmyelinated afferent nerves is confirmed. The presence on the perineurium and the abundant immunoreactivity of the smooth muscle is more puzzling. Possibly vanilloidreceptor blockade by intravesical capsaicin or resiniferatoxin not only results in an indirect smooth muscle relaxation by desensitisation of the afferent branch of a spinal C-fiber mediated reflex arc, but also in a direct relaxation by interaction with the smooth muscle itself.

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72

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Title (type in CAPITAL LETTERS, leave one blank line before the text):
EXTRAMURAL AMBULATORY CYSTOMETRY AS A RESEARCH TOOL TO QUANTIFY BLADDER OVERACTIVITY IN RANDOMIZED CLINICAL TRIALS

Introduction

Bladder overactivity is poorly defined by the ICS. Descriptive terms like 'a disorder of the filling/storage phase', 'involuntary bladder contractions, while the patient is attempting to inhibit', 'detrusor hyperreflexia' or 'detrusor instability' are today's confusing clinical practice. In this study *"Bladder overactivity is a dysfunction of the bladder, in which a subject has no or decreased control over sudden occurring contractions of the musculus detrusor, in such a way that this leads to premature passing of urine".*