

selective 5-HT<sub>1A</sub> receptor antagonist is endowed with favourable effects on the bladder, inducing increase of bladder capacity without derangement of bladder contractility.

#### REFERENCES

- 1) Urology 50 (Suppl. 6A): 36-52, 1997.
- 2) J Auton Nervous System Supp 393-397, 1986.
- 3) Eur J Pharmacol 334: 1-23, 1997.
- 4) J Pharmacol Exp Ther 290: 1258-1269, 1999.

## 70

Author(s): T Yamanishi, CR Chapple, K Yasuda\*, R Chess-Williams

Institution, city, country:

Royal Hallamshire Hospital and University of Sheffield, Sheffield, UK, Koshigaya Hospital, Dokkyo University, Saitama, Japan\*

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<sub>3</sub>-receptors, but M<sub>2</sub>-mediated contraction has been demonstrated following M<sub>3</sub>-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 [<sup>3</sup>H]QNB with 4-DAMP (M<sub>3</sub>-selective antagonist) and methoctramine (M<sub>2</sub>-selective antagonist) determined the M<sub>2</sub>:M<sub>3</sub> 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<sub>3</sub>-inactivation (incubation with 40 μM 4-DAMP mustard in the presence of 1 μM methoctramine to "protect" M<sub>2</sub>-receptors), precontraction with 50 mM KCl and relaxation with isoprenaline (30 μM).

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

**Conclusions:** Bladder dome and base have similar distribution of muscarinic receptor subtypes, the M<sub>3</sub>: M<sub>2</sub> ratio being 3:1. Urethra appears to have predominantly M<sub>2</sub> receptor. *In vitro*, the M<sub>2</sub>-subtype appears to mediate contraction of the normal pig bladder dome and base, and an involvement of M<sub>2</sub>-receptors in contraction was noted following selective M<sub>3</sub>-inactivation and cAMP elevation. Contraction of the pig urethra appears to be mediated by M<sub>2</sub> and M<sub>3</sub> receptors.

## 71

Author(s): D. De Ridder, T. Roskams, D. Ost, H. Van Poppel, L. Baert

Institution, city, country: Dept. of Urology and Dept. of Pathology, Katholieke Universiteit Leuven, Leuven, Belgium

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:** So far 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