Residual volumes did not increase even after 100 μg/kg of administration. On the other hand, when procaterol were administrated i.v., bladder capacity was not increased even at 100 μg/kg (Fig. 1B). CL316243 slightly decreased blood pressure and increased heart rates only at high doses (10 -100 μg/kg i.v.: Fig. 1A). The maximal decrease in blood pressure was less than 10 % and the maximal increase in heart rates was only 10 %. On the other hand, procaterol (1-100 μg/kg) decreased blood pressure and increased heart rates in a dose-dependent manner (Fig. 1B). These effects were significant from 1 μg/kg. At 100 μg/kg, blood pressure decreased by 20 % and the effect lasted at least for 30 minutes. Procaterol given at 10 and 100 μg/kg increased heart rates by 40 %.

CONCLUSIONS

The results of the present study using conscious rats indicate that the selective β3-adrenoceptor agonist, CL316243, can inhibit the PGE2-induced bladder hyperactivity with minimal cardiovascular effects. In contrast to CL316243, procaterol, a selective β2-adrenoceptor agonist, showed negligible effects on the bladder hyperactivity, but had significant effects on the cardiovascular system. A β3-adrenoceptor agonist might be used for controlling bladder hyperactivity without affecting the cardiovascular system.

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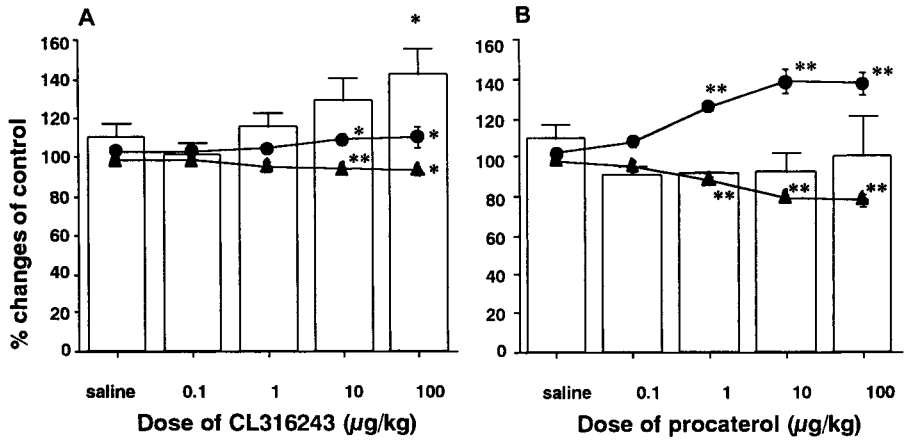

Fig. 1. Effects of CL316243 (A) and procaterol (B) on bladder capacity (open bars), heart rates (closed circles) and mean blood pressure (closed triangles).

Each value represents the mean ± S.E. of 6-7 animals. *,** ; $p<0.05$, $p<0.01$ significantly different from control, respectively by paired *t*-test.

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EFFECT OF ANTAGONISTS FOR SEVERAL 5-HT RECEPTORS ON THE MICTURITION REFLEX IN RATS

AIMS OF STUDY

Several neurotransmitters have been identified as inhibitory transmitters in the micturition reflex pathways at both spinal and supraspinal sites, including 5-HT, GABA, glycine, dopamine, acetylcholine, enkephalins and other peptides (1). With regard to 5-HT, it has been demonstrated that electrical stimulation of 5-HT-containing neurons in the caudal raphe and activation of postsynaptic 5-HT receptors in the spinal cord of cats, *via* the release of 5-HT, inhibit bladder contractions (2). Multiple 5-HT receptors have been characterized in mammalian species based on their affinity for different 5-HT agonists and antagonists and/or gene structure (3). Our previous studies (4) have shown that 5-HT_{1A} receptor neutral antagonists influence central control of lower urinary tract function, decreasing

the frequency of bladder voiding contractions and increasing bladder capacity. In order to better define the role of various 5-HT receptors on the micturition reflex, we have tested the effects of several antagonists of different 5-HT families in the anaesthetised rat bladder voiding contractions model, and on cystometrographic parameters in conscious rats.

METHODS

Urinary bladder of female anaesthetised rats was catheterised *via* the urethra and filled with physiological saline until spontaneous bladder contractions occurred. Intravesical pressure was measured by a pressure transducer and displayed continuously on a chart recorder. The time of bladder quiescence (disappearance time of rhythmic contractions in min) observed after injection of the different compounds tested was recorded. Cystometry was performed in conscious rats with chronically (infravesical) implanted catheters to continuously record bladder capacity (evaluated as amount of saline infused between two voiding cycles) and maximal micturition pressure.

RESULTS

Intravenous injection of low doses of the 5-HT_{1A} antagonist p-MPPI induced a dose-dependent disappearance of bladder contractions (longer than 10 min). The dose-response curve was bell-shaped. The amplitude of bladder contractions was not markedly altered. The tested antagonists of 5-HT_2 (ketanserin, partially selective for the 2A subtype, and SB 242084, 2C selective), 5-HT_3 and 5-HT_4 serotoninergic families, as well as the 5-HT_{1B} antagonist GR 55562 were poorly or not active in this model (Table 1). Only the compounds showing activity in the voiding contractions model (induction of a block of contractions exceeding 4 min) were also evaluated in cystometry in conscious rats. Generally, the doses eliciting the maximal effect in the voiding contractions were tested in the cystometry model. The i.v. administration of 1 mg/kg of zatosetron (5-HT_3 antagonist) and RS 39604 (5-HT_4 antagonist) was practically devoid of effects both on BVC and MP, whereas p-MPPI induced a consistent increase of bladder capacity with no relevant activity on micturition pressure (Fig. 1).

Table 1.

Effects of tested compounds on rhythmic (isovolumic) bladder voiding contractions after intravenous administration in anaesthetised rats. Data represent the time of bladder quiescence (disappearance of rhythmic contractions: min ± SE) observed after i.v. injection of the indicated doses.

	5-HT_1 antagonists		5-HT_2 antagonists		5-HT_3 antagonist	5-HT_4 antagonist
Dose µg/kg	p-MPPI	GR 55562	Ketanserin	SB 242084	Zatosetron	RS 39604
3					2.20 ± 0.49	
10	1.08 ± 0.15				5.35 ± 1.97	
30	7.52 ± 0.99				3.39 ± 1.43	
100	15.98 ± 2.40	1.20 ± 0.27	2.55 ± 0.51	1.58 ± 0.64	4.49 ± 2.27	2.65 ± 1.52
300	11.75 ± 0.87		2.76 ± 1.09	0.68 ± 0.06		3.63 ± 0.77
1000	7.97 ± 1.05	1.30 ± 0.57	2.57 ± 0.77	1.58 ± 0.39	4.77 ± 1.51	5.54 ± 0.84

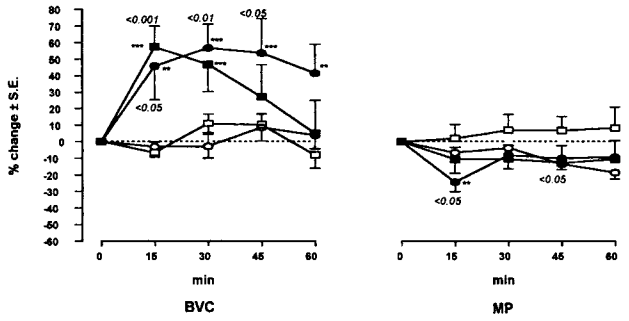

Figure 1

Time-course of the effects of i.v. injection of saline (open symbols) and p-MPPI (0.3 mg/kg: closed circles; 1 mg/kg: closed squares) on BVC and MP in conscious rats. Data represent the % changes versus basal values at different times during infusion. Significativity (<...) shown is "P between treatments" (ANOVA of contrast variables), indicating the difference between the trend observed in the control (saline) and treated groups at each time. ** = $p<0.01$, *** = $p<0.001$ versus basal values (within treatment).

CONCLUSIONS

These findings show that, among the tested antagonists and in agreement with our previous findings (4), only the

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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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.

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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