Characterization of the 5-HT receptor potentiating neuromuscular cholinergic transmission in strips of human isolated detrusor muscle

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In human isolated detrusor strips, submaximal contractile responses evoked by electrical stimulation were resistant to hexamethonium (30 μ M) and abolished by tetrodotoxin (0.6 μ M) and hyoscine (1 μ M), indicating the activation of postganglionic cholinergic nerves. In methysergide (1 μ M) and ondansetron (3 μ M) pretreated tissues, 5-hydroxytryptamine (5-HT) (0.3 nM–1 μ M) caused a concentration-dependent increase in the amplitude of contractions (pEC_{50} = 8.1), which was antagonized by the selective 5-HT₄ receptor antagonist GR 113808 (3, 10 and 30 nM) in a competitive manner. Schild analysis yielded a pA_2 estimate of 8.9, a value comparable to that reported for GR 113808 in other animal and human peripheral tissues (8.8–9.7). Our findings indicate that neuromuscular cholinergic transmission in human isolated detrusor muscle is facilitated by neural 5-HT receptors belonging to the 5-HT₄ subtype. The human urinary bladder can thus be regarded as an additional site in which 5-HT₄ receptors are distributed.

Keywords: Human urinary bladder; detrusor strips; neuromuscular cholinergic transmission; 5-hydroxytryptamine; GR 113808; 5-HT₄ receptors

Introduction Excitatory effects elicited by 5-hydroxytryptamine (5-HT) have been described in both unstimulated (Klarskov & Hørby-Petersen, 1986) and electrically stimulated human detrusor strips (Hindmarsh *et al.*, 1977; Corsi *et al.*, 1991). In unstimulated preparations, the 5-HT-induced contractile response is probably mediated by 5-HT_{2A} receptors located on the effector cells, as demonstrated by ketanserin antagonism and by tetrodotoxin resistance (Klarskov & Hørby-Petersen, 1986). In stimulated preparations, 5-HT facilitates neuromuscular cholinergic transmission via receptors different from the 5-HT₁, 5-HT_{2A} and 5-HT₃ subtypes. In fact, the 5-HT₁/5-HT₂ receptor antagonist methysergide and ketanserin were only slightly effective or ineffective (Hindmarsh *et al.*, 1977; Corsi *et al.*, 1991), as was the 5-HT₃ receptor antagonist ondansetron (Corsi *et al.*, 1991). Conversely, the 5-HT potentiating effect was sensitive to micromolar concentrations of tropisetron (Corsi *et al.*, 1991), a low affinity competitive antagonist at central and peripheral 5-HT₄ receptors (Tonini *et al.*, 1991). However, the reported pA_2 value for tropisetron (7.0 instead of 6.5) and a marked reduction of the 5-HT response maximum, have raised some uncertainty as to the type of antagonism observed, as well as on the definition of receptors involved. The need for a careful characterization of this 'atypical' 5-HT receptor has been recently stressed (Ford & Clarke, 1993). The availability of high affinity antagonists at 5-HT₄ receptors, such as GR 113808 (Kaumann, 1993; Gale *et al.*, 1994), prompted us to investigate whether these receptors are involved in the potentiating effect of 5-HT on human isolated detrusor strip contractions to electrical field stimulation.

Methods Specimens from the anterior part of the dome of the urinary bladder were obtained from patients undergoing total cystectomy due to bladder base malignancy. Muscular

strips (20 mm long, 4 mm wide) were prepared by removing the serosal and mucosal layers and were mounted isometrically (tension: 20 mN) in a 5 ml bath containing modified Krebs solution gassed with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C. Methysergide (1 μ M) and ondansetron (3 μ M) were added to the solution in order to isolate pharmacologically putative 5-HT₄ receptors. After 90 min equilibration, the strips were field stimulated by means of two platinum electrodes connected to a MARB ST 87 stimulator. Trains of electrical pulses at 5 Hz and 5 s in duration were delivered at 1 min intervals, at 0.1 ms pulse width and 60 V. This stimulation evoked reproducible submaximal contractions, the amplitude of which was approximately 30% of that obtained by 20 Hz stimulation (Corsi *et al.*, 1991). Cumulative concentration-response curves to 5-HT were obtained using half logarithmic dosing increments until the maximum effect was reached. Two agonist concentration-response curves were constructed in each tissue. Preliminary experiments showed that the second concentration-response curve was superimposable, providing that there were frequent solution changes (every 5 min) and 60 min intervals between curves. The potentiating effect of 5-HT, either in the absence or in the presence of GR 113808 (3, 10 and 30 nM) (incubation time: 30 min) was studied. Antagonist pA_2 estimate was calculated following Schild regression analysis, using 5-HT concentration-ratios determined at EC_{50} levels in control and test curves. Confidence limits (CL) at 95% probability for the slope of the regression were evaluated by using a computer programme (PHARM/PCS, Version 4.1) based on a manual of pharmacological calculations (Tallarida & Murray, 1986). Results were expressed as means $\pm$ s.e. mean of *n* observations. The modified Krebs solution (pH 7.4) had the following composition (mM): NaCl 120, KCl 4.7, MgSO₄·7H₂O 0.6, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2.0 and glucose 10. Aqueous solutions of the following drugs were used: hyoscine hydrochloride, hexamethonium bromide and 5-hydroxytryptamine creatinine sulphate (all from Sigma Chemical Co.), tetrodotoxin (TTX) (Sankyo), ondansetron and GR 113808 ([1-[2-[(methylsulphonyl)amino]ethyl]4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate) (both synthesized

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Figure 1 Concentration-response curves for the potentiation of electrically-induced cholinergic contractions of the human isolated detrusor strips by 5-HT. In (a) control curve (O); curves constructed in the presence of 3 (●), 10 (■) and 30 nM (▲) GR 113808. (b) Schild plot of the data in (a). Values are mean $\pm$ s.e.mean, $n = 5-9$.

by Boehringer Ingelheim Italy, Milan). Methysergide maleate (Sandoz) was dissolved in methanol and then diluted in distilled water.

Results Electrical field stimulation (5 Hz for 5 s every 60 s, 0.1 ms pulse duration, 60 V) evoked reproducible submaximal contractions, which were unaffected by hexamethonium (30 μ M, $n = 5$) and abolished by TTX (0.6 μ M, $n = 5$) and hyoscine (1 μ M, $n = 5$). Cumulative administration of 5-HT (0.3 nM–1 μ M) produced a concentration-dependent increase (maximal increase: $86.6 \pm 9.6\%$, $n = 20$) in the amplitude of stimulated contractions, with a pEC_{50} value of 8.1 ± 0.06

($n = 20$). In the presence of 5-HT (1 μ M), the resulting activity was still resistant to hexamethonium (30 μ M, $n = 5$) and completely antagonized by hyoscine (1 μ M, $n = 5$). GR 113808 (3, 10 and 30 nM) had no effect on basal contractions, but caused a parallel rightward shift of the 5-HT-induced response, without depression of the maximum (Figure 1a). Schild regression analysis afforded a line (Figure 1b) with a slope of 0.9 (95% CL 0.7–1.1), which was not different from unity, and a pA_2 value of 8.9 ± 0.04 (slope constrained to 1).

Discussion In human isolated detrusor strips, trains of electrical pulses at low frequency (5 Hz) evoked hexamethonium-resistant, TTX- and hyoscine-sensitive contractions, indicating the activation of postganglionic cholinergic nerves (see Maggi *et al.*, 1989). Such contractile responses were enhanced by 5-HT in a concentration-dependent manner, through a hexamethonium-insensitive mechanism and were completely antagonized by hyoscine. This suggests that in the detrusor strips the excitatory effect of 5-HT is dependent on the release of acetylcholine (ACh) from cholinergic motor neurones. GR 113808 produced a concentration-dependent and parallel rightward displacement of the 5-HT response, without depression in maxima. The surmountable nature of antagonism, the unity slope of the Schild plot and the affinity estimate ($pA_2 = 8.9$) are entirely consistent with competitive antagonism at 5-HT₄ receptors. In fact, the latter value is in the range of the affinity estimates of GR 113808 at 5-HT₄ receptors observed in guinea-pig and rat alimentary canal (9.0–9.7) (Gale *et al.*, 1994). Using the same antagonist to counteract the positive inotropic effect caused by 5-HT₄ receptor stimulation in human isolated atrial strips, Kaumann (1993) found a pA_2 value of 8.8. The similarity of pA_2 estimates in isolated detrusor and atrial strips suggests 5-HT₄ receptor homogeneity in human peripheral tissues. Recently, the presence of 5-HT₄ receptors has also been demonstrated in the urinary bladder of two monkeys of the macaque family (Waikar *et al.*, 1994). At variance with human bladder, in monkey bladder these receptors are located post-junctionally, where they cause muscular relaxation through a mechanism antagonized with high affinity by GR 113808 ($pA_2 = 9.5$). In conclusion, our findings indicate that, in electrically stimulated human detrusor strips, the 'atypical' (Corsi *et al.*, 1991) excitatory 5-HT receptor, which promotes ACh release from cholinergic nerves, clearly belongs to the 5-HT₄ subtype. Based on this evidence, selective agonists at 5-HT₄ receptors could be valuable for the pharmacological treatment of micturition disturbances associated with detrusor hypomotility.

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