British Journal of Pharmacology (2009), 157, 271–280

0007-1188/09
www.brjpharmacol.org

# RESEARCH PAPER

# 5-hydroxytryptamine induced relaxation in the pig urinary bladder neck

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**Background and purpose:** 5-Hydroxytryptamine (5-HT) is one of the inhibitory mediators in the urinary bladder outlet region. Here we investigated mechanisms involved in 5-HT-induced relaxations of the pig bladder neck.
**Experimental approach:** Urothelium-denuded strips of pig bladder were mounted in organ baths for isometric force recordings of responses to 5-HT and electrical field stimulation (EFS).
**Key results:** After phenylephrine-induced contraction, 5-HT and 5-HT receptor agonists concentration-dependently relaxed the preparations, with the potency order: 5-carboxamidotryptamine (5-CT) > 5-HT = RS67333 > (±)-8-hydroxy-2-dipropylaminotetralinhydrobromide > m-chlorophenylbiguanide > α-methyl-5-HT > ergotamine. 5-HT and 5-CT relaxations were reduced by the 5-$HT_7$ receptor antagonist (2R)-1-[(3-hydroxyphenyl)sulphonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride and potentiated by (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY 100135) and cyanopindolol, 5-$HT_{1A}$ and 5-$HT_{1A/1B}$ receptor antagonists respectively. Inhibitors of 5-$HT_{1B/1D}$, 5-$HT_2$, 5-$HT_{2B/2C}$, 5-$HT_3$, 5-$HT_4$, 5-$HT_{5A}$ and 5-$HT_6$ receptors failed to modify 5-HT responses. Blockade of monoamine oxidase A/B, noradrenergic neurotransmission, α-adrenoceptors, muscarinic and purinergic receptors, nitric oxide synthase, guanylate cyclase and prostanoid synthesis did not alter relaxations to 5-HT. Inhibitors of $Ca^{2+}$-activated $K^+$ and ATP-dependent $K^+$ channels failed to modify 5-HT responses but blockade of neuronal voltage-gated $Na^+$-, $Ca^{2+}$- and voltage-gated $K^+$ ($K_v$)-channels potentiated these relaxations. Adenylyl cyclase activation and cAMP-dependent protein kinase (PKA) inhibition potentiated and reduced, respectively, 5-HT-induced responses. Under non-adrenergic, non-cholinergic, non-nitrergic conditions, EFS induced neurogenic, frequency-dependent, relaxations which were resistant to WAY 100135 and cyanopindolol.
**Conclusions and implications:** 5-HT relaxed the pig urinary bladder neck through muscle 5-$HT_7$ receptors linked to the cAMP-PKA pathway. Prejunctional 5-$HT_{1A}$ receptors and $K_v$ channels modulated 5-HT-induced relaxations whereas postjunctional $K^+$ channels were not involved in such responses. 5-$HT_7$ receptor antagonists could be useful in the therapy of urinary incontinence produced by intrinsic sphincter deficiency.
*British Journal of Pharmacology* (2009) **157**, 271–280; doi:10.1111/j.1476-5381.2009.00144.x; published online 20 March 2009

**Keywords:** 5-HT; muscle 5-$HT_7$ receptors; 5-$HT_{1A}$ receptors; adenylyl cyclase-dependent mechanism; $K^+$ channels; neuronal $K_v$ channels; pig urinary bladder neck

**Abbreviations:** 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-dipropylaminotetralinhydrobromide; 8-SPT, 8-(p-sulphophenyl)theophylline; GR 113808, 1-methyl-1H-indole-3-carboxylic acid, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl] methyl ester; GR 127935, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride; IbTX, iberiotoxin; $K_{ATP}$, ATP-dependent $K^+$; $K_{Ca}$, $Ca^{2+}$-activated $K^+$; $K_V$, voltage-gated $K^+$; L-NOARG, $N^G$-nitro-L-arginine; NOS, nitric oxide synthase; ODQ, 1H-[1,2,4]-oxadiazolo[4, 3-a]quinoxalin-1-one; PKA, cAMP-dependent protein kinase; Rp-8-CPT-cAMPS, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp-isomer; RS 67333, 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride; SB 258585, 4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl] benzenesulphonamide hydrochloride; SB 269970, (2R)-1-[(3-hydroxyphenyl)sulphonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride; SB 699551, N-[2-(dimethylamino)ethyl]-N-[[4′-[[(2-phenylethyl)amino]methyl][1,1′-biphenyl]-4-yl]methyl]cyclopentanepropanamide dihydrochloride; SDZ

SER 082, (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-bc][2,6]-naphthyridine fumarate; TTX, tetrodotoxin; WAY 100135, (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride; ω-CgTX, ω-conotoxin GVIA; Y 25130, N-(1-azabicyclo[2,2,2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride

## Introduction

In the urinary tract, 5-hydroxytryptamine (5-HT) plays an important role in the control of micturition through regulation of the parasympathetic emptying of the bladder and the somatic outflow to the external urethral sphincter (Ramage, 2006). Seven major subtypes of 5-HT receptors have been characterized by pharmacological, signal transductional and structural criteria and some have been further subdivided by overlapping pharmacological properties and second messenger coupling pathway. The $5\text{-HT}_1$ (A, B, D, E, F), $5\text{-HT}_2$ (A, B, C), $5\text{-HT}_4$, $5\text{-HT}_5$ (A, B), $5\text{-HT}_6$ and $5\text{-HT}_7$ receptors couple to G-proteins, whereas the $5\text{-HT}_3$ receptors are 5-HT-gated ion channels (Boess and Martin, 1994; Hoyer *et al.*, 2002; receptor nomenclature follows Alexander *et al.*, 2008). $5\text{-HT}_1$ and $5\text{-HT}_{5A}$ receptors are negatively coupled to adenylyl cyclase by $G_i$, $5\text{-HT}_2$ are positively coupled to PLC by $G_{q/11}$, $5\text{-HT}_3$ receptors are coupled to ligand-gated cation channel and $5\text{-HT}_4$, $5\text{-HT}_6$ and $5\text{-HT}_7$ receptors are positively coupled to adenylyl cyclase by $G_s$ (Hoyer *et al.*, 2002; Nelson, 2004).

5-HT stimulates micturition in intact animals and promotes contraction of isolated urinary bladder strips from several species including man (Klarskov and Hørby-Petersen, 1986). 5-HT induces detrusor muscle contraction via 5-HT receptors on both postjunctional (smooth muscle) and prejunctional (autonomic excitatory nerves) sites. The 5-HT-induced contractions are mediated mainly through muscle $5\text{-HT}_2$ receptors (Saxena *et al.*, 1985; Klarskov and Hørby-Petersen, 1986; Cohen, 1990). In addition, indirect contractile effects through the enhancement of excitatory neurotransmission involving several 5-HT subtypes have been reported in the guinea pig (Messori *et al.*, 1995), rat (Palea *et al.*, 2004) and human (Tonini *et al.*, 1994; D'Agostino *et al.*, 2006) detrusor muscle, and in the intravesical ureter of the pig (Hernández *et al.*, 2003).

In the bladder neck, nitric oxide (NO) (Thornbury *et al.*, 1992; Hernández *et al.*, 2007) and peptides such as pituitary adenylyl cyclase-activating polypeptide (Hernández *et al.*, 2006a,b) are involved in the non-adrenergic non-cholinergic (NANC) inhibitory neurotransmission producing relaxation of smooth muscle through neuronal and non-neuronal mechanisms. At this level, a relaxant effect of 5-HT has also been reported in pig (Hills *et al.*, 1984; Klarskov and Hørby-Petersen, 1986) and in man (Klarskov and Hørby-Petersen, 1986). However, there is no information concerning the mechanisms involved in these responses. As the bladder neck together with the proximal urethra are the integral part of the urine bladder outflow region (Hedlund, 2005; De Groat, 2006), understanding the nature of the transmitters and/or modulators and of the mechanisms involved in the control of the smooth muscle tone in this structure is essential in order to correct urinary continence (English *et al.*, 1999). For this reason, the current study was designed to investigate the mechanisms involved in the relaxations elicited by 5-HT in the pig urinary bladder neck.

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Received 24 October 2008; accepted 5 December 2008

## Methods

*Dissection and mounting*

Adult pigs of either sex with no lesions in their urinary tract were selected from the local slaughterhouse. Urinary bladders were removed immediately after death and kept at 4°C in chilled physiological saline solution [PSS; composition ($mmol \cdot L^{-1}$): NaCl 119, KCl 4.6, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11, $CaCl_2$ 1.5, $KH_2PO_4$ 1.2, EDTA (ethylenediamine tetraacetic acid) 0.027]. The adjacent connective and fatty tissues were removed carefully and strips were dissected out from the bladder neck as previously described (Hernández *et al.*, 2006b). Strips 4–6 mm long and 2–3 mm wide were suspended horizontally with one end connected to an isometric force transducer (Grass FT 03C) and the other one to a micrometer screw, in 5 mL organ baths containing PSS at 37°C gassed with carbogen (95% $O_2$ and 5% $CO_2$), to obtain a final pH of 7.4. The signal was continuously recorded on a polygraph (Graphtec Multicorder MC 6621). Passive tension of 2 g was applied to the strips and they were allowed to equilibrate for 60 min.

*Experimental procedure*

The contractile ability of the strips was determined by exposure to potassium-rich (124 $mmol \cdot L^{-1}$) PSS (KPSS; PSS with KCl exchanged for NaCl on an equimolar basis.). The mechanisms involved in the 5-HT-induced relaxations were carried out in strips precontracted with 1 $\mu mol \cdot L^{-1}$ phenylephrine. A first concentration-response curve to 5-HT and 5-HT receptor agonists was performed, the bath solution was changed every 20 min for a total period of 80 min, the preparations were incubated for 30 min with the 5-HT receptor antagonists or the inhibitors of neuronal voltage-gated $Na^+$- and $Ca^{2+}$-channels, $Ca^{2+}$-activated $K^+$ ($K_{Ca}$) channels, ATP-dependent $K^+$ ($K_{ATP}$) channels, voltage-gated $K^+$ (Kv) channels, nitric oxide synthase (NOS), guanylyl cyclase or cAMP-dependent protein kinase (PKA), and then a second relaxation concentration-response curve was constructed. For irreversible inhibition of monoamine oxidase (MAO) A/B activity, pargyline was incubated with the tissue for 1 h, then removed from the organ bath. In the experiments using electrical field stimulation (EFS), the strips were treated with guanethidine (10 $\mu mol \cdot L^{-1}$), atropine (1 $\mu mol \cdot L^{-1}$) and $N^G$-nitro-L-arginine (L-NOARG), for

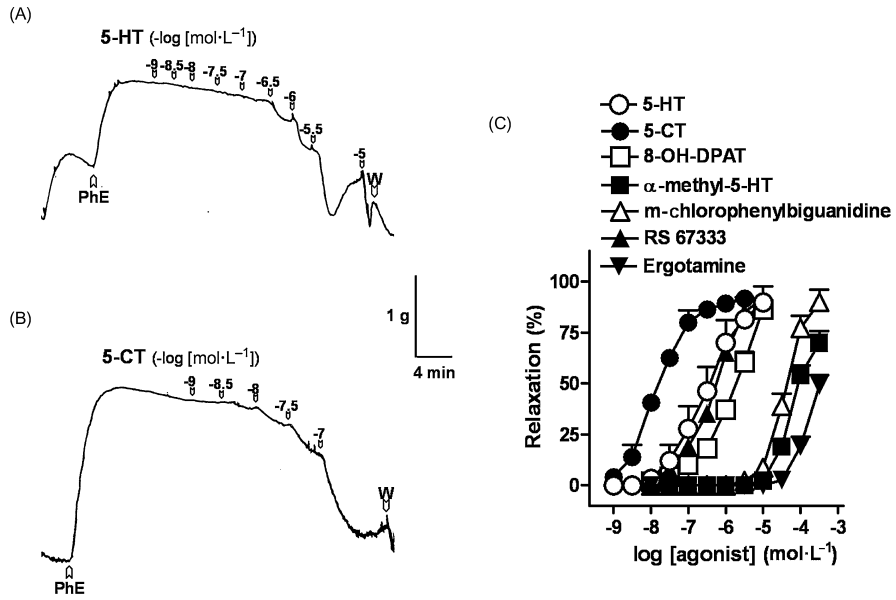


**Figure 1** Isometric force recordings showing the relaxations evoked by 5-hydroxytryptamine (5-HT, 1 nmol·L$^{-1}$–10 μmol·L$^{-1}$) (A) and 5-carboxamidotryptamine (5-CT, 1–100 nmol·L$^{-1}$) (B) on 1 μmol·L$^{-1}$ phenylephrine (PhE)-precontracted pig urinary bladder neck strips. The vertical bar shows tension (g) and the horizontal bar time (min). (C) Log concentration-response relaxation curves to 5-HT and 5-HT receptor agonists. Results are expressed as a percentage of the phenylephrine-induced contraction and represent mean ± SEM of six to twelve preparations. 8-OH-DPAT, (±)-8-hydroxy-2-dipropyl aminotetralin hydrobromide; RS 67333, 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride.

a period of 1 h, washing them every 20 min. These drugs were present throughout the experiment to block noradrenergic neurotransmission, muscarinic receptors and NOS respectively. Under such conditions, EFS was performed by delivering rectangular pulses (1 ms duration, 1–16 Hz, 20 s trains, with constant current output adjusted to 75 mA), at 4 min intervals, from a Cibertec CS20 stimulator (Barcelona, Spain). A first control curve was obtained to EFS and a second curve was performed after incubation with 5-HT$_1$ receptor antagonists for 30 min. Control curves were run in parallel.

*Data analysis and statistics*

Sensitivity to 5-HT and 5-HT agonists is expressed in terms of $pD_2$, where $pD_2 = -\log EC_{50}$ and $EC_{50}$ is the agonist concentration needed to produce half-maximal response. $pD_2$ was estimated by computerized non-linear regression analysis (GraphPad Prism, San Diego, CA, USA). Differences were analyzed by Student's *t*-test for paired and unpaired observations and by analysis of variance and *a posteriori* Bonferroni method for multiple comparisons. Differences were considered significant with a probability level of $P < 0.05$.

*Drugs and solutions*

The following drugs were used: 4-aminopyridine, apamin, atropine, ω-conotoxin GVIA (ω-CgTX), 1,9-dideoxy-forskolin, ergotamine, forskolin, guanethidine, 5-hydroxytryptamine (5-HT), iberiotoxin (IbTX), α-methyl-5-HT, L-NOARG, pargyline, phenylephrine, phentolamine, ritanserin, 8-(p-sulphophenyl)theophylline (8-SPT), suramin and tetrodotoxin (TTX) all from Sigma (USA). (±)-8-Hydroxy-2-dipropylaminotetralinhydrobromide (8-OH-DPAT), 5-carboxamidotryptamine (5-CT), m-chlorophenylbiguanide, cyanopindolol, glibenclamide, 1-methyl-1H-indole-3-carboxylic acid, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl] methyl ester (GR 113808), N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride (GR 127935), 1H-[1,2,4]-oxadiazolo[4, 3-a]quinoxalin-1-one (ODQ), 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride (RS 67333), 4-Iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzenesulphonamide hydrochloride (SB 258585), (2R)-1-[(3-hydroxyphenyl)sulphonyl]-2-[2-(4-methyl-1-piperidinyl) ethyl]pyrrolidine hydrochloride (SB 269970), N-[2-(dimethylamino)ethyl]-N-[[4′-[[(2-phenylethyl)amino]methyl]

**Table 1** Relaxation induced by 5-HT and 5-HT receptor agonists in the pig urinary bladder neck

| | n | $pD_2$ | Emax (%) |
|---|---|---|---|
| 5-HT | 12 | 6.5 ± 0.2 | 89.7 ± 7.8 |
| 5-CT | 9 | 7.9 ± 0.1* | 91.6 ± 4.3 |
| 8-OH-DPAT | 7 | 5.5 ± 0.2*,# | 86.4 ± 4.1 |
| α-methyl-5-HT | 6 | 4.2 ± 0.1*,# | 69.9 ± 5.9*,# |
| M-chlorophenylbiguanidine | 6 | 4.4 ± 0.1*,# | 89.9 ± 6.1 |
| RS 67333 | 9 | 6.3 ± 0.2# | 91.7 ± 5.9 |
| Ergotamine | 6 | 3.8 ± 0.1*,# | 49.1 ± 5.1*,# |

Results are expressed as percentage of the phenylephrine (1 μmol·L$^{-1}$)-induced contraction, and represent the mean ± SEM of n preparations.
*,#$P < 0.05$ versus 5-HT and 5-CT respectively (analysis of variance followed by Bonferroni method). Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax. 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-dipropyl aminotetralin hydrobromide.

[1,1′-biphenyl]-4-yl]methyl]cyclopentanepropanamide dihydrochloride (SB 699551), (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY 100135) and N-(1-azabicyclo[2,2,2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride (Y 25130) were provided by Tocris (UK). 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp-isomer (Rp-8-CPT-cAMPS) was provided by Biolog (Germany). Indomethacin was dissolved in 96% ethanol. Cyanopindolol, 1,9-dideoxy-forskolin, forskolin, GR 113808, ODQ, Rp-8-CPT-cAMPS, SB 269970, SB 699551 and WAY 100135 were dissolved in dimethyl sulphoxide. The other drugs were dissolved in distilled water. The solvents used had no effect on the contractility of the bladder neck preparations. Stock solutions were prepared daily in distilled water.

## Results

Urothelium-denuded strips of pig urinary bladder neck were allowed to equilibrate to a passive tension of 1.7 ± 0.1 g ($n = 77$). Under these conditions, KPSS (124 mmol·L$^{-1}$) produced a contraction of 2.2 ± 0.3 g ($n = 77$). The strips were precontracted with 1 μmol·L$^{-1}$ phenylephrine which induced a sustained contraction above basal tension of 1.9 ± 0.4 g ($n = 71$).

*Relaxations induced by 5-HT and 5-HT receptor agonists*

On phenylephrine-induced tone, 5-HT and the 5-HT receptor agonists produced concentration-dependent relaxations with the following order of potency: 5-CT > 5-HT = RS 67333 > 8-OH-DPAT > m-chlorophenylbiguanide > α-methyl-5-HT > ergotamine (Figure 1, Table 1).

*Effects of 5-HT receptor antagonists on relaxations to 5-HT*

The 5-HT$_7$ receptor selective antagonist SB 269970 (100 nmol·L$^{-1}$ and 300 nmol·L$^{-1}$) caused maximal rightwards displacements of the relaxation concentration-response curve to 5-HT (Figure 2A,B, Table 2) and 5-CT (Figure 2C, Table 2). WAY 100135 (1 μmol·L$^{-1}$) (Figure 3A,C, Table 2) and cyanopindolol (2 μmol·L$^{-1}$) (Figure 3B, Table 2), 5-HT$_{1A}$- and 5-HT$_{1A/1B}$-receptor antagonists, respectively, potentiated the relaxations to 5-HT. However, GR 127935 (100 nmol·L$^{-1}$), ritanserine (100 nmol·L$^{-1}$), (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-bc][2,6]-naphthyridine fumarate (SDZ SER 082) (1 μmol·L$^{-1}$), Y 25130 (1 μmol·L$^{-1}$), GR 113808 (1 μmol·L$^{-1}$), SB 699551 (100 nmol·L$^{-1}$) and SB 258585 (100 nmol·L$^{-1}$), selective antagonists of the 5-HT$_{1B/1D}$-, 5-HT$_2$-, 5-HT$_{2B/2C}$-, 5-HT$_3$-, 5-HT$_4$-, 5-HT$_{5A}$- and 5-HT$_6$-receptors, respectively, failed to modify the relaxations to 5-HT (Table 2).

*Role of the PKA pathway and of the K$^+$ channels on relaxations to 5-HT*

A threshold concentration (30 nmol·L$^{-1}$) of the adenylyl cyclase activator forskolin evoked a leftward displacement

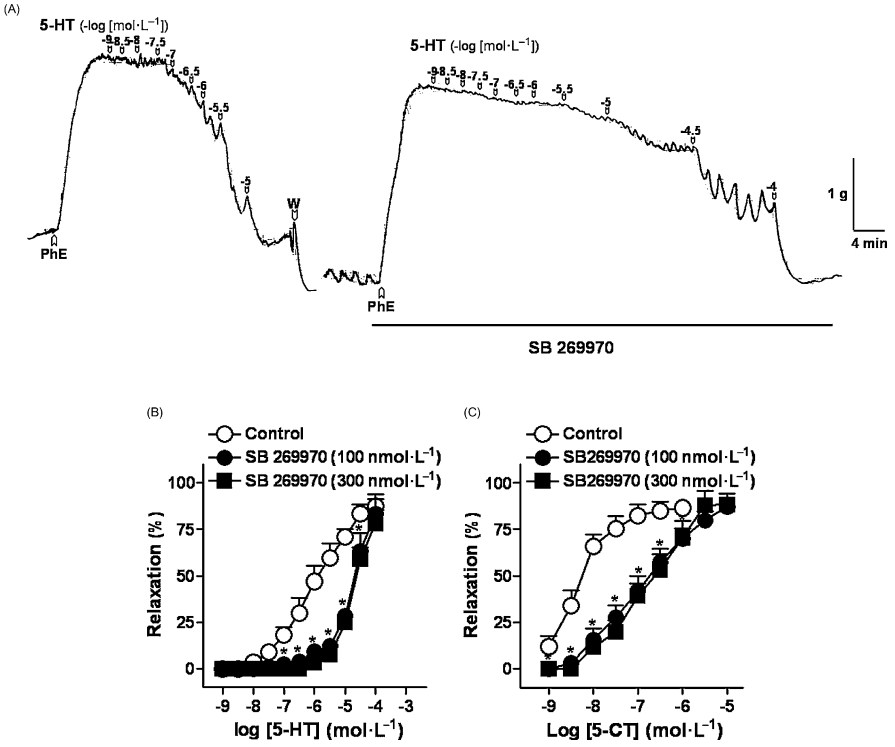


**Figure 2** (A) Isometric force recordings showing the relaxations evoked by 5-hydroxytryptamine (5-HT, 1 nmol·L$^{-1}$–10 μmol·L$^{-1}$) in the absence or presence of (2R)-1-[(3-hydroxyphenyl)sulphonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB 269970, 100 nmol·L$^{-1}$), on 1 μmol·L$^{-1}$ phenylephrine (PhE)-precontracted pig urinary bladder neck strips. Vertical bar shows tension in g and horizontal bar time in min. Log concentration-response relaxation curves to 5-HT (B) and 5-carboxamidotryptamine (5-CT) (C) in control conditions (open circles) and in the presence of 100 nmol·L$^{-1}$ (closed circles) and of 300 nmol·L$^{-1}$ (closed squares) of SB 269970. Results are expressed as a percentage of the-induced contraction and represent mean ± SEM of eight preparations. *$P < 0.05$, versus control (analysis of variance followed by Bonferroni method).

**Table 2** Effect of blockade of 5-$HT_{1A}$, 5-$HT_{1A/1B}$, 5-$HT_{1B/1D}$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_{5A}$ or 5-$HT_6$ receptors on relaxations to 5-HT, and of inhibition of 5-$HT_7$ receptors on relaxations to 5-HT and 5-CT

| | | *5-HT* | |
|---|---|---|---|
| | n | *pD₂* | *Emax (%)* |
| Control | 7 | 6.1 ± 0.2 | 81.7 ± 5.8 |
| WAY 100135 (1 μmol·L$^{-1}$) | 7 | 6.9 ± 0.1* | 82.1 ± 5.1 |
| Control | 7 | 5.9 ± 0.1 | 73.4 ± 7.5 |
| Cyanopindolol (2 μmol·L$^{-1}$) | 7 | 7.1 ± 0.1* | 75.9 ± 12.6 |
| Control | 6 | 6.1 ± 0.1 | 83.8 ± 12.2 |
| GR 127935 (100 nmol·L$^{-1}$) | 6 | 5.8 ± 0.2 | 88.1 ± 10.2 |
| Control | 6 | 6.1 ± 0.2 | 84.7 ± 4.5 |
| Ritanserin (100 nmol·L$^{-1}$) | 6 | 6.0 ± 0.1 | 83.9 ± 5.9 |
| Control | 6 | 5.9 ± 0.1 | 87.7 ± 5.5 |
| SDZ SER 082 (1 μmol·L$^{-1}$) | 6 | 6.0 ± 0.2 | 89.1 ± 7.5 |
| Control | 6 | 6.3 ± 0.1 | 91.1 ± 5.9 |
| Y 25130 (1 μmol·L$^{-1}$) | 6 | 6.3 ± 0.1 | 92.5 ± 4.5 |
| Control | 7 | 5.9 ± 0.1 | 79.2 ± 12.4 |
| GR 113808 (1 μmol·L$^{-1}$) | 7 | 6.1 ± 0.2 | 79.4 ± 10.0 |
| Control | 7 | 6.4 ± 0.1 | 86.2 ± 6.3 |
| SB 699551 (100 nmol·L$^{-1}$) | 7 | 6.4 ± 0.1 | 93.5 ± 5.1 |
| Control | 7 | 6.4 ± 0.1 | 78.9 ± 5.1 |
| SB 258585 (100 nmol·L$^{-1}$) | 7 | 6.5 ± 0.2 | 82.5 ± 4.8 |
| Control | 8 | 6.0 ± 0.2 | 87.7 ± 6.2 |
| SB 269970 (100 nmol·L$^{-1}$) | 8 | 4.7 ± 0.1* | 83.3 ± 8.0 |
| SB 269970 (300 nmol·L$^{-1}$) | 8 | 4.6 ± 0.1* | 77.9 ± 7.5 |
| | | *5-CT* | |
| Control | 8 | 8.4 ± 0.1 | 86.7 ± 4.1 |
| SB 269970 (100 nmol·L$^{-1}$) | 8 | 6.9 ± 0.1* | 87.2 ± 3.7 |
| SB 269970 (300 nmol·L$^{-1}$) | 8 | 6.8 ± 0.1* | 88.3 ± 5.9 |

Results are expressed as mean ± SEM of *n* preparations.
*$P < 0.05$ versus the control value (paired *t*-test and analysis of variance followed by Bonferroni method for multiple comparisons). Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax.
5-CT, 5-carboxamidotryptamine; GR 113808, 1-methyl-1H-indole-3-carboxylic acid, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl] methyl ester; GR 127935, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl l-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride; SB 258585, 4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzene-sulphonamide hydrochloride; SB 269970, (2R)-1-[(3-hydroxyphenyl) sulphonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride; SB 699551, N-[2-(dimethylamino)ethyl]-N-[[4′-[[(2-phenylethyl)amino]methyl] [1,1′-biphenyl] -4-yl]methyl]cyclopentanepropanamide dihydrochloride; SDZ SER 082, (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-bc][2,6]-naphthyridine fumarate; WAY 100135, (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride; Y 25130, N-(1-azabicyclo[2,2,2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride.

of the relaxation concentration-response curve to 5-HT (Figure 4A,B, Table 3) whereas 100 nmol·L$^{-1}$ 1,9-dideoxy-forskolin, a forskolin derivative that does not activate adenylyl cyclase, failed to modify these responses (Figure 4C, Table 3). The PKA inhibitor Rp-8-CPT-cAMPS (100 μmol·L$^{-1}$) induced a rightwards displacement of the relaxation concentration-response curve to 5-HT (Figure 4D, Table 3).

4-aminopyridine (1 mmol·L$^{-1}$), a $K_v$ channel inhibitor, produced a leftwards displacement of the 5-HT relaxation curve (Figure 5D, Table 4). However, either the blocker of $K_{Ca}$, tetraethylammonium (3 mmol·L$^{-1}$) or IbTX (100 nmol·L$^{-1}$), apamin (0.5 μmol·L$^{-1}$) and glibenclamide (1 μmol·L$^{-1}$), inhibitors of the large- and small-conductance $K_{Ca}$ and of $K_{ATP}$ channels, respectively, did not change the relaxations to 5-HT (Table 4).

*Effects of blockade of MAO A/B activity, noradrenergic-, cholinergic muscarinic-, nitrergic- and purinergic-systems and of prostanoids synthesis on relaxations to 5-HT*

Pargyline (100 μmol·L$^{-1}$), guanethidine (10 μmol·L$^{-1}$), phentolamine (1 μmol·L$^{-1}$), atropine (1 μmol·L$^{-1}$), L-NOARG (100 μmol·L$^{-1}$), ODQ (5 μmol·L$^{-1}$), suramin (100 μmol·L$^{-1}$), 8-SPT (100 μmol·L$^{-1}$) and indomethacin (10 μmol·L$^{-1}$), inhibitors of MAO A/B activity, noradrenergic neurotransmission, α-adrenergic and muscarinic receptors, NOS, guanylyl cyclase, $P_2$- and $P_1$-purinergic receptors and cyclooxygenase, respectively, failed to modify the relaxations to 5-HT (Table 5).

*Effects of 5-$HT_1$ receptor antagonists on relaxations to transmural nerve stimulation*

Under NANC and non-nitrergic conditions, EFS (1–16 Hz) evoked frequency-dependent relaxations (maximal relaxation of 83.3±8.9% of the phenylephrine-induced contraction, $n = 12$, at 16 Hz). These relaxations were not changed by WAY 100135 (1 μmol·L$^{-1}$) (Figure 3C) and cyanopindolol (2 μmol·L$^{-1}$), antagonists of the 5-$HT_{1A}$- and 5-$HT_{1A/1B}$- receptors respectively (Table 6).

*Involvement of neuronal voltage-gated cation channels on relaxations to 5-HT*

ω-conotoxin GVIA (1 μmol·L$^{-1}$) (Figure 5A,B, Table 7) and TTX (1 μmol·L$^{-1}$) (Figure 5C, Table 7), inhibitors of neuronal voltage-gated $Ca^{2+}$- and $Na^+$-channels, respectively, potentiated the relaxations to 5-HT.

## Discussion and conclusions

Our results suggest that 5-HT relaxes the bladder through activation of muscle 5-$HT_7$ receptors coupled to adenylyl cyclase activation. Prejunctional 5-$HT_{1A}$ receptors and $K_v$ channels could modulate 5-HT relaxations, whereas postjunctional $K^+$ channels seem not be involved in such responses.

Our experimental protocol has been carried out in urothelium-denuded preparations, thus indicating that receptors located at the smooth muscle are involved in the 5-HT-induced relaxations. This result agrees with that found in the urinary bladder of several species where 5-HT evokes part of its effect by activation of smooth muscle 5-HT receptors (Saxena *et al.*, 1985; Klarskov and Hørby-Petersen, 1986; Cohen, 1990). In the pig upper urinary tract, a high MAO activity reducing the responses to 5-HT has been reported (Hernández *et al.*, 2003). In the current study, pargyline, an irreversible MAO A/B inhibitor, did not change the relaxations to 5-HT, suggesting the absence of a MAO activity precluding the access of 5-HT to its receptor(s). In some vascular beds such as in the human umbilical artery, prostanoids modulate the effects induced by 5-HT (Karlsson *et al.*, 1998). In our study, the cyclooxygenase inhibitor indomethacin

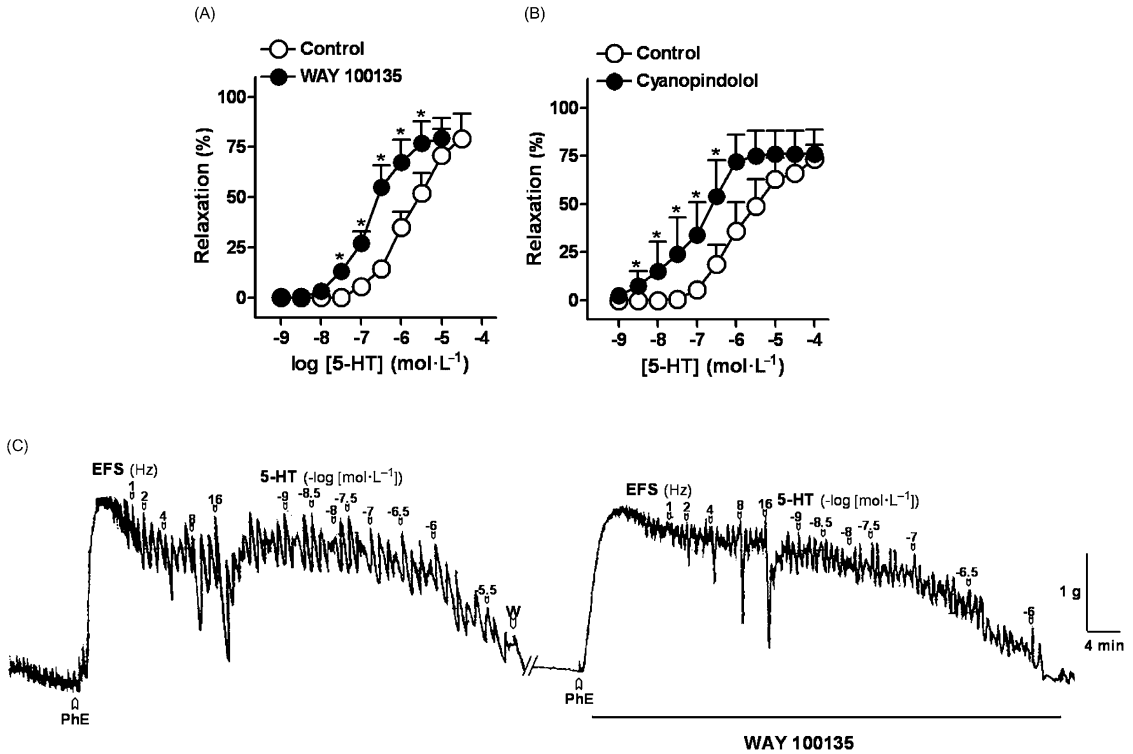


**Figure 3** Log concentration-response relaxation curves to 5-HT on 1 μmol·L$^{-1}$ phenylephrine (PhE)-precontracted pig urinary bladder neck strips in control conditions (open circles) and in the presence (closed circles) of WAY 100135 (1 μmol·L$^{-1}$) (A) or cyanopindolol (2 μmol·L$^{-1}$) (B). Results are expressed as a percentage of the PhE-induced contraction and represent mean ± SEM of seven preparations. *$P < 0.05$, versus control (paired *t*-test). (C) Isometric force recordings showing the relaxations evoked by electrical field stimulation (EFS, 1 ms duration, 1–16 Hz, 20 s trains) and 5-hydroxytryptamine (5-HT, 1 nmol·L$^{-1}$–3 μmol·L$^{-1}$) in the absence or presence of (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride (WAY 100135, 1 μmol·L$^{-1}$), on 1 μmol·L$^{-1}$ phenylephrine (PhE)-precontracted pig urinary bladder neck strips treated with guanethidine (10 μmol·L$^{-1}$), atropine (0.1 μmol·L$^{-1}$) and N$^{G}$-nitro-L-arginine (L-NOARG, 100 μmol·L$^{-1}$). Vertical bar shows tension in g and horizontal bar time in min. Note that WAY 100135 did not change the relaxations to EFS but potentiated the responses to 5-HT.

failed to modify the 5-HT relaxations, suggesting that prostanoids are not involved in these responses.

The order of potency for 5-HT and the 5-HT receptor agonists (5-CT > 5-HT = RS 67333 > 8-OH-DPAT > m-chlorophenylbiguanide > α-methyl-5-HT > ergotamine) is in line with that reported for 5-HT$_7$ receptors in the porcine myometrium and oviduct (Kitazawa *et al.*, 1998; Inoue *et al.*, 2003). However, other receptors could also be implicated.

The possible involvement of the 5-HT$_1$ receptors in bladder neck relaxation was initially considered as 5-CT is more potent than 5-HT at 5-HT$_1$ receptors (Boess and Martin, 1994; Hoyer *et al.*, 2002). 5-HT$_1$ receptors, however, are coupled to the inhibition of adenylyl cyclase by G$_i$, thus reducing cAMP intracellular levels and producing smooth muscle contraction (Boess and Martin, 1994; Hoyer *et al.*, 2002). In the present study, 8-OH-DPAT, an agonist which shows high and moderate affinity for 5-HT$_{1A}$ and 5-HT$_7$, respectively, produced a less potent relaxation than that exhibited by 5-CT and 5-HT. This, together with the fact that GR 127935, a 5HT$_{1B/1D}$ receptor antagonist, failed to modify the 5-HT relaxations, initially rules out the involvement of these receptors in the relaxant responses to 5-HT. WAY 100135 and cyanopindolol, antagonists of the 5-HT$_{1A}$ and 5-HT$_{1A/1B}$ receptors, respectively, potentiated the 5-HT relaxations but failed to modify those to NANC nerve stimulation. On the other hand, the potentiation of the 5-HT responses caused by ω-CgTX and TTX, blockers of the neuronal voltage-gated $Ca^{2+}$ and $Na^{+}$ channels, respectively, suggests that in addition to the mediation of muscular 5-HT receptors, there is a neuronal modulation of the 5-HT relaxations. Prejunctional 5-HT$_{1A}$ receptors have been implicated in a facilitatory modulation of the noradrenergic neurotransmission (Cohen *et al.*, 1999). In fact, in some urinary tract structures, such as in the intravesical ureter, part of the 5-HT contraction is produced via NA release from nerves (Hernández *et al.*, 2003). In the current study, guanethidine and phentolamine, blockers of noradrenergic neurotransmission and of the α-adrenergic receptors, respectively, failed to modify the 5-HT relaxations, thus ruling out a 5-HT$_{1A}$ receptor-mediated modulation of NA release from intramural nerves.

5-HT$_2$ receptors mediate 5-HT contraction in human (Klarskov and Hørby-Petersen, 1986) and dog (Cohen, 1990) urinary bladder. 5-HT$_3$ and 5-HT$_4$ receptors have been described in the rabbit (Chen, 1990), guinea-pig (Messori *et al.*, 1995) and human (Tonini *et al.*, 1994; D'Agostino *et al.*, 2006) bladder, where they induce indirect contractile effects, which are due to neurally released ACh and ATP. In the human detrusor, 5-HT potentiates the neurogenic contractions by activating prejunctional 5-HT$_4$ receptors implicated in the control of ACh release (Corsi *et al.*, 1991). In the

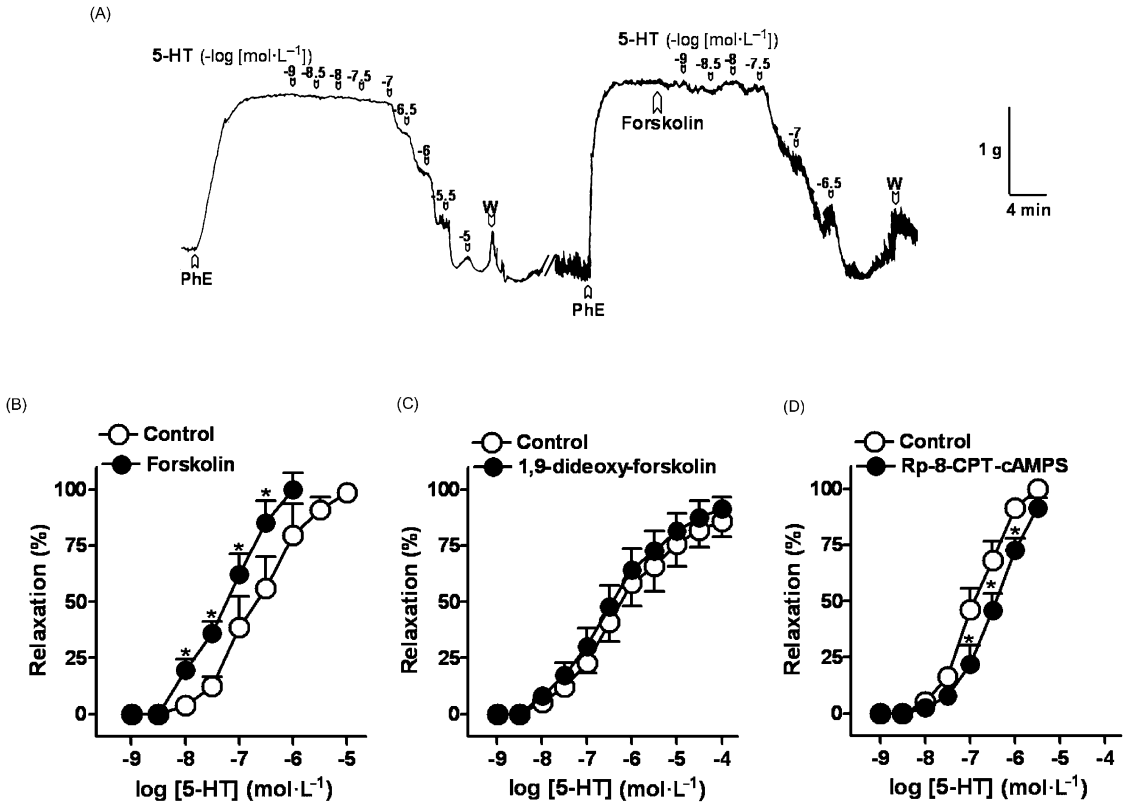


**Figure 4** (A) Isometric force recordings showing the relaxations evoked by 5-hydroxytryptamine (5-HT, 1 nmol·L$^{-1}$–10 μmol·L$^{-1}$) in the absence or presence of forskolin (30 nmol·L$^{-1}$). Vertical bar shows tension in g and horizontal bar time in min. Log concentration-response relaxation curves to 5-HT in control conditions (open circles) and in the presence (closed circles) of forskolin (30 nmol·L$^{-1}$) (B), of 1,9-dideoxy-forskolin (100 nmol·L$^{-1}$) (C) and of 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp-isomer (Rp-8-CPT-cAMPS, 100 μmol·L$^{-1}$) (D). Results are expressed as a percentage of the PhE-induced contraction and represent mean ± SEM of six–eight preparations. *$P < 0.05$, versus control (paired *t*-test).

**Table 3** Effect of adenylyl cyclase activation, of negative control of adenylyl cyclase activation and of inhibition of the PKA pathway on relaxations to 5-HT

| | n | $pD_2$ | *Emax (%)* |
|---|---|---|---|
| Control | 7 | 6.6 ± 0.1 | 98.5 ± 3.4 |
| Forskolin (30 nmol·L$^{-1}$) | 7 | 7.2 ± 0.2* | 97.9 ± 5.3 |
| Control | 8 | 6.4 ± 0.1 | 85.9 ± 7.0 |
| 1,9-dideoxy-forskolin (100 nmol·L$^{-1}$) | 8 | 6.5 ± 0.1 | 91.6 ± 5.1 |
| Control | 6 | 6.8 ± 0.1 | 100 ± 0 |
| Rp-8-CPT-cAMPS (100 μmol·L$^{-1}$) | 6 | 6.3 ± 0.1* | 91.4 ± 4.5 |

Results are expressed as mean ± SEM of n preparations.
*$P < 0.05$ versus the control value (paired *t*-test). Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax.
PKA, cAMP-dependent protein kinase; Rp-8-CPT-cAMPS, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp-isomer.

current study, α-methyl-5-HT, m-chlorophenylbiguanide and RS 67333, agonists of the 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors, respectively, were less effective than 5-CT in promoting relaxation. This fact together with the lack of inhibitory effect shown by ritanserin, SDZ SER 082, Y 25130 and GR 113808, 5-HT$_2$, 5HT$_{2B/2C}$, 5-HT$_3$ and 5-HT$_4$ receptor antagonists, on 5-HT responses, suggests that it is unlikely that 5-HT$_2$ 5-HT$_3$ and 5-HT$_4$ receptors were involved in the 5-HT responses. The lack of inhibitory effect exhibited by atropine, suramin and 8-SPT, inhibitors of muscarinic- and purinergic $P_2$- and $P_1$-receptors, respectively, on 5-HT responses, seems to discount a possible interaction of 5-HT on ACh and ATP release. Therefore, the potentiation produced by 5-HT$_{1A}$ and 5-HT$_{1A/1B}$ receptor antagonists of the 5-HT relaxations along with the enhancing effect of neuronal voltage-gated channel blockers, and the resistance to treatment with guanethidine, phentolamine and atropine, suggest the presence of prejunctional facilitatory 5-HT$_{1A}$ receptors on NANC excitatory neurotransmission in pig bladder neck.

5-HT$_{5\ A,\ B}$ receptors are essentially restricted in distribution to the CNS with a higher incidence of 5-HT$_{5A}$ receptors. 5-HT$_{5A}$ receptors are coupled to $G_i$ proteins to inhibit adenylyl cyclase activity whereas 5-HT$_{5B}$ receptor mechanisms are still unclear (Nelson, 2004). 5-CT and ergotamine are more potent than 5-HT in both 5-HT$_5$ receptor subtypes (Nelson, 2004). In the current study, ergotamine produced consistent relaxations only at high concentrations (up to 100 μmol·L$^{-1}$). This fact, together with the lack of effect of the 5-HT$_{5A}$ receptor antagonist SB 699551, suggests that 5-HT$_5$ receptors seem not to be involved in relaxations to 5-HT.

5-HT$_6$ and 5-HT$_7$ receptors are positively coupled to adenylyl cyclase and can be discriminated by comparing the potency of 5-HT and 5-CT. 5-HT is more potent than 5-CT in cloned rat and human 5-HT$_6$ receptors, while 5-CT is 10 times

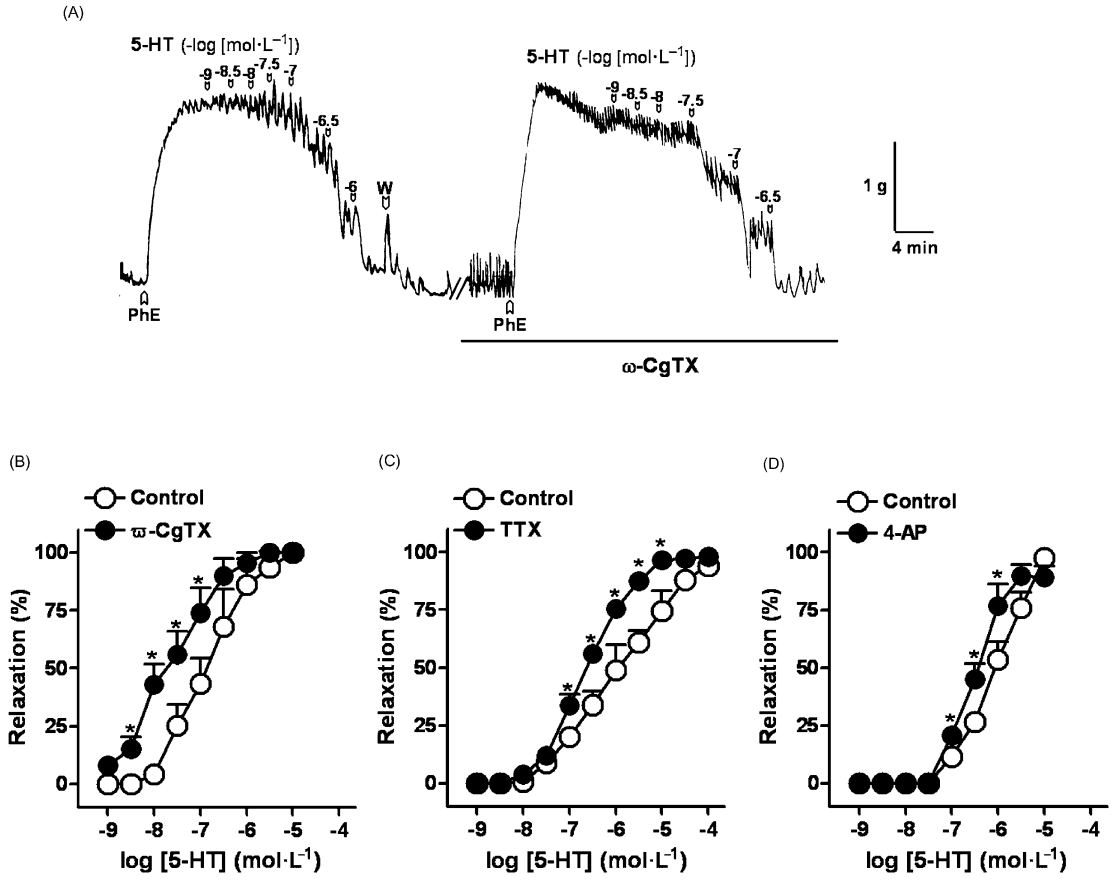


**Figure 5** (A) Isometric force recordings showing the relaxations evoked by 5-hydroxytryptamine (5-HT, 1 nmol·L$^{-1}$–1 μmol·L$^{-1}$) in the absence or presence of ω-conotoxin GVIA (ω-CgTX, 1 μmol·L$^{-1}$). Vertical bar shows tension in g and horizontal bar time in min. Log concentration-response relaxation curves to 5-HT in control conditions (open circles) and in the presence (closed circles) of ω-CgTX (1 μmol·L$^{-1}$) (B) tetrodotoxin (TTX, 1 μmol·L$^{-1}$) (C), or 4-aminopyridine (4-AP, 1 mmol·L$^{-1}$) (D). Results are expressed as a percentage of the PhE-induced contraction and represent mean ± SEM of six–nine preparations. *$P < 0.05$, versus control (paired *t*-test).

**Table 4** Effects of blockade of $K_{Ca}$, $K_{ATP}$ and $K_V$ channels on relaxations to 5-HT

| | n | *pD₂* | *Emax (%)* |
|---|---|---|---|
| Control | 8 | 6.0 ± 0.2 | 92.6 ± 5.9 |
| TEA (3 mmol·L$^{-1}$) | 8 | 5.7 ± 0.2 | 83.9 ± 11.5 |
| Control | 6 | 6.1 ± 0.1 | 68.6 ± 10.4 |
| IbTX (100 nmol·L$^{-1}$) | 6 | 6.0 ± 0.2 | 69.9 ± 8.6 |
| Control | 6 | 6.2 ± 0.1 | 85.8 ± 9.0 |
| Apamin (0.5 μmol·L$^{-1}$) | 6 | 6.4 ± 0.2 | 83.6 ± 10.1 |
| Control | 6 | 5.9 ± 0.1 | 71.3 ± 11.3 |
| Glibenclamide (1 μmol·L$^{-1}$) | 6 | 6.1 ± 0.1 | 74.3 ± 8.8 |
| Control | 9 | 5.9 ± 0.1 | 97.5 ± 2.5 |
| 4-AP (1 mmol·L$^{-1}$) | 9 | 6.5 ± 0.2* | 89.2 ± 4.9 |

Results are expressed as mean ± SEM of n preparations.
*$P < 0.05$ versus the control value (paired *t*-test). Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax.
4-AP, 4-aminopyridine; IbTX, iberiotoxin; $K_{ATP}$, ATP-dependent $K^+$; $K_{Ca}$, $Ca^{2+}$-activated $K^+$; $K_V$, voltage-gated $K^+$; TEA, tetraethylammonium.

more potent than 5-HT in human cloned 5-HT$_7$ receptors (Hoyer *et al.*, 2002). In the current study, 5-CT was more potent that 5-HT in promoting bladder neck relaxation and the 5-HT$_6$ receptor antagonist SB 258585 failed to modify the relaxation to 5-HT, thus ruling out a mediation of 5-HT$_6$ receptors and suggesting an involvement of the 5-HT$_7$ subtype in such responses. 5-HT$_7$ receptors are involved in the central control of micturition, as SB 269970 can attenuate distension-evoked bladder contraction (Read *et al.*, 2003). In rat detrusor, 5-HT$_7$ receptors are involved in the modulation of bladder contraction both *in vitro* and *in vivo* (Palea *et al.*, 2004). In the current study, the potent relaxation induced by the 5-HT$_7$ receptor agonist 5-CT, and the rightwards displacement of the 5-CT and 5-HT CRC by the selective 5-HT$_7$ receptor antagonist SB 269970, suggest the involvement of 5-HT$_7$ receptors in the 5-HT relaxations of the bladder neck.

Muscle 5-HT$_7$ receptor activation is positively coupled to adenylyl cyclase leading to an elevation of the cytoplasmic cAMP which is thought to mediate the 5-HT relaxations (Kitazawa *et al.*, 1998; Inoue *et al.*, 2003). cAMP-dependent relaxant effects in smooth muscle are generally mediated by activation of PKA. The involvement of the cAMP-PKA pathway in pig bladder neck muscle relaxation has previously been demonstrated (Hernández *et al.*, 2006a). In the current study, the reduction by the PKA inhibitor Rp-8-CPT-cAMPS of the 5-HT responses, their potentiation by the adenylyl cyclase activator forskolin and the lack of effect of 1,9-dideoxy-forskolin, a forskolin derivative that does not activate adeny-

lyl cyclase, suggest that 5-HT produces relaxation of bladder neck through activation of 5-$HT_7$ receptors linked to the cAMP-PKA pathway.

$K_{Ca}$, $K_{ATP}$ and $K_v$ channels play an essential role in the regulation of urinary bladder smooth muscle contractility (Brading, 1992; Herrera *et al.*, 2000; Thorneloe and Nelson, 2003). In fact, alterations in the expression of $K^+$ channels may produce motor dysfunctions such as overactive bladder and urinary incontinence (Herrera *et al.*, 2005). 5-HT modulates the activity of $K^+$ channels in both physiological and pathophysiological conditions. Thus, 5-$HT_{1A}$ receptor agonists modulate the activity of low conductance $K_{Ca}$ channels in *Xenopus* oocytes (Grunnet *et al.*, 2004). $K_{ATP}$ channels modulate the 5-HT-induced pressor response under hypoxic conditions in canine pulmonary arteries (Barman, 1997) and $K_v$ channel inhibition mediates the 5-HT vasoconstriction in rat pulmonary arteries (Cogolludo *et al.*, 2006). In the current study, $K_{Ca}$ and $K_{ATP}$ channel blockers failed to modify the 5-HT relaxations, thus ruling out the mediation of these channels at postjunctional sites.

**Table 5** Effects of blockade of monoamine oxidase A/B activity, noradrenergic neurotransmission, α-adrenoceptors and muscarinic receptors, NOS, guanylyl cyclase, $P_2$- and $P_1$-purinergic receptors and prostanoid synthesis on relaxations to 5-HT

| | n | $pD_2$ | Emax (%) |
|---|---|---|---|
| Control | 6 | 6.1 ± 0.1 | 87.9 ± 5.9 |
| Pargyline (100 μmol·$L^{-1}$) | 6 | 6.0 ± 0.2 | 91.1 ± 7.7 |
| Control | 7 | 5.9 ± 0.2 | 90.9 ± 7.8 |
| Guanethidine (10 μmol·$L^{-1}$) | 7 | 6.0 ± 0.2 | 92.0 ± 8.0 |
| Control | 6 | 6.8 ± 0.1 | 99.3 ± 3.1 |
| Phentolamine (1 μmol·$L^{-1}$) | 6 | 6.9 ± 0.2 | 99.9 ± 2.3 |
| Control | 6 | 6.1 ± 0.1 | 91.1 ± 5.0 |
| Atropine (1 μmol·$L^{-1}$) | 6 | 6.1 ± 0.2 | 88.7 ± 3.9 |
| Control | 6 | 6.8 ± 0.1 | 99.5 ± 2.5 |
| L-NOARG (100 μmol·$L^{-1}$) | 6 | 6.8 ± 0.1 | 98.9 ± 2.3 |
| Control | 7 | 6.8 ± 0.1 | 83.8 ± 8.5 |
| ODQ (5 μmol·$L^{-1}$) | 7 | 6.9 ± 0.2 | 82.8 ± 9.5 |
| Control | 6 | 6.5 ± 0.1 | 92.1 ± 5.5 |
| Suramin (100 μmol·$L^{-1}$) | 6 | 6.7 ± 0.1 | 94.4 ± 5.9 |
| Control | 6 | 6.5 ± 0.1 | 93.2 ± 6.8 |
| 8-SPT (100 μmol·$L^{-1}$) | 6 | 6.3 ± 0.2 | 94.6 ± 5.4 |
| Control | 6 | 6.6 ± 0.1 | 93.9 ± 5.2 |
| Indomethacin (10 μmol·$L^{-1}$) | 6 | 6.6 ± 0.1 | 95.5 ± 4.7 |

Results are expressed as mean ± SEM of preparations. Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax.
L-NOARG, $N^G$-nitro-L-arginine; NOS, nitric oxide synthase; ODQ, 1H-[1,2,4]-oxadiazolo[4, 3-a]quinoxalin-1-one.

Nitric oxide plays an essential role in the pig bladder neck inhibitory neurotransmission, relaxing smooth muscle through guanylyl cyclase-dependent mechanisms (Hernández *et al.*, 2008). Thus, the 5-HT-induced relaxation could, in part, be produced indirectly through NO release. However, the blockade of NOS and guanylyl cyclase with L-NOARG and ODQ, respectively, failed to modify the 5-HT responses, thus suggesting an NO-independent relaxation of 5-HT. In this structure, a modulation of prejunctional $K_v$ channels on relaxations induced by NO release from intramural nerves has been reported recently (Hernández *et al.*, 2008). The present results showing that blockade of $K_v$ channels enhances 5-HT-induced relaxations suggest that, in addition to the inhibition on nitrergic neurotransmission, $K_v$ channels might have a modulatory role in the 5-HT responses.

Due to the facilitatory action of 5-$HT_4$ and 5-$HT_7$ receptors on cholinergic neurotransmission, antagonists of these subtypes have been proposed in the treatment of overactive bladder (D'Agostino *et al.*, 2006). In bladder neck, the fact that 5-HT promotes a potent relaxation through muscle 5-$HT_7$ receptors suggests that in addition to the therapy of overactive bladders, antagonists of these receptors could also be useful in the therapy of urinary incontinence produced by intrinsic sphincter deficiency.

In conclusion, our present results suggest that 5-HT promotes relaxation of the pig urinary bladder neck through neuronal and non-neuronal mechanisms. Thus, 5-HT produces relaxation through muscular 5-$HT_7$ receptors coupled to the adenylyl cyclase activation pathway. Prejunctional 5-$HT_{1A}$ receptors and $K_v$ channels could modulate the 5-HT relaxations, whereas postjunctional $K^+$ channels seem not to be involved in such responses.

**Table 7** Effects of blockade of neuronal voltage-gated $Ca^{2+}$- and $Na^+$-channels on relaxations to 5-HT

| | n | $pD_2$ | Emax (%) |
|---|---|---|---|
| Control | 7 | 6.8 ± 0.1 | 100 ± 0 |
| ω-CgTX (1 μmol·$L^{-1}$) | 7 | 7.6 ± 0.1* | 100 ± 0 |
| Control | 6 | 5.9 ± 0.2 | 94.0 ± 3.2 |
| TTX (1 μmol·$L^{-1}$) | 6 | 6.6 ± 0.1* | 98.1 ± 3.7 |

Results are expressed as mean ± SEM of n preparations.
*$P < 0.05$ versus the control value (paired *t*-test). Emax is the maximal relaxation, expressed as a percentage of the phenylephrine-induced contraction, obtained for each drug. $pD_2 = -\log EC_{50}$, where $EC_{50}$ is the concentration of agonist producing 50% of the Emax.
TTX, tetrodotoxin; ω-CgTX, ω-conotoxin GVIA.

**Table 6** Effects of 5-$HT_{1A}$ and 5-$HT_{1A/1B}$ receptor blockers on relaxations evoked by electrical field stimulation (EFS, 1–16 Hz, 1 ms, 20 s trains)

| | n | EFS (Hz) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 16 |
| Control | 6 | 8.1 ± 3.9 | 23.0 ± 5.8 | 43.3 ± 8.9 | 68.3 ± 8.9 | 89.1 ± 8.5 |
| WAY 100135 (1 μmol·$L^{-1}$) | 6 | 7.4 ± 4.5 | 21.6 ± 5.1 | 36.6 ± 9.9 | 65.5 ± 9.1 | 81.1 ± 7.5 |
| Control | 6 | 4.0 ± 2.5 | 17.1 ± 5.0 | 34.6 ± 7.7 | 53.1 ± 9.7 | 77.5 ± 10.3 |
| Cyanopindolol (2 μmol·$L^{-1}$) | 6 | 4.2 ± 2.9 | 18.0 ± 5.7 | 31.3 ± 7.9 | 50.1 ± 9.1 | 70.9 ± 9.3 |

Results are expressed as percentage of the phenylephrine-induced contraction, and represent the mean ± SEM of n preparations.
WAY 100135, (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide dihydrochloride.

## Acknowledgements

Authors wish to thank to Ms Rosana Barahona-Gomariz, who improved the English language and Mr Francisco Puente and Mr Manuel Perales for their technical assistance. They also thank *Industrias Cárnicas Vaquero* slaughterhouse (Madrid) for kindly donating the urinary bladders.

## Conflict of interest

None.

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