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5-HT_7 receptor-mediated relaxation of the oviduct in nonpregnant proestrus pigs

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Abstract

The effects of 5-hydroxytryptamine (5-HT) on the muscle tonus of the ampulla and isthmus of the oviduct isolated from nonpregnant proestrus pigs were investigated, and the 5-HT receptor subtype and mechanisms of the responses were analyzed. 5-HT (1 nM–10 μM) caused a relaxation of longitudinal and circular muscles of the isthmus in a concentration-dependent manner. Tetrodotoxin did not change the relaxation, indicating a direct action of 5-HT on smooth muscle cells. The EC_{50} value in the longitudinal muscle was significantly lower than that in the circular muscle but the maximum relaxations were similar. 5-HT also caused a relaxation of both muscle layers in the ampulla but the maximum relaxation of both muscles was smaller than that of the isthmus. 5-Carboxamidotryptamine (5-CT), 5-methoxytryptamine (5-MeOT) and (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) mimicked the relaxation of the isthmic longitudinal muscle by 5-HT, and the ranking order was 5-CT>5-HT>5-MeOT>8-OH-DPAT. On the other hand, oxymethazoline, 2-methyl-5-hydroxytryptamine (2-methyl-5-HT), α-methyl-5-hydroxytryptamine (α-methyl-5-HT), [endo-*N*-8-methyl-8-azabicyclo-(3,2,1) oct-3-yl]-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazol-1-carboxamide (BIMU-1), ergotamine and dihydroergotamine were less effective. The relaxation by 5-HT was not decreased by ketanserin, 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino)ethyl ester (tropisetron) or [1[2-(methylsulphonyl) amino ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate (GR113808) but was antagonized by the following compounds in a competitive manner (with pK_b values in parentheses): 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydro-benzo[*cd*]indol-2(1*H*)-one (DR4004, 9.31), methiothepin (8.91), methysergide (7.95), metergoline (7.98), mianserin (7.69), mesulergine (8.4), spiperone (6.86) and clozapine (7.4). The correlation of these pK_b values with pK_i values of cloned 5-HT_7 receptor or pA_2 values of porcine uterus was high and significant. 4-(3-Butoxy-4-methoxybenzyl)-imidazolidin-2-one (Ro20-1724) significantly enhanced the relaxation by 5-HT but zaprinast, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) and L-nitroarginine methylester (L-NAME) did not change the responses to 5-HT. 5-HT increased cyclic AMP in the isthmic oviduct. Ampulla and isthmus contained a single class of [^{3}H]5-CT binding sites with a similar K_d value (0.4 nM), but the density of the receptors in the isthmus was 2.4 times higher than that in the ampulla. A significant correlation was found between the pK_i values in the oviduct and those of the cloned 5-HT_7 receptors. Isoprenaline, sodium nitroprusside, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide were less effective in causing the relaxation of the oviduct. In conclusion, the 5-HT receptor, functionally correlated to the 5-HT_7 type, mediates the relaxation of the porcine oviduct by 5-HT through an increase in intracellular cyclic AMP. The degrees of 5-HT-induced relaxation in the isthmus and ampulla of the oviduct were different due to the heterogeneous distribution of 5-HT_7 receptors. The strongest relaxation through 5-HT_7 receptor activation suggests that 5-HT plays an important physiological role in the regulation of porcine oviduct contractility.

Keywords: Porcine oviduct; 5-HT (5-hydroxytryptamine, serotonin); Relaxation; 5-HT_7 receptor; cAMP; [^{3}H]5-CT ([^{3}H]5-carboxamidotryptamine) binding

1. Introduction

Receptors for 5-hydroxytryptamine (5-HT) have been classified by pharmacological, signal transductional and structural criteria into seven major subtypes: 5-HT_1 (negatively coupled to adenylate cyclase by G_i), 5-HT_2 (positively coupled to phospholipase C by $G_{q/11}$), 5-HT_3 (coupled to ligand-gated cation channel), 5-HT_4 (positively coupled to adenylate cyclase by G_s), 5-ht_5 (unknown), 5-HT_6 (positively coupled to adenylate cyclase by G_s) and 5-HT_7 (positively coupled to adenylate cyclase by G_s). Subclasses of 5-HT_1 (A, B, D, E, F), 5-HT_2 (A, B, C) and 5-ht_5 (A, B) receptors have also been defined (Boess and Martin, 1994; Hoyer et al., 1994). The presence of these 14 5-HT

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receptors diversifies the action of 5-HT on smooth muscles (contraction, relaxation and mixture of both responses). Since 5-ht$_5$, 5-HT$_6$ and 5-HT$_7$ receptors have recently been cloned from the central nervous system, clarification of the physiological roles of these receptors is now in progress. In peripheral vascular and nonvascular smooth muscles, the 5-HT$_7$ receptor is thought to mediate the 5-HT-induced relaxation through an elevation of cytoplasmic cyclic AMP (monkey jugular vein, dog coronary artery, canine cerebral artery, human colonic circular muscle and porcine pial vein) (Terron, 1996; Leung et al., 1996; Prins et al., 1999; Terron and Falcon-Neri, 1999; Ishine et al., 2000). We have demonstrated that 5-HT$_7$-type receptors are present in the uterus of nonpregnant proestrus pigs and that they mediate the 5-HT-induced inhibition of myometrial contractility (Kitazawa et al., 1998, 2000). Decreases in cytosolic Ca^{2+} concentration and Ca^{2+} sensitivity of the contractile elements followed by an elevation of cyclic AMP have been suggested to be the mechanisms of inhibitory responses (Kitazawa et al., 1999). The distribution of 5-HT$_7$ receptors (more abundant in circular muscle than in longitudinal muscle) is different from that of β_2-adrenoceptors (more abundant in longitudinal muscle than in circular muscle), which mediates the inhibition of myometrial contractility by catecholamines. These findings suggest that 5-HT$_7$ receptors and β_2-adrenoceptors are both involved in the regulation of myometrial contraction of pigs in a muscle layer-dependent manner (Kitazawa et al., 2001).

Gamete and embryo transport in the mammalian oviduct results from a complex and still poorly understood interaction of smooth muscle contractions, ciliary activity and secretory function. Oviductal smooth muscle motility in mammals is regulated by autonomic nerves (adrenergic nerves), bioactive substances and sex steroid hormones. Noradrenaline, a transmitter of adrenergic nerves, causes the contraction of oviducts through α-adrenoceptors, and on the other hand, relaxes the oviduct through activation of β-adrenoceptors (Hodgson et al., 1973; Owman and Sjoberg, 1972; Rodriguez-Martinez, 1984). Recently, peptidergic (vasoactive intestinal peptide), nitroxidergic and serotoninergic (5-HT) innervations in the oviduct have also been demonstrated in several animals (Amenta et al., 1992; Costagliola et al., 1997; Czaja et al., 1996; Ekerhovd et al., 1997). The oviduct contains a large number of mast cells that contain bioactive substances such as prostaglandins, histamine and 5-HT (Amenta et al., 1992; DuBois et al., 1980). It has been reported that prostaglandin $F_{2\alpha}$ stimulates and that prostaglandin E_2 or E_1 inhibits the contraction of oviduct in many mammalian species, including pigs (Gimeno et al., 1984, 1985). Although endogenous 5-HT was demonstrated to be present in the oviduct (Amenta et al., 1992), there have been very few studies concerning the effect of 5-HT on the motility of the oviduct.

While examining the effect of 5-HT, we found that 5-HT inhibited the spontaneous contractility of the oviduct and relaxed the oviduct similar to the case in the spontaneously contracting porcine uterus (Kitazawa et al., 1998). These findings suggest that 5-HT is involved in regulation of the motility of female reproductive tracts (vagina, uterus and oviduct) in pigs. Although it has been shown that 5-HT relaxed the porcine oviduct, the characteristics of the 5-HT receptor involved in the relaxation have not been determined yet. Therefore, the present study was designed to identify the 5-HT receptor subtype and mechanisms of the relaxation. For this purpose, a mechanical study using 5-HT receptor agonists, antagonists and some enzyme inhibitors, a cyclic AMP assay and a study on [^{3}H]5-carboxamidotryptamine (5-CT) binding to oviduct membrane were carried out.

2. Methods

2.1. Tissue preparations

Fresh oviducts, with the ovaries and uteri intact, from 80 sexually matured crossbred virgin gilts (about 6 months old) were obtained from a local abattoir and were used for experiments within 3–4 h. The estrous cycles of the pigs were judged to be in proestrus according to the results of gross examination of the follicle size (<3 mm) and to the appearance of the corpora lutea (McDonald, 1975). The isthmic portion of the oviduct (20–30 mm distal from the cornual apex) and the ampullary portion of the oviduct (90–120 mm distal from the cornual apex) were carefully isolated from the utero-ovarian ligament, mesosalpinx and other extraneous tissues while being immersed in dishes containing Krebs solution. Although the wall of the porcine oviduct consists of longitudinal and circular muscles, it was difficult to separate the two muscle layers mechanically. Therefore, after each oviduct had been cut open in the longitudinal direction and the endometrium removed by fine scissors, strips in either the longitudinal or circular direction were prepared under a binocular microscope and used as longitudinal (10 × 1 mm) and circular muscle preparations (4 × 1 mm). The thickness of each preparation was less than 0.3 mm. One or two isthmic preparations and one ampullary preparation were made from the oviduct of each pig. Muscle preparations were suspended vertically in an organ bath (5 ml) containing 37 °C Krebs solution (NaCl, 118.4 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM and glucose, 11.5 mM) bubbled with 95% O_2 + 5% CO_2 (pH = 7.4). A force–displacement transducer (SB-1T, Nihon Kohden), equipped with a pen-writing recorder (Recticorder, Nihon Kohden) was used to measure the mechanical activity of the smooth muscle preparations. The muscle strips were loaded at 0.5 g as an initial tension and allowed to equilibrate for 1 h. During the equilibration, each strip showed spontaneous contractile activity and established considerable muscle tonus (0.3–0.6 g) for about 3 h.

2.2. Experimental protocol and data analysis

After establishing spontaneous contraction and steady resting tonus of oviduct preparations, 5-HT and 5-HT receptor agonists were applied cumulatively to an organ bath. Effects on amplitude of spontaneous contractions and decrease in the resting tonus (relaxation) of muscles induced by agonists were analyzed. In the case of spontaneous contraction, the minimum (inhibition) and maximum (potentiation) amplitudes of spontaneous contraction during each 5-min cycle of application were measured and expressed as percentages of those in the absence of 5-HT. The relaxation by 5-HT receptor agonists was expressed as a percentage of 100 μM papaverine-induced response tested at the end of each experiment. In the case of papaverine-exposed preparations, recovery of contractile tonus was difficult despite repeated washing out. Therefore, only one concentration–response curve of the agonists was constructed in the oviduct preparation from one pig. The EC_{50} value (concentration of agonist that caused 50% of the maximum relaxation) and the maximum response were determined by least-squares nonlinear regression analysis of the concentration–response curve.

The effects of several 5-HT receptor antagonists on the 5-HT-induced relaxation were examined to characterize receptor subtypes. Two longitudinal muscle preparations were isolated from close portion of the isthmus, and each muscle preparation was suspended vertically. One preparation was used as control and the other was treated with 5-HT receptor antagonists for 30 min. 5-HT was applied cumulatively to each preparation, and the EC_{50} values for 5-HT in the absence and presence of the antagonist were determined from the concentration–response curves. The apparent dissociation constant (K_b) of each antagonist was determined according to the following equation (Van Rossum, 1963): K_b = antagonist concentration/(CR − 1), where CR is the concentration ratio of EC_{50} (EC_{50} of 5-HT in the presence of an antagonist divided by EC_{50} of 5-HT in the absence of an antagonist). These results were then expressed as the negative logarithm of pK_b ($-\log K_b = pK_b$). Correlations between pK_b values obtained in the oviduct and pK_i values of 5-HT receptor antagonists at various 5-HT receptor subtypes were compared.

2.3. Measurement of cyclic AMP level

Isolated fresh isthmic oviducts weighing approximately 20–30 mg were used in the cyclic AMP study. After equilibration in warmed (37 °C) and gassed (95% O_2 + 5% CO_2) Krebs solution for 1 h, the oviduct strips were exposed to 100 nM 5-HT for 5 min. After incubation, the strips were quickly frozen in liquid nitrogen and homogenized in 6% trichloroacetic acid solution with a Polytron. The homogenate was centrifuged at 2000 × *g* for 20 min (two times) and the resulting supernatant was collected. After removing trichloroacetic acid in the supernatant by washing three times with water-saturated ether, cyclic AMP in the extract was assayed using an enzyme immunoassay kit (Amersham). Tissue cyclic AMP levels were expressed as pmol/g tissue wet weight.

2.4. Radioligand binding study

For further characterization of 5-HT receptors in the porcine oviduct, a receptor binding study using [^{3}H]5-CT (37 MBq/ml, NEN Life Science Products) was carried out. [^{3}H]5-CT has been used previously to label 5-HT_7 receptors in transfected cells (To et al., 1995) and porcine myometrium (Kitazawa et al., 2000). As previously mentioned, it was difficult to completely separate the longitudinal and circular muscles of the oviduct. Therefore, determination of the distribution of 5-HT_7 receptors in the isthmus and ampulla of the oviducts, and pharmacological characterization of [^{3}H]5-CT binding sites were aims of the present study. The membrane of the porcine oviduct was prepared by methods described previously (Kitazawa et al., 2000). After removing the endometrium, the isthmus and ampulla of the porcine oviduct were cut into small pieces and homogenized in 10 volumes of ice-cold Tris–EDTA buffer (Tris, 50 mM; Na_2EDTA, 0.5 mM; $MgSO_4$, 10 mM; $CaCl_2$, 2 mM; pargyline, 0.01 mM; ascorbic acid, 0.1%, neutralized with HCl to pH 7.4 at 4 °C) with the use of a Polytron. The homogenate was filtered through a single layer of nylon mesh (pore size, 250 μm) and centrifuged at 1200 × *g* for 20 min at 4 °C, and the pellet was discarded. The supernatant was centrifuged at 40,000 × *g* for 30 min at 4 °C. The resulting pellets were washed twice and suspended in the Tris–EDTA buffer and used as a crude membrane preparation for determination of [^{3}H]5-CT binding. Protein in the membrane preparation was measured according to the method of Lowry et al. (1951).

The membrane preparations (isthmus: 300 μg protein/tube, ampulla: 500 μg protein/tube) were incubated with eight increasing concentrations of [^{3}H]5-CT (0.088–11 nM) in 500 μl of Tris–EDTA buffer (at 37 °C for 60 min). The binding reaction was stopped by adding ice-cold Tris–EDTA buffer (4 ml), and the mixture was then filtered through a 0.3% polyethylenimine-presoaked glass fiber filter (GF/B; Whatman) under a vacuum to trap the crude membrane. The filter was then rapidly washed four times with 4 ml of ice-cold incubation buffer and placed in 20-ml glass scintillation vials with scintillation fluid (Scintisol EX-H, Dojin). Radioactivity trapped on the filter paper was measured by a liquid scintillation spectrometer (LCS-700; Aloka). Specific binding was calculated by subtracting nonspecific binding from total binding. Nonspecific binding was determined in the presence of 100 μM 5-HT. The maximum number of binding sites per milligram of protein (B_{max}, concentration of receptors) and the equilibrium dissociation-binding constant (K_d) were estimated by Scatchard analysis. Lines of the best fit were calculated using linear regression analysis by the method of least squares.

To characterize the [^{3}H]5-CT binding sites in the porcine oviduct, a competition study was performed using several 5-HT receptor agonists and antagonists. [^{3}H]5-CT and the crude membrane preparation of the isthmus were incubated with various concentrations of 5-HT receptor agonists and antagonists for 60 min at 37 °C. After incubation, [^{3}H]5-CT bound to membrane 5-HT receptors was separated by filtration through 0.3% polyethylenimine-presoaked glass fiber filters, and the radioactivity on the filters was measured. From the IC_{50} value (concentration of agent that inhibited 50% of the control binding obtained in the absence of a competitor), the inhibition constant (K_i) was calculated by the equation of Cheng and Prusoff (1973): $K_i = IC_{50}/(1+[L]/K_d)$, where $[L]$ is the concentration of [^{3}H]5-CT (0.2–0.3 nM) used in the competition study.

2.5. Chemicals

The following chemicals were used in this study: 5-carboxamidotryptamine maleate (5-CT, RBI), dihydroergotamine tartrate (Tokyo Kasei), ergotamine hydrochloride (Tokyo Kasei), 5-hydroxytryptamine creatinine sulfate (5-HT, Wako), (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, RBI), isoprenaline hydrochloride (Sigma), ketanserin tartrate (5-HT_2 receptor antagonist, RBI), L-nitroarginine methylester (L-NAME, nitric oxide synthase inhibitor, Sigma), metergoline (RBI), methiothepin hydrochloride (RBI), 5-methoxytryptamine hydrochloride (5-MeOT, Sigma), methysergide hydrochloride (RBI), 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, 5-HT_3 receptor agonist, RBI), α-methyl-5-hydroxytryptamine (α-methyl-5-HT, 5-HT_2 receptor agonist, RBI), mianserin hydrochloride (RBI), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ, guanylate cyclase inhibitor, Sigma), oxymetazoline hydrochloride (RBI), papaverine hydrochloride (Tokyo Kasei), pargyline hydrochloride (RBI), 4-(3-butoxy-4-methoxybenzyl)-imidazolidin-2-one (Ro20-1724, cyclic AMP specific phosphodiesterase inhibitor, RBI), pituitary adenylate cyclase activating peptide (Peptide Ins., Osaka), sodium nitroprusside (nitric oxide donor, Wako), spiperone hydrochloride (RBI), vasoactive intestinal peptide (Peptide Ins.) and zaprinast (cyclic GMP specific phosphodiesterase inhibitor, RBI). The

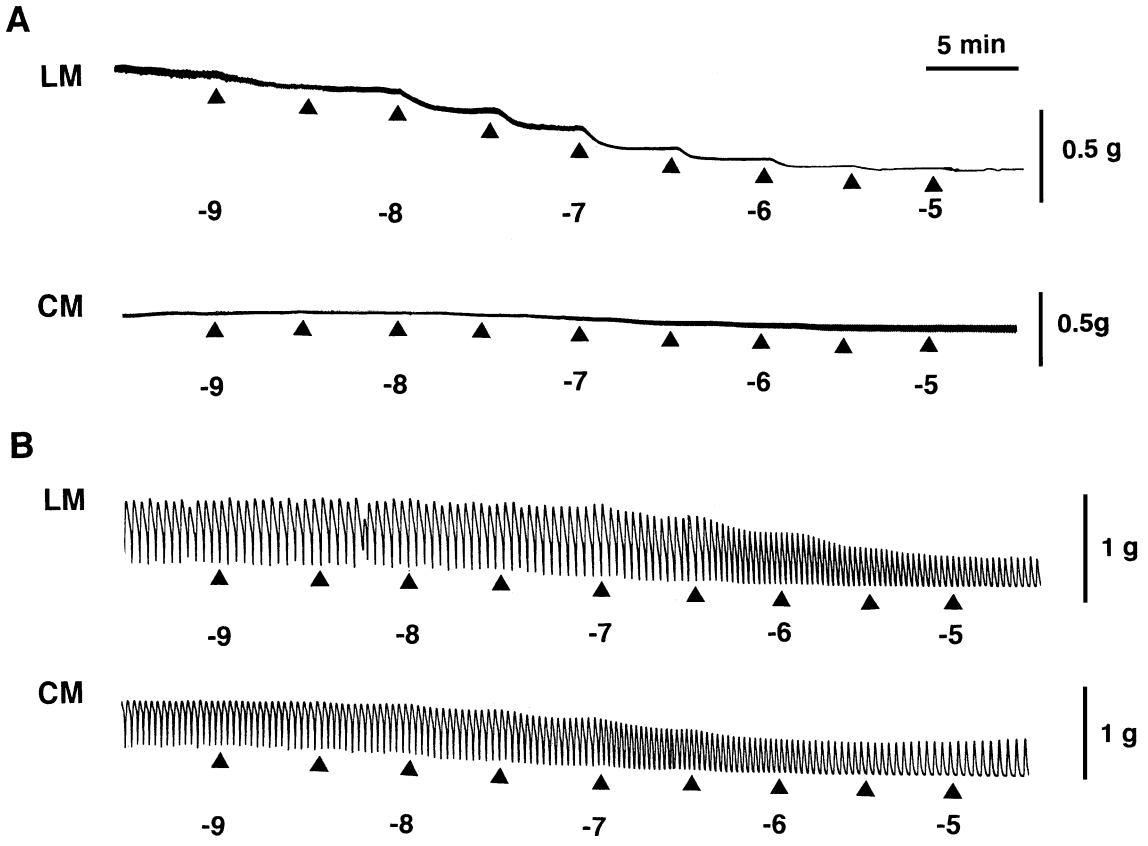

Fig. 1. Representative effects of 5-HT on mechanical activity of the porcine oviduct. Typical responses of 5-HT in longitudinal muscles (LM) and circular muscles (CM) isolated from the isthmic (A) and ampullary portions (B). In the isthmic LM and CM preparations (A), frequency of spontaneous contraction was too high to trace the individual contraction wave under this recording speed. Vertical width of mechanical activity corresponded to the amplitude of spontaneous contraction. 5-HT decreased the amplitude of spontaneous contraction in the LM but increased it in the CM contrarily. Numerals under contractile curves indicate the concentrations of 5-HT (log *M*). The horizontal bar shows the time scale for 5 min and the vertical bar shows the scale for 0.5 or 1 g.

following drugs were kindly donated: 2*a*-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2*a*,3,4,5-tetrahydro-benzo[*cd*]indol-2(1*H*)-one (DR4004, 5-HT_7 receptor antagonist, Meiji Seika, Yokohama, Japan, Kikuchi et al., 1999), [endo-*N*-8-methyl-8-azabicyclo-(3,2,1) oct-3-yl]-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazol-1-carboxamide (BIMU-1, 5-HT_4 receptor agonist, Boehringer Ingelheim, Milano, Italy, Grossman et al., 1993), [1[2-(methylsulphonyl) amino ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate (GR113808, 5-HT_4 receptor antagonist, Glaxo, Greenford, Middlesex, UK, Grossman et al., 1993) and 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino)ethyl ester (tropisetron, 5-HT_3/5-HT_4 receptor antagonist, Novartis, Basel, Switzerland, Grossman et al., 1993). Drugs except for ODQ, DR4004, metergoline, mianserin and spiperone were dissolved in distilled water. ODQ, DR4004, metergoline, mianserin and spiperone were dissolved in dimethylsulfoxide. The maximum concentrations of dimethylsulfoxide in the organ bath solutions or incubation buffers were set below 0.2%, concentrations that did not change the spontaneous contracting activity, muscle tonus of the porcine oviduct or [^{3}H]5-CT binding.

2.6. Statistical analysis

The results of the experiments are expressed as means ± S.E.M. of more than four oviduct preparations obtained from different animals. The relaxation by 5-HT and [^{3}H]5-CT binding in the different preparations were compared by using unpaired Student's *t*-test. A *P* value of 0.05 or less (two-tailed) was considered statistically significant.

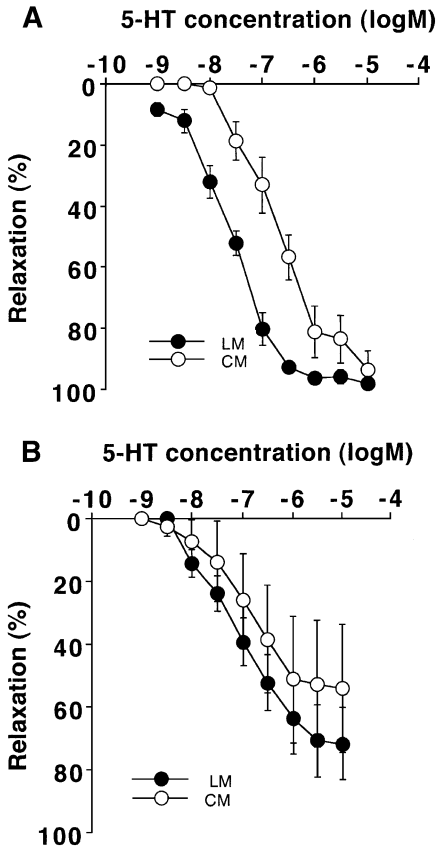

Fig. 2. Muscle layer- and region-dependent relaxation of the porcine oviduct induced by 5-HT. Each symbol shows the concentration–response curve for 5-HT in longitudinal muscles (LM, ●) and circular muscles (CM, ○) isolated from the isthmus (A) and ampulla (B). Ordinate: relative amplitude of relaxation expressed as percentage of the response induced by 100 μM papaverine. Abscissa: concentration of 5-HT (log *M*). Points represent the means of four or more experiments with S.E.M. shown by vertical lines.

3. Results

3.1. Effects of 5-HT on motility of the porcine oviduct

Fig. 1A shows a typical mechanical activity and the actions of 5-HT in longitudinal and circular muscle strips of the isthmus of the porcine oviduct. Frequent spontaneous contractions were observed in both muscle layers (longitudinal muscle: 12.3 ± 0.9 contractions/min, $n=8$; circular muscle: 11.3 ± 1.5 contractions/min, $n=8$). 5-HT applied cumulatively caused a concentration-dependent relaxation and a decrease in the amplitude of spontaneous contractile activity of the longitudinal muscles. On the other hand, although 5-HT decreased the tonus of the circular muscle (Figs. 1A and 2A), the amplitude of spontaneous contractions increased (Figs. 1A and 3A). The EC_{50} values and maximum degrees of relaxation (relative to 100 μM papaverine-induced relaxation) were 35 ± 8.4 nM and 98 ± 1.3% ($n=6$) in the longitudinal muscle, and 208 ±45 nM ($P<0.05$ compared with longitudinal muscle) and 94 ± 4.5% ($n=5$) in the circular muscle, respectively (Fig. 2). Tetrodotoxin was added to the organ bath to clarify the action site of 5-HT. Tetrodotoxin (1 μM) did not affect the spontaneous contractions, smooth muscle tonus or the 5-HT-induced relaxation ($EC_{50}=134\pm57$ nM, maximum relaxation = 94 ± 2.6%, $n=4$) in the longitudinal muscle of the isthmus.

The regional differences in the responses to 5-HT were examined using longitudinal and circular muscle strips isolated from the ampulla. The ampullary oviduct also showed spontaneous contractions with low frequency compared with the isthmus (longitudinal muscle: 3.1 ± 0.5 contractions/min, $n=6$; circular muscle: 2.9 ± 0.3 contractions/min, $n=6$). 5-HT applied to the organ bath (10 nM–10 μM) caused relaxation of the oviductal smooth muscle (Fig. 1B). The EC_{50} values and the maximum responses in the longitudinal and circular muscles were 96 ± 26 nM and 72.6 ± 11.4% ($n=6$) and 178 ± 58 nM and 54.5 ± 20.4% ($n=4$), respectively (Fig. 2B). 5-HT also decreased the amplitude of spontaneous contractions in

both muscle layers, but the inhibition was small (25–30%) and was not different between the two muscle layers (Figs. 1B and 3B).

3.2. Effects of 5-HT receptor agonists

Since the longitudinal muscle of the isthmus was most sensitive to 5-HT among the preparations examined, the effects of several 5-HT receptor agonists were examined in this preparation to estimate the 5-HT receptor subtype involved in the relaxation. As indicated in Fig. 4, oxymethazoline, α-methyl-5-HT, 2-methyl-5-HT, BIMU-1, ergotamine and dihydroergotamine did not cause marked relaxation even at 10 μM (less than 40% of the papaverine-induced relaxation). However, 5-CT and 5-MeOT relaxed the oviduct in a concentration-dependent manner. The EC_{50} values and the maximum degrees of relaxation were 0.8 ± 0.34 nM and 98 ± 2% for 5-CT ($n = 8$) and 670 ± 160 nM and 95 ± 2.3% for 5-MeOT ($n = 6$), respectively. 8-OH-DPAT also caused relaxation, but the response did not reach

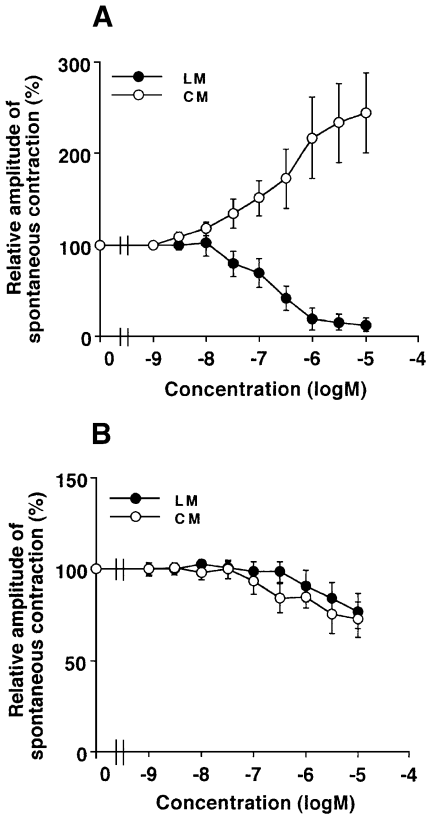

Fig. 3. Effects of 5-HT on amplitude of spontaneous contraction in the porcine oviduct. Each symbol shows the concentration–response curve for 5-HT in spontaneous contracting longitudinal muscles (LM, ●) and circular muscles (CM, ○) isolated from the isthmus (A) and ampulla (B). Ordinate: relative amplitude of spontaneous contraction (before treatment = 100%). Abscissa: concentration of 5-HT (log *M*). Points represent the means of four or more experiments with S.E.M. shown by vertical lines.

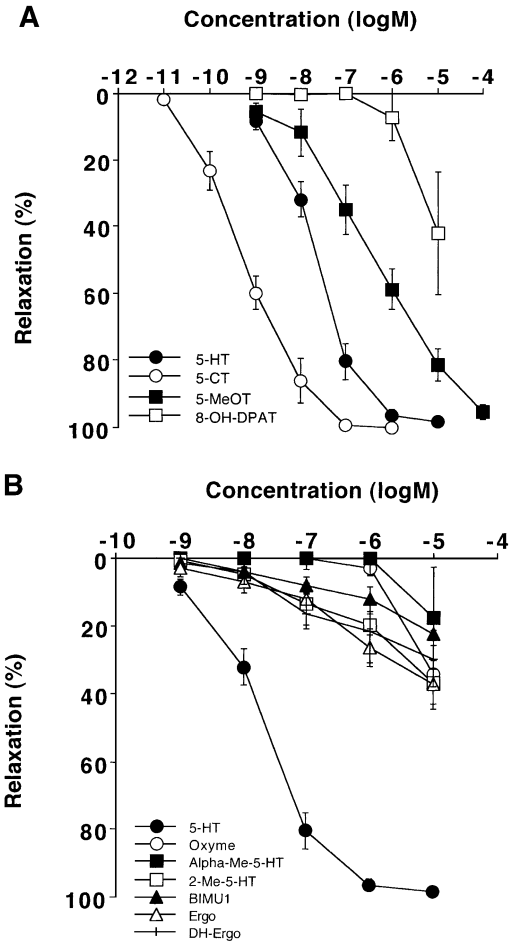

Fig. 4. Concentration–response curves of some 5-HT receptor agonists in the longitudinal muscle of the isthmic portion of the oviduct. (A) Symbols show relaxation of the oviduct induced by 5-CT (○), 5-HT (●), 5-MeOT (■) and 8-OH-DPAT (□). (B) Comparison of the degrees of relaxation induced by 5-HT (●), oxymethazoline (Oxyme, ○), α-methyl-5-HT (■), 2-methyl-5-HT (□), BIMU-1 (▲), ergotamine (Ergo, △) and dihydroergotamine (DH-Ergo, +). Ordinate: relative amplitude of relaxation expressed as percentage of the response induced by 100 μM papaverine. Abscissa: concentration of 5-HT receptor agonists (log *M*). Points represent the means of four or more experiments with S.E.M. shown by vertical lines.

a plateau at 10 μM (42.1 ± 18.4%, $n = 4$). Therefore, the EC_{50} value was not calculated. According to the concentration–response curves, the ranking order of the potency was estimated as follows: 5-CT>5-HT>5-MeOT>8-OH-DPAT (Fig. 4).

3.3. Effects of 5-HT receptor antagonists on the responses to 5-HT

For further characterization of the relaxatory 5-HT receptor in the oviduct, the effects of some 5-HT receptor antagonists on 5-HT-induced relaxation (control, $EC_{50} = 33 \pm 5.6$ nM, maximum response = 99 ± 0.6%, $n = 40$) were investigated in the longitudinal muscle of the isthmus. Ketan-

serin (1 μM), tropisetron (10 μM) and GR113808 (10 μM) did not reduce relaxation induced by 5-HT (data not shown, $n=2–4$). On the other hand, DR4004 (10 nM) ($EC_{50}=716\pm347$ nM, maximum response $=99\pm0.3\%$, $n=5$), methiothepin (10 nM) ($EC_{50}=300\pm230$ nM, maximum response $=98\pm1.9\%$, $n=5$), metergoline (100 nM) ($EC_{50}=348\pm120$ nM, maximum response $=99\pm1.3\%$, $n=4$), methysergide (100nM) ($EC_{50}=330\pm57$ nM, maximum response $=100\pm0\%$, $n=5$), mesulergine (100 nM) ($EC_{50}=870\pm279$ nM, maximum response $=97\pm2.8\%$, $n=5$), clozapine (1 μM) ($EC_{50}=853\pm203$ nM, maximum response $=99\pm1.1\%$, $n=6$), spiperone (1 μM) ($EC_{50}=277\pm41$ nM, maximum response $=100\pm0\%$, $n=6$) and mianserin (300 nM) ($EC_{50}=518\pm420$ nM, maximum response $=99\pm0.5\%$, $n=5$) antagonized the relaxation of 5-HT and shifted the concentration–response curve for 5-HT to the right without affecting the maximum relaxation (competitive manner). On the basis of changes in mean EC_{50} values caused by the antagonists, pK_b values were estimated as follows: DR4004 (9.31), methiothepin (8.91), methysergide (7.95), metergoline (7.98), mianserin (7.69), mesulergine (8.4), spiperone (6.86) and clozapine (7.4). Table 1 shows the correlation of pK_b in the porcine oviduct with pA_2 values of porcine uterine circular muscle and pK_i values of cloned 5-HT_4, 5-ht_5, 5-HT_6 and 5-HT_7 receptors. A significant correlation ($P<0.05$) was found when pK_b values of the oviduct were compared with pA_2 values of porcine uterine circular muscle and 5-HT_7 receptors (both mouse and human). However, the correlation between 5-HT_4, 5-ht_{5A}, 5-ht_{5B} or 5-HT_6 receptors was low and not significant (Table 1).

Examination of the inhibitory actions of 5-HT receptor antagonists on the 5-HT-induced relaxation showed that some 5-HT receptor antagonists caused a slow developing contraction of the oviduct. Fig. 5 shows the relative amplitude of contraction induced by treatment of respective antagonists for 20 min. Compared with the time-matched control ($1.1\pm5.1\%$, $n=5$), methiothepin (10 nM, $110\pm81\%$, $n=4$), metergoline (300 nM, $177\pm50.1\%$, $n=4$), methysergide (100 nM, $70.5\pm22.7\%$, $n=4$), mesulergine (100 nM, $45\pm18.3\%$, $n=4$), mianserin (300 nM,

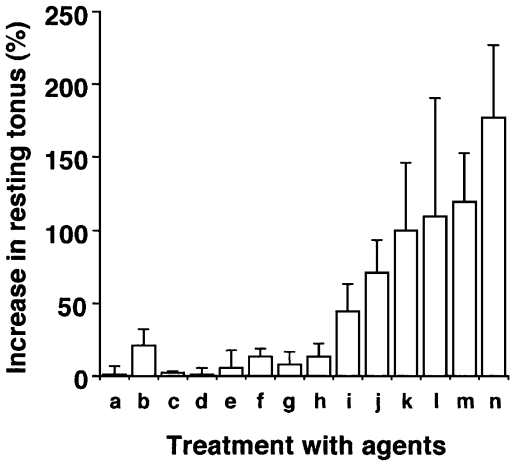

Fig. 5. 5-HT receptor antagonist-induced increase in resting tonus of the longitudinal muscle isolated from the isthmic portion of the oviduct. Each column shows increase in the resting tonus (contraction) after 20 min of treatment with respective agents. a: Time control, b: 1 μM tetrodotoxin, c: 100 μM L-NAME, d: 3 μM ODQ, e: 300 nM ketanserin, f: 1 μM tropisetron, g: 1 μM clozapine, h: 100 nM DR4004, i: 100 nM mesulergine, j: 100 nM methysergide, k: 1 μM spiperone, l: 10 nM methiothepin, m: 300 nM mianserin, and n: 300 nM metergoline. Ordinate: relative increase in resting tonus of the oviduct expressed as percentage of the resting tonus before treatment with each agent. Points represent the means of four or more experiments with S.E.M. shown by vertical lines.

Table 1
Pharmacological characterization of the 5-HT receptor present in the longitudinal muscle of the porcine isthmic oviduct using some 5-HT receptor antagonists

Index	Oviducts (pK_b)	Uterine CM (pA_2)	Mouse 5-HT_7 (pK_i)	Human 5-HT_7 (pK_i)	5-HT_6 (pK_i)	5-ht_{5A} (pK_i)	5-ht_{5B} (pK_i)	5-HT_4 (pK_i)
Reference		Kitazawa et al., 1998, 2001	Plassat et al., 1993	Bard et al., 1993	Monsma et al., 1993	Plassat et al., 1992	Matthes et al., 1993; Erlander et al., 1993	Hoyer et al., 1994
Methiothepin	8.91	8.05	8.2	8.43	8.74	7.0	7.8	inactive[a]
Metergoline	7.98	7.4	7.5	8.19	7.52	<6[a]	<6[a]	–
Methysergide	7.95	7.92	7.9	7.08	6.6	7.6	6.9	5.0
Mesulergine	8.4	–	7.6	7.74	5.76	<6[a]	<6[a]	inactive[a]
Clozapine	7.4	7.06	7.4	–	7.89	–	–	–
Mianserin	7.69	7.08	7.0	–	7.34	–	–	–
Spiperone	6.86	6.86	7.2	6.96	–	5.6	–	inactive[a]
DR4004	9.31	8.86	–	8.67[b]	6.28[b]	–	–	<6.0[a,b]
Correlation coefficient		0.93	0.77	0.85	0.18			
Significance		$P<0.01$	$P<0.05$	$P<0.05$	$P>0.1$			

Data are the means of four or more separate experiments in the porcine oviduct and uterus or mean values from radioligand displacement studies with recombinant 5-HT_4, 5-ht_{5A}, 5-ht_{5B}, 5-HT_6 and 5-HT_7 receptors expressed in mammalian cells. Correlations to the oviducts were determined, and the coefficients and significance were indicated.

[a] In the case of 5-ht_5 and 5-HT_4 receptors, correlation was not determined, because of the indefinite pK_i values.

[b] pK_i values of DR4004 for 5-HT_4, 5-HT_6 and 5-HT_7 receptors were from Kikuchi et al. (1999).

120 ± 33.5%, $n=4$) and spiperone (1 μM, 100 ± 46.6%, $n=4$) were effective in causing contraction of longitudinal muscle of the isthmus (more than 40% of the resting tonus). On the other hand, DR4004 (100 nM, 13 ± 9.5%, $n=4$), clozapine (1 μM, 8 ± 8%, $n=4$), ketanserin (300 nM, 6 ± 12%, $n=4$), tropisetron (1 μM, 14 ± 4.8%, $n=4$) and GR113808 (100 nM, 5 ± 4.1%, $n=5$) were ineffective 5-HT receptor antagonists (Fig. 5).

3.4. Effects of Ro20-1724, zaprinast, L-NAME and ODQ on the responses to 5-HT

The possible involvement of cyclic AMP or cyclic GMP in the 5-HT-induced relaxation was investigated using Ro20-1724 or zaprinast. After obtaining steady tonus of the isthmic longitudinal muscles of the oviduct, strips were treated with a vehicle (control) or the inhibitors for 20 min, and then concentration–response relationships of 5-HT were determined. As shown in Table 2, treatment with Ro20-1724 (30 μM) potentiated the relaxation by 5-HT and shifted the concentration–response curve to the left (EC_{50} values and maximum relaxation: 67 ± 23.6 nM and 100 ± 0% for the control and 13 ± 6.3 nM and 100 ± 0% for Ro20-1724, respectively, $n=5$). On the other hand, zaprinast (30 μM) failed to change the relaxation induced by 5-HT (EC_{50} values and maximum relaxation: 38 ± 14.3 nM and 99.7 ± 0.3% for the control and 78 ± 50 nM and 97 ± 1.8% for the zaprinast, respectively, $n=5$), suggesting that cyclic GMP did not mediate the response of 5-HT in the oviducts. To confirm this assumption, effects of L-NAME (100 μM) and ODQ (3 μM) were examined. Neither L-NAME nor ODQ affected the resting tonus and relaxation induced by 5-HT (Fig. 5 and Table 2).

Table 2
Effects of Ro20-1724, zaprinast, L-NAME and ODQ on the 5-HT-induced relaxation in the longitudinal muscle of the porcine isthmic oviduct

	Relative relaxation (%)[a]					
	1	10	100 nM	1	10	100 μM
Control	10 ± 4.5	35 ± 7.2	60 ± 7.5	85 ± 6.3	93 ± 3.4	100 ± 0
Ro20-1724 (30 μM)	18 ± 8.0	55 ± 10.5	92 ± 5.2[b]	100 ± 0[b]	100 ± 0[b]	
Control	4 ± 3.2	34 ± 8.5	77 ± 9.6	89 ± 5.3	97 ± 1.2	100 ± 0.4
Zaprinast (30 μM)	5 ± 2.7	46 ± 12.3	72 ± 12.3	85 ± 7.9	91 ± 5.9	96 ± 2.4
Control	3 ± 1.5	25 ± 10.0	54 ± 20	76 ± 12.9	91 ± 6.3	97 ± 2.7
L-NAME (100 μM)	8 ± 5.9	19 ± 12.3	47 ± 14.6	64 ± 14.3	88 ± 4.2	100 ± 0
Control	3 ± 0.9	15 ± 3.9	61 ± 5.5	89 ± 2.9	96 ± 1.6	98 ± 1.7
ODQ (3 μM)	7 ± 1.5	32 ± 13.0	57 ± 21.3	71 ± 20.3	97 ± 2.6	100 ± 0

Values are means ± S.E.M. of at least four preparations from different animals.
[a] Amplitude of the relaxation expressed as a percentage of the response induced by 100 μM papaverine.
[b] Significantly different from the corresponding control values, $P<0.05$.

3.5. Effect of 5-HT on cyclic AMP level

The ability of 5-HT to produce cyclic AMP was examined in the isthmus of the oviduct. The resting cyclic AMP level was 260 ± 22 pmol/g tissue wet weight ($n=5$), and treatment with 100 nM 5-HT for 5 min significantly increased the tissue cyclic AMP content (661 ± 141 pmol/g tissue wet weight, $n=6$).

3.6. [^{3}H]5-CT binding sites in the isthmus and ampulla of the oviduct

A [^{3}H]5-CT binding study (saturation and competition) was carried out to examine the distributions of 5-HT_7 receptors in the isthmus and ampulla. Specific binding of [^{3}H]5-CT to the crude membrane increased upon increment of the free concentration of the ligand (0.088–5.5 nM) and almost reached a plateau at 11 nM. Scatchard plots of saturation-binding parameters of both portions fitted one straight line and revealed the presence of a single class of binding sites in both regions (Fig. 6). From the regression line, K_d and B_{max} values were estimated and compared. The K_d value in the isthmus (0.4 ± 0.12 nM, $n=6$) was the same as that in the ampulla (0.42 ± 0.09 nM, $n=6$). On the other hand, the B_{max} value in the isthmus (20 ± 2.9 fmol/mg protein, $n=6$) was 2.4 times higher than that in the ampulla (8.5 ± 1.2 fmol/mg protein, $n=6$) in association with the regional difference in 5-HT-induced relaxation. Hill plots of the binding data were linear with Hill coefficients of 0.95 ± 0.03 ($n=6$) in the isthmus and 1.01 ± 0.05 ($n=6$) in the ampulla. These values were not significantly different from unity, suggesting that there was no positive or negative cooperativity in binding profiles in the oviduct.

Competition between four 5-HT receptor agonists and competition between four 5-HT receptor antagonists in the [^{3}H]5-CT binding were examined for identification of the binding sites. In the membrane preparations obtained from the isthmic region, all agonists and antagonists concentration-dependently inhibited the specific [^{3}H]5-CT binding and finally displaced it completely. The ranking order of potency (pK_i) for agonists in competing for specific binding was 5-CT (9.4 ± 0.11, $n=4$)>5-MeOT (8.6 ± 0.13, $n=4$) = 5-HT (8.5 ± 0.1, $n=4$)>8-OH-DPAT (6.6 ± 0.14, $n=4$). In the case of antagonists, the ranking order was metergoline (7.7 ± 0.05, $n=4$)> methysergide (7.6 ± 0.09, $n=4$)>spiperone (7.4 ± 0.27, $n=4$)>mianserin (6.4 ± 0.11, $n=4$). pK_i values of the four 5-HT receptor agonists and four 5-HT receptor antagonists were significantly correlated with cloned mouse ($r=0.96$, $P<0.001$), rat ($r=0.98$, $P<0.001$), human ($r=0.93$, $P<0.01$) and guinea pig 5-HT_7 receptors ($r=0.94$, $P<0.001$)

(Plassat et al., 1993; Shen et al., 1993; Bard et al., 1993; To et al., 1995).

3.7. Effects of isoprenaline, sodium nitroprusside, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide

To compare the inhibitory response to 5-HT with other smooth muscle relaxant agents, effects of isoprenaline, sodium nitroprusside, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide on the longitudinal muscle of the isthmus were examined. As shown in Fig. 7, isoprenaline (10 nM–10 μM) caused relaxation of the oviduct but the response was weak even at 10 μM (30.7 ± 7.2%, $n=4$). Sodium nitroprusside (100 nM–100 μM) also relaxed the longitudinal muscle of the isthmus, but this agent was about 200 times less potent than 5-HT and the relaxation at 100 μM was 68 ± 7.9% ($n=4$). The relaxatory neuropeptides, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide did not cause relaxation at the concentrations up to 1 μM (Fig. 7).

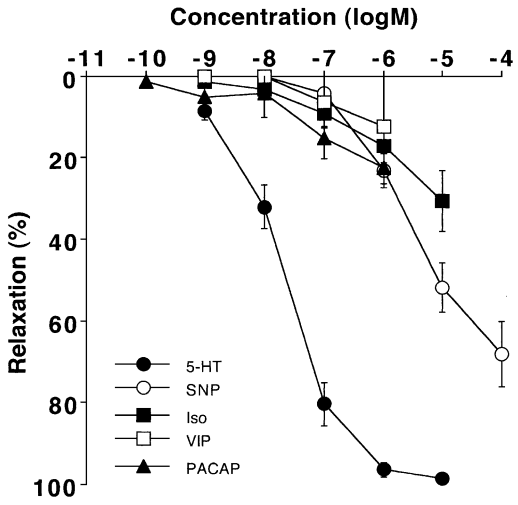

Fig. 7. Comparison of concentration–response curves for sodium nitroprusside, isoprenaline, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide with 5-HT. Symbols show relaxation of oviduct induced by 5-HT (●), sodium nitroprusside (SNP, ○), isoprenaline (Iso, ■), vasoactive intestinal peptide (VIP, □) and pituitary adenylate cyclase activating peptide (PACAP, ▲) in the longitudinal muscle of the isthmic oviduct. Ordinate: relative amplitude of relaxation expressed as percentage of the response induced by 100 μM papaverine. Abscissa: concentration of each agent (log *M*). Points represent the means of four or more experiments with S.E.M. shown by vertical lines.

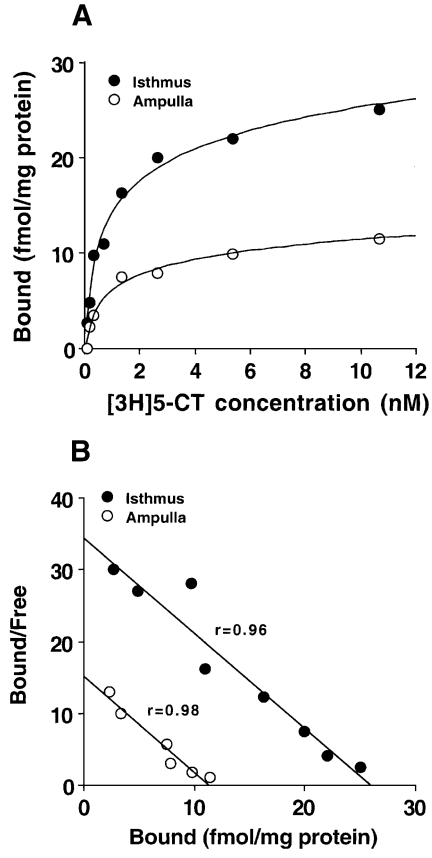

Fig. 6. [^{3}H]5-CT binding to the isthmus and ampulla of the porcine oviduct. (A) Crude membrane preparations obtained from the isthmus and ampulla were incubated with increasing concentrations of [^{3}H]5-CT for 60 min at 37 °C. Specific binding was determined as the difference between total and nonspecific bindings obtained in the presence of 100 μM 5-HT. Abscissa: [^{3}H]5-CT concentration (nM). Ordinate: specific [^{3}H]5-CT bound (fmol/mg protein). (B) Scatchard plot of the binding data in the isthmus and ampulla. The line was determined by linear regression analysis (correlation coefficients shown). The points shown are from one of similar experiments.

4. Discussion

We have already reported that 5-HT$_7$ receptors were present on myometrial cells and mediate a muscle layer-dependent (circular muscle>longitudinal muscle) inhibition of porcine uterine contractility due to heterogeneous expression of the receptors (more abundant in circular muscle than in longitudinal muscle) (Kitazawa et al., 1998, 2000, 2001). Among the species so far examined, the presence of 5-HT$_7$ receptors in the uterus is characteristics of pigs, suggesting a species-specific mechanism of regulation of uterine contractility by 5-HT. In the present study, we examined the effect of 5-HT on motility of the porcine oviduct and demonstrated that 5-HT$_7$ receptors are also present in the oviduct and mediate the relaxation induced by 5-HT probably through an increase in cytoplasmic cyclic AMP. Since the degree of 5-HT-induced relaxation in the oviduct was not decreased by tetrodotoxin, the relaxatory 5-HT$_7$ receptor was thought to be present on smooth muscle cells. Recently, nitric oxide has been suggested to be an inhibitory modulator in the oviduct (Costagliola et al., 1997; Ekerhovd et al., 1997), and it has been shown that sodium nitroprusside

relaxed the porcine oviduct. However, L-NAME and ODQ failed to decrease the relaxation induced by 5-HT. Among the smooth muscle relaxatory agents examined (5-HT, isoprenaline, sodium nitroprusside, vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide), 5-HT was the strongest agent. Therefore, it is thought that 5-HT and 5-HT_7 receptor-linked cyclic AMP mechanisms play an important role in the regulation of motility of not only the uterus but also the oviduct in the pig. As previously reported in the myometrium (Kitazawa et al., 2001), there was also a regional difference in the degrees of relaxation induced by 5-HT (isthmus>ampulla) probably due to the heterogeneous distribution of 5-HT_7 receptors (isthmus/ampulla = 2.4:1), suggesting different contribution of the 5-HT and 5-HT_7 receptor mechanisms to the regulation of ampullary and isthmic motility. Although there was a smooth muscle layer-dependent difference in the responses to 5-HT of the isthmus and ampulla (longitudinal muscle>circular muscle), the difference was opposite to that reported in the uterus (circular muscle>longitudinal muscle) (Kitazawa et al., 2000) and not so conspicuous.

Of the 5-HT receptor agonists examined, 5-CT was the most potent agonist causing relaxation. Among the well-defined 5-HT receptor subtypes (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4), involvement of the 5-HT_1 receptor in relaxation was suggested, firstly, because 5-CT is more potent than 5-HT at 5-HT_1 receptors (Boess and Martin, 1994; Hoyer et al., 1994). However, generally, the 5-HT_1 receptor family causes the inhibition of adenylate cyclase activity by G_i and assists the contraction of smooth muscle, reducing cytoplasmic cyclic AMP (Boess and Martin, 1994; Hoyer et al., 1994). Therefore, it seems unlikely that 5-HT_1 receptors mediate the smooth muscle relaxation. Additionally, in the present study, oxymethazoline (a full agonist for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors with a pEC_{50} value of 7.35–7.73, Schoeffter and Hoyer, 1991) and dihydroergotamine (a potent 5-HT_{1D} receptor agonist with a pK_i value of 9.87, Weinshank et al., 1992) did not cause the marked relaxation even at 10 μM. Two newly cloned 5-HT_1 receptors (5-HT_{1E} and 5-HT_{1F}) have been reported (Barone et al., 1993; Lovenberg et al., 1993), but 5-CT is less effective on 5-HT_{1E} and 5-HT_{1F} receptors than 5-HT (Boess and Martin, 1994). The above results rule out the possible involvement of the 5-HT_1 receptor family in 5-HT-induced relaxation of the porcine oviduct.

α-Methyl-5-HT, 2-methyl-5-HT and BIMU-1 were less effective in causing relaxation of the porcine oviduct even at 10 μM (15–30% of the maximum relaxation by 5-HT). In addition, 1 μM ketanserin (a 5-HT_{2A} and 5-HT_{2C} receptor antagonist with pK_b values of 9.3 and 6.5, respectively) (Hoyer et al., 1994), 10 μM tropisetron (pK_i values of 8.7 for 5-HT_3 receptor and 7.5 for 5-HT_4 receptor) (Boess and Martin, 1994) and 10 μM GR113808 (pK_i value of 9.5 for 5-HT_4 receptor) (Grossman et al., 1993) failed to antagonize the relaxation induced by 5-HT. Moreover, the correlation between the porcine oviduct 5-HT receptor and 5-HT_4 receptor was very low, because methiothepin, spiperone and mesulergine showed no antagonistic actions on the 5-HT_4 receptor (Table 1). These pharmacological findings suggest that the relaxatory 5-HT receptor in the smooth muscle of the porcine oviduct is not the 5-HT_2, 5-HT_3 or 5-HT_4 subtype.

In the present experiments, the 5-HT-induced relaxation of the oviduct was potentiated by Ro20-1724 but not by zaprinast, and 5-HT increased the tissue cyclic AMP contents. These results suggest that the 5-HT receptors present in the porcine oviduct are positively coupled to adenylate cyclase and that stimulation of these receptors increases the intracellular cyclic AMP followed by decrease in cytoplasmic [Ca^{2+}] and Ca^{2+} sensitivity of the contractile apparatus (Karaki et al., 1997). Of the 5-HT receptor subtypes, except for 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4, 5-HT_6 and 5-HT_7 receptors are positively coupled with adenylate cyclase and can be discriminated by comparison of the potency of 5-HT and 5-CT. 5-HT is more potent than 5-CT in the cloned rat and human 5-HT_6 receptors (Monsma et al., 1993) but 5-CT is 10 times more potent compared with 5-HT in the cloned rat, mouse and human 5-HT_7 receptors (Bard et al., 1993; Ruat et al., 1993; Shen et al., 1993). The higher potencies of 5-CT than that of 5-HT in the present mechanical study (pEC_{50}, 9.1 vs. 7.5) and the [^{3}H]5-CT binding competition study (pK_i, 9.4 vs. 8.5) suggest that the 5-HT_7 receptor mediates the relaxation of the oviduct induced by 5-HT. 5-MeOT and 8-OH-DPAT also act as 5-HT_7 receptor agonists, and the ranking order of potency is 5-CT>5-HT ≧ 5-MeOT>8-OH-DPAT (Shen et al., 1993; Leung et al., 1996; Terron, 1996). In the porcine oviduct, the ranking order of relaxation (5-CT>5-HT>5-MeOT>8-OH-DPAT) and of inhibition of [^{3}H]-5-CT binding (5-CT>5-MeOT ≧ 5-HT>8-OH-DPAT) were similar to that of the 5-HT_7 receptor. Although the ranking orders of 5-HT and 5-MeOT in contraction and competition studies were slightly different, low responsiveness of 5-MeOT on 5-HT_7 receptors has already been found in vitro functional studies using the monkey jugular veins (pEC_{50} = 5.7, Leung et al., 1996) and dog coronary arteries (pEC_{50} = 4.4, Terron, 1996). The conclusion that the 5-HT_7 receptor mediates the relaxation induced by 5-HT is strongly supported by the results of the antagonist study. DR4004, methiothepin, methysergide, metergoline, clozapine, mianserin and spiperone inhibited the 5-HT-induced relaxation and caused a parallel shift of the concentration–response curve to the right. These pK_b values were significantly correlated with pA_2 values in the porcine uterus (Kitazawa et al., 1998, 2001) and with pK_i values in the cloned human and mouse 5-HT_7 receptors (Plassat et al., 1993; Bard et al., 1993). On the other hand, correlation with 5-HT_6 receptor was weak (not significant). In the competitive binding study, 5-HT receptor agonists (5-HT, 5-CT, 5-MeOT and 8-OH-DPAT)

and antagonists (metergoline, methysergide, spiperone, mianserin) inhibited the [^{3}H]5-CT binding to the oviduct membrane and pK_i values of these agents showed significant correlation with those of 5-HT$_7$ receptors of several animal species (mouse, rat, human and guinea pig). These results also indicate the presence of 5-HT$_7$ receptors and contribution of 5-HT$_7$ receptors to the relaxation of the oviduct by 5-HT.

Some 5-HT receptor antagonists antagonized the relaxation induced by 5-HT in the oviduct (except for DR4004 and clozapine) caused a slowly developing contraction of isthmus of the oviduct quite different from the case in the porcine uterus (Kitazawa et al., 1998). These phenomena were interesting, but the mechanisms underlying could not be clarified from the results of the present experiments. One possible explanation is elimination of the tonic inhibition of endogenous 5-HT by 5-HT receptor antagonists. However, DR4004, a potent 5-HT$_7$ receptor antagonist (Kikuchi et al., 1999) was less effective in causing a slow contraction and the presence of endogenous 5-HT in the porcine oviduct has not been detected yet. Clarification of the mechanisms of antagonist-induced contraction might be important to determine the physiological roles of endogenous 5-HT and 5-HT$_7$ receptors in the maintenance of porcine oviduct tone.

The 5-ht$_5$ receptor is another type of 5-HT receptor, whose signal transduction mechanism and functional roles have not yet been clearly identified (Hoyer et al., 1994). Similar to the case in cloned 5-HT$_7$ receptor, 5-CT is more potent than 5-HT in the cloned mouse 5-ht$_5$ receptors (Plassat et al., 1992; Matthes et al., 1993). However, since ergotamine, a potent agonist of the 5-ht$_5$ receptor (pK_i value of 8.4–8.5, Plassat et al., 1992; Matthes et al., 1993) did not cause relaxation of the oviduct and the correlation between pK_b values in the oviduct and pK_i value for the 5-ht$_5$ receptor (both 5-ht$_{5A}$ and 5-ht$_{5B}$) is relatively weak (see Table 1), it is unlikely that 5-ht$_5$ receptors mediate the relaxation induced by 5-HT in the porcine oviduct.

In conclusion, this is the first in vitro mechanical study showing 5-HT-induced relaxation of the porcine oviduct. On the basis of (1) ranking order of 5-HT receptor agonists (5-CT>5-HT>5-MeOT>8-OH-DPAT), (2) significant correlations of antagonist pK_b values with pK_i for the 5-HT$_7$ receptor and (3) significant correlations of agonist and antagonist pK_i values with pK_i for the 5-HT$_7$ receptor, the 5-HT$_7$ receptor has been demonstrated to mediate the 5-HT-induced relaxation of the porcine oviduct through an increase in intracellular cyclic AMP. Distribution of 5-HT$_7$ receptors is dependent on the oviduct regions (more abundant in the isthmus than in the ampulla), and this heterogeneity causes a region-dependent difference in the 5-HT-induced relaxation (isthmus>ampulla). The strongest relaxation induced by 5-HT and 5-HT$_7$ receptor antagonist-induced contraction suggests that 5-HT$_7$ receptors play an important physiological role in the regulation of oviduct contractility in pigs.

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