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European Journal of Pharmacology 442 (2002) 115–123

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In vitro pharmacological characterization of the prostanoid receptor population in the non-pregnant porcine myometrium

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Received 6 December 2001; received in revised form 18 February 2002; accepted 26 February 2002

Abstract

In order to characterize prostanoid receptors present in the non-pregnant porcine uterus, the effects of naturally occurring prostaglandins (D_2, E_2, $F_{2\alpha}$, I_2) and synthetic prostanoid receptor agonists on contractility of the longitudinal and circular muscles were examined in vitro. The potent contractile actions of prostaglandin $F_{2\alpha}$ and cloprostenol indicate the presence of excitatory FP receptors in the porcine uterus. The longitudinal muscle was more sensitive to FP receptor agonists than was the circular muscle. Prostaglandin D_2 produced an excitatory response in the longitudinal muscle but completely inhibited the spontaneous contraction of the circular muscle. BW-245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin, 1 nM–10 μM, a DP receptor agonist) inhibited the spontaneous contractions of both muscles, but the inhibition was conspicuously stronger in the circular muscle. Prostaglandin I_2 caused excitatory and inhibitory responses in the longitudinal and circular muscles, respectively, at relatively high concentrations (10–100 μM). Cicaprost, an IP receptor agonist caused inhibition of the contraction in the circular muscle but contracted the longitudinal muscle. Iloprost, an EP_1/IP receptor agonist, caused excitatory responses in both muscles at relative high concentrations. Prostaglandin E_2 caused excitatory responses at 1–100 nM and inhibitory responses at 100 nM–10 μM in both muscle layers. ONO-DI-004 ((17*S*)-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E_1, an EP_1 receptor agonist) and ONO-AE-248 ((16*S*)-9-deoxy-9β-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2, an EP_3 receptor agonist) contracted the longitudinal muscle but had little effect on the circular muscle. ONO-AE1-259 (11,15-*O*-dimethyl prostaglandin E_2, an EP_2 receptor agonist) inhibited the spontaneous contractions of both muscle layers to almost the same degree, but ONO-AE1-329 (16-(3-methoxymethyl)phenyl-ω-tetranor-3,7-dithia prostaglandin E_1, an EP_4 receptor agonist) did not inhibit the myometrial contraction. The present results indicate that contractile (FP, EP_1, EP_3) and relaxatory (DP, IP, EP_2) prostanoid receptors are present in the non-pregnant porcine uterus. There are marked muscle layer-related differences in the degree of responsiveness of prostanoid receptor agonists, and these differences suggest that there is a heterogeneous distribution of prostanoid receptors in the longitudinal and circular muscles (FP, EP_1 and EP_3, longitudinal muscle > circular muscle; DP, circular muscle>longitudinal muscle).

Keywords: Prostanoid receptor; Uterus, porcine; DP receptor; FP receptor; EP receptor; Smooth muscle contractility

1. Introduction

Prostanoids produce a wide variety of mechanical responses (contraction, relaxation or a mixture of both responses) in vascular and non-vascular smooth muscles (trachea, gastrointestinal tracts, uterus) of several mammalian species. These tissue- and species-related variations in prostanoid-induced responses are caused by the presence of five types of prostanoid receptors, named EP, FP, IP, DP and TP, and by the low specificity of naturally occurring prostanoids (prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2 and thromboxane A_2) to each type of receptor. Moreover, the results of studies using selective agonists and antagonists have shown that the EP receptor can be arbitrarily subdivided into EP_1, EP_2, EP_3 and EP_4 receptors (Narumiya et al., 1999). The prostanoid receptors belong to a family of seven membrane-spanning receptors that transduce signals through G protein and stimulate phospholipase C-mediated hydrolysis of phosphoinositides (FP, TP and EP_1 receptors) and adenylate cyclase-mediated synthesis of cyclic AMP (DP, IP, EP_2 and EP_4 receptors). On the other hand, the EP_3 receptor causes inhibition of adenylate cyclase activity via G_i. On the basis of the intracellular signal transduction pathways, prostanoid receptors can be functionally divided into three groups: relaxant receptors (DP, IP, EP_2 and EP_4), which produce smooth muscle relaxation through elevation of cytoplasmic cyclic

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01489-9

AMP; contractile receptors (TP, FP and EP_1), which produce contraction through an increase in intracellular Ca^{2+}; and an EP_3, which is a contractile receptor inhibiting the smooth muscle relaxation through a decrease in adenylate cyclase activity (Narumiya et al., 1999).

It is thought that prostaglandins and prostanoid receptors play significant physiological roles in the induction of parturition. This speculation is based on findings that prostaglandins modify uterine contractility both in vivo and in vitro, application of exogenous prostaglandin induces parturition but treatment with a prostaglandin synthesis inhibitor delays labor, levels of prostaglandins and their metabolites increase sharply in amniotic fluid and maternal plasma during labor, and normal parturition does not occur in FP receptor knockout mice (Keirse, 1979; Chan, 1983; Challis and Olson, 1988; Romero et al., 1996; Sugimoto et al., 1997). Prostanoid receptors present in the myometrium have been investigated in contraction studies using selective prostanoid analogues, and these studies have demonstrated the existence of a heterogeneous population of excitatory and inhibitory prostanoid receptors in the human uterus (FP, TP, EP_1, EP_2, EP_3, DP and IP receptors) (Senior et al., 1991, 1992; Senchyna and Crankshaw, 1996; Hillock and Crankshaw, 1999; Popat and Crankshaw, 2001), rat uterus (FP, EP_3 and IP receptors) (Goureau et al., 1992) and sheep uterus (FP, EP_1, EP_3 and TP) (Crankshaw and Gaspar, 1995). Recent molecular biological studies have also indicated that several prostanoid receptors (FP, EP_3, EP_4) are expressed in the uterus of some species (human, rat, sheep and baboon) and that their expressions are regulated up or down manner during pregnancy and labor (Matsumoto et al., 1997; Smith et al., 1998; Brodt-Eppley and Myatt, 1998; Ma et al., 1999). Dong and Yallampalli (2000) reported that the expression of EP_2 and FP receptors in the rat uterus was modulated by exogenous steroid hormones (estradiol-17β and progesterone) and that sex hormones regulate uterine activity during pregnancy and labor through these mechanisms.

Prostaglandin $F_{2\alpha}$ analogues have been used clinically for induction of artificial parturition in pigs (Chantaraprateep et al., 1986; Friendship et al., 1990). Prostaglandin $F_{2\alpha}$ contracts the uterine muscle indirectly through increase in responsiveness of oxytocin followed by the FP receptor mediated regression of corpora lutea (decrease in plasma progesterone levels) (Sugimoto et al., 1997; Narumiya et al., 1999). However, possible direct action of prostaglandin $F_{2\alpha}$ on myometrial cells is supposed to assist the uterine contraction. But there have been no systematic studies on the effects of prostanoids on uterine contractility of domestic animals at present. The aim of this study was, therefore, to characterize the functional prostanoid receptor population present in the non-pregnant porcine myometrium. For this purpose, the mechanical actions of naturally occurring prostaglandins (D_2, E_2, $F_{2\alpha}$, I_2) and selective synthetic prostanoid receptor agonists on uterine contractility were examined in vitro. On the basis of the elicited mechanical responses (contraction or inhibition) to synthetic agonists and the rank order of responses to prostaglandins, subtypes of prostanoid receptors were identified. Since it was easy to mechanically separate the longitudinal and circular muscles of the porcine uterus, it was possible to compare prostanoid receptors present in both muscle layers.

2. Methods

2.1. Tissue preparations

Fresh uteri, with the ovaries intact, from 120 sexually mature crossbred virgin gilts (about 6 months old) were obtained from a local abattoir and were used for experiments on the day of slaughter. The animals were judged to be in proestrus (about 4 days in 21 days oestrus cycle) according to the results of gross examination of the follicle size (<2 mm) and to the appearance of the corpora lutea (McDonald, 1975). Longitudinal and circular muscle preparations were isolated surgically from the antimesometrial coat of the adtubal region (10 cm distal from the apex) in either the left or right cornu. As described previously (Taneike et al., 1991; Kitazawa et al., 2000), after removal of the endometrium, each muscle layer was cut through the muscle coat in either the longitudinal and circular muscle directions. The unwanted muscle layers were then removed from each muscle strip by meticulously cutting them away with fine scissors under a binocular microscope, thereby isolating the remaining layers for experimental use.

2.2. Experimental protocol and data analysis

Each muscle strip (1×10 mm) was suspended vertically in an organ bath (5 ml) containing 37 °C Krebs solution (mM) (NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11.5) 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 myometrial preparations. The muscle strips were loaded at $0.3-0.5 \times g$ as an initial tension and allowed to equilibrate for 60 min and to establish a steady spontaneous contractile activity. Frequencies of the spontaneous contraction in the longitudinal muscle (6.8 ± 0.4/10 min, $n = 24$) were different from those in the circular muscle (18 ± 0.7/10 min, $n = 22$). Each muscle layer was subjected to three or four stimulations with 50 mM high-K^+ solution every 15 min. Then, 0.5 or 1.0 logarithm concentration increments of prostaglandins and prostanoid receptor agonists were applied cumulatively to the organ bath at 5-min intervals. Stimulant-induced excitatory responses (contraction) were analyzed as the area between the contraction curve and the baseline (area under the curve) during a 5-min period after application of agonists. The change in contractile activity was expressed as a percentage of the area under the curve of 50 mM high-K^+-

induced contraction (for 5 min), calculated by the following equation: Relative response (%) = 100 × (area under the curve of stimulant-induced response)/(area under the curve of high-K^+-induced response). The excitatory responses to agonists were also expressed as a percentage of the area under the curve of the spontaneous contraction (for 5 min) to compare magnitude of the induced excitatory responses in the longitudinal and circular muscles. The areas under the curve of the spontaneous contraction in the longitudinal and circular muscles were 39.5 ± 4.2% (n = 45) and 106 ± 6.1% (n = 30) of the 50 mM high K^+-induced response, respectively. When inhibition of the spontaneous contraction was induced by prostaglandins and the agonists, these responses were analyzed by the minimum amplitude of the spontaneous contractions during application. The inhibition was expressed as a percentage of the spontaneous contraction in the absence of agonists, and IC_{50} values (concentrations of agonists that caused 50% inhibition of the spontaneous contraction) were calculated from the concentration–response relationships.

In some studies, the contractile responses to prostaglandins and prostanoid receptor agonists were examined in Kumagai solution (Kumagai et al., 1952) (mM) (NaCl, 150.6; KCl, 5.4; $CaCl_2$, 0.4; $MgCl_2$, 0.2; $NaHCO_3$, 4.8; Na_2HPO_4, 0.6; KH_2PO_4, 0.1; and glucose, 2.8) saturated with 95%O_2 + 5%CO_2 at 28 °C. Under experimental conditions of a low Ca^{2+} concentration and a low bathing temperature, spontaneous myometrial contractions were completely abolished and the excitatory responses were analyzed by the amplitude of contractile response. Prostaglandins and the receptor agonists were applied cumulatively at 2-min intervals, and the amplitude of the contraction was expressed as a percentage of the 50 mM high-K^+-induced response. The EC_{50} values (concentrations of agonists that caused 50% of the maximum contraction) and maximum contractions were estimated from the concentration–response curves.

2.3. Chemicals

The following drugs were used in the present experiments: cloprostenol (Sigma), prostaglandin $F_{2\alpha}$ (Wako), prostaglandin E_2 (Wako), prostaglandin D_2 (Wako), prostaglandin I_2 sodium salt (Sigma) and tetrodotoxin (Wako). Iloprost and cicaprost, as liquid for injection were kindly donated by Schering and were diluted by distilled water. EP receptor subtype selective agonists, (17*S*)-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E_1 (ONO-DI-004, EP_1 receptor), (16*S*)-9-deoxy-9β-chloro-15-deoxy-16-hyfroxy-17,17-trimethylene-19,20-didehydro prostaglandin F_2 sodium salt (ONO-AE1-259, EP_2 receptor), 11,15-*O*-dimethyl prostaglandin E_2 (ONO-AE-248, EP_3 receptor) and 16-(3-methoxymethyl)phenyl-ω-tetranor-3,7-dithia prostaglandin E_1 (ONO-AE1-329, EP_4 receptor) (Suzawa et al., 2000) were gifts from Ono Pharmaceutical, and BW-245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin) was from GlaxoWellcome. Prostaglandin I_2 and ONO-AE1-259 were dissolved in distilled water. Prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin D_2 were prepared in absolute ethanol. Cloprostenol, ONO-DI-004, ONO-AE-248 and ONO-AE1-329 were dissolved in dimethylsulfoxide. These prepared solutions were stored at −80 °C until use and were diluted by distilled water. The maximum concentrations of dimethylsulfoxide and ethanol in the bathing solution were set below 0.5% and 0.05%, respectively, concentrations that did not change the spontaneous contractile activity of the porcine uterus.

2.4. Statistical analysis

The results of the experiments are expressed as means ± S.E.M. of more than four experiments. Statistical analysis was performed by paired and unpaired *t*-tests, with $P < 0.05$ as the criterion of statistical significance.

3. Results

3.1. Mechanical effects of naturally occurring prostaglandins in longitudinal and circular muscles

Prostaglandin $F_{2\alpha}$ (1 nM–10 μM) applied cumulatively caused concentration-dependent increases in area under the curve of the longitudinal muscle without markedly affecting

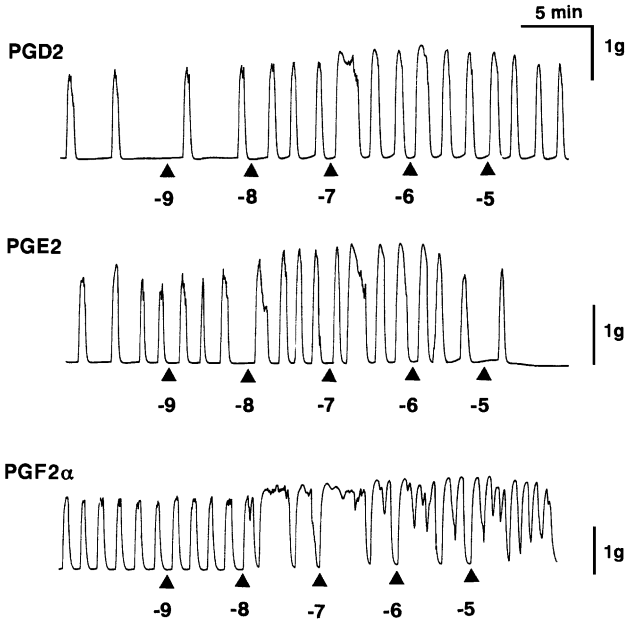

Fig. 1. Representative mechanical responses to prostaglandin D_2 (PGD_2) prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the longitudinal muscle of the porcine uterus. Five increasing concentrations (1, 10, 100 nM, 1 and 10 μM) of prostaglandins were applied cumulatively at 5-min intervals. Numbers under each trace indicate the concentrations of prostaglandins (log*M*).

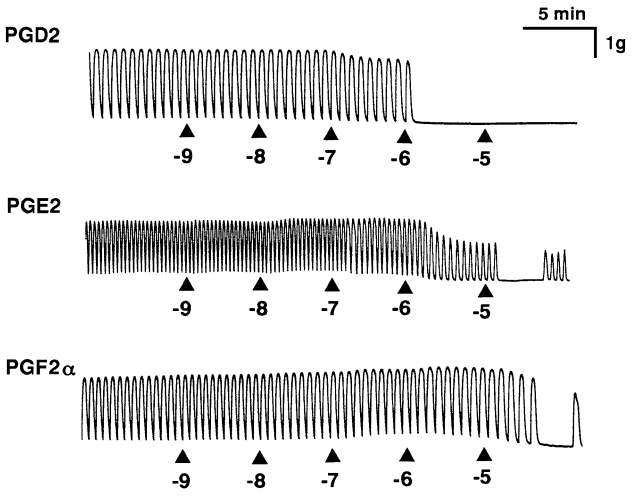

Fig. 2. Representative mechanical responses to prostaglandin D_2 (PGD_2) prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the circular muscle of the porcine uterus. Five increasing concentrations (1, 10, 100 nM, 1 and 10 μM) of prostaglandins were applied cumulatively at 5-min intervals. Numbers under each trace indicate the concentrations of prostaglandins (log*M*).

the amplitude of the spontaneous contractions (Figs. 1 and 3A). Prostaglandin D_2 was 10-times and prostaglandin I_2 was 100-times less potent agonists compared with prostaglandin $F_{2\alpha}$ (Figs. 1 and 3A). On the other hand, prostaglandin E_2 caused an excitatory response at low concentrations (1–100 nM) but inhibited the spontaneous contractions at higher concentrations (100 nM–10 μM). The excitatory responses to prostaglandins and the inhibitory response to prostaglandin E_2 were not affected by tetrodotoxin (1 μM) (data not shown), suggesting that prostaglandins have direct action on uterine smooth muscle cells. The EC_{100} values (concentrations of prostaglandins that caused the same excitatory responses as those of 50 mM high-K^+) for prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin D_2 and prostaglandin I_2 were estimated to be 17 ± 8 nM ($n=5$), 24 ± 11 nM ($n=7$), 108 ± 40 nM ($n=6$) and 3200 ± 670 nM ($n=5$), respectively (Fig. 3A). Four prostaglandins also contracted the longitudinal muscle in Kumagai solution, and the maximum responses were about 110–130% of the 50 mM high-K^+-induced contraction (Fig. 3B). Although concentration–response curves for prostaglandin $F_{2\alpha}$, prostaglandin D_2 and prostaglandin I_2 were monophasic (sigmoid), that of prostaglandin E_2 was bell-shaped (contraction at low concentrations and relaxation at higher concentrations), similar to the responses in Krebs solution (Fig. 3B). EC_{50} values were 27 ± 6.3 nM (prostaglandin $F_{2\alpha}$, $n=5$), 20 ± 2 nM (prostaglandin E_2, $n=6$), 400 ± 150 nM (prostaglandin D_2, $n=7$) and 990 ± 240 nM (prostaglandin I_2, $n=5$), respectively. The EC_{100} (Krebs solution) and EC_{50} values (Kumagai solution) indicated that the rank order of prostaglandins in the longitudinal muscle was prostaglandin $F_{2\alpha} \geqq$ prostaglandin E_2>prostaglandin D_2>prostaglandin I_2.

In the circular muscle preparations, prostaglandin D_2 inhibited the spontaneous contractions and finally abolished

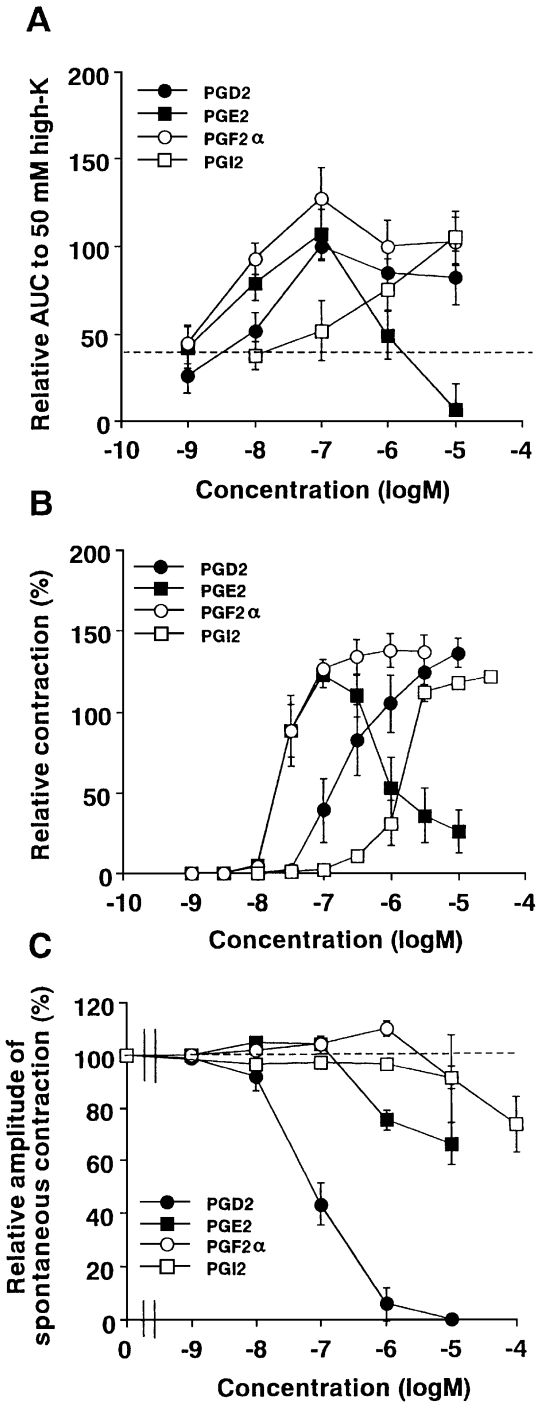

Fig. 3. Concentration–response relationships of four prostaglandins (PGs) in the longitudinal muscle (A, B) and circular muscle (C) of the porcine uterus. (A) Excitatory responses induced by prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2 in Krebs solution are shown as percentages of the 50 mM high-K^+-induced area under the curve (AUC for 5 min). The dotted line indicates the mean AUC level of spontaneous contractile activity ($39.5 \pm 4.2\%$, $n=45$). (B) Concentration–response curves for prostaglandins in Kumagai solution. Amplitudes of contraction are shown as percentages of the 50 mM high-K^+-induced contraction. (C) Effect of prostaglandins on spontaneous contraction of the circular muscle in Krebs solution. The ordinate represents the relative amplitude of spontaneous contraction (in the absence of prostaglandins = 100%). Each point is the mean ± S.E.M. of four to eight separate experiments.

Table 1
Excitatory effects of selective prostanoid receptor agonists (cloprostenol, ONO-DI-004 and ONO-AE-248) on the spontaneous contractions of the longitudinal and circular muscles of the porcine uterus

Agonist (receptor)	Relative mechanical response (%)[a]				
	1 nM	10 nM	100 nM	1 μM	10 μM
Cloprostenol (FP)					
Longitudinal muscle	96 ± 1.9	255 ± 43.1	380 ± 76.6	308 ± 65.5	276 ± 41.7
Circular muscle	98 ± 8.0	99 ± 8.3[b]	114 ± 7.5[b]	135 ± 6.2[b]	156 ± 2.6[b]
ONO-DI-004 (EP_1)					
Longitudinal muscle	108 ± 10	94 ± 18.5	152 ± 10.7	211 ± 37.9	337 ± 56.0
Circular muscle	98 ± 3.6	99 ± 5.1	100 ± 2.2[b]	104 ± 2.1[b]	100 ± 3.5[b]
ONO-AE-248(EP_3)					
Longitudinal muscle	109 ± 7.5	122 ± 7.5	157 ± 31.1	211 ± 36.7	213 ± 25.2
Circular muscle	103 ± 3.0	100 ± 3.4[b]	97 ± 3.4[b]	128 ± 6.8[b]	143 ± 12.4[b]

Values are means ± S.E.M. of over four separate experiments.
[a] Mechanical responses to each receptor agonist were expressed as a percentage of the area under the curve of spontaneous contraction (for 5 min, 100%).
[b] Significantly different from the corresponding responses in the longitudinal muscle.

them completely (IC_{50} = 110 ± 50 nM, n = 6). Low concentrations of prostaglandin E_2 (1–100 nM) and prostaglandin $F_{2\alpha}$ (1 nM–1 μM) caused slight increases in the amplitude of the spontaneous contraction (5–15%), but high concentrations (10 μM) caused inhibition of the spontaneous contractions (Figs. 2 and 3C). Prostaglandin I_2 inhibited the spontaneous contraction at 10–100 μM but the inhibition was weak even at high concentrations (10 μM, 8.7 ± 4.4%; 100 μM, 26 ± 11%, n = 6). From the concentration–response curves, the rank order of inhibition was prostaglandin $D_2 \gg$ prostaglandin E_2 > prostaglandin $F_{2\alpha}$ = prostaglandin I_2 (Fig. 3C). The inhibitory responses to prostaglandins were also resistant to tetrodotoxin (1 μM) (data not shown).

3.2. Effect of a FP receptor agonist

Cloprostenol (1 nM–1 μM) caused excitatory responses of the longitudinal muscle in a concentration-dependent manner. The maximum response obtained at 100 nM was 380 ± 76.6% (n = 5) of the area under the curve of spontaneous contraction. Although cloprostenol also caused excitatory responses in the circular muscle, a 100-times higher concentration (100 nM) was required to increase the muscle contractility and the responses were considerably weak (Table 1).

3.3. Effect of a DP receptor agonist

BW-245C (1 nM–10 μM) concentration-dependently inhibited the spontaneous contractions (both amplitude and frequency) of the longitudinal and circular muscles. However, there was a muscle layer-dependent difference in the responsiveness of BW-245C. In the circular muscle, inhibition by BW-245C was induced by a low concentration and the spontaneous contraction was abolished at 100 nM in all preparations examined (IC_{50}, 17 ± 4 nM, n = 9) (Table 2). On the other hand, the inhibitory response to BW-245C in the longitudinal muscle was relatively weak and the inhibition was only 56 ± 11.9%

Table 2
Inhibitory effects of selective prostanoid receptor agonists (BW-245C, ONO-AE1-259 and ONO-AE1-329) on the spontaneous contractions of the longitudinal and circular muscles of the porcine uterus

Agonist (receptor)	Relative amplitude of spontaneous contraction (%)[a]				
	1 nM	10 nM	100 nM	1 μM	10 μM
BW-245C (DP)					
Longitudinal muscle	100 ± 1.1	97 ± 3.2	74 ± 11.5	53 ± 13.4	44 ± 11.9
Circular muscle	98 ± 1.0	67 ± 6.2[b]	0 ± 0[b]	0 ± 0[b]	ND
ONO-AE1-259 (EP_2)					
Longitudinal muscle	99 ± 0.7	94 ± 4.0	63 ± 10.8	16 ± 15.9	0 ± 0
Circular muscle	99 ± 0.6	95 ± 1.4	72 ± 2.7	25 ± 11	0 ± 0
ONO-AE1-329 (EP_4)					
Longitudinal muscle	100 ± 2.3	99 ± 3.0	93 ± 5.0	100 ± 9.0	101 ± 8.0
Circular muscle	101 ± 8.4	101 ± 2.9	99 ± 4.1	100 ± 6.2	86 ± 8.6

Values are means ± S.E.M. of over four separate experiments.
ND, not determined.
[a] Inhibitory effects of the agonists were expressed as a relative amplitude of spontaneous contraction (before treatment = 100%).
[b] Significantly different from the corresponding responses in the longitudinal muscle.

($n=8$) even at 10 μM. The concentration that caused 50% of the maximum inhibition was 120 ± 29 nM ($n=8$) (Table 2).

3.4. Effects of IP receptor agonists

To investigate the presence of IP receptors, effects of iloprost and cicaprost on the spontaneous contractions were examined. In the longitudinal muscle, both agonists caused slight inhibition of the spontaneous contraction at low concentrations (1–10 nM) but contracted the myometrial strips at higher concentrations (100 nM–1μM). Relative areas under the curve of the responses (spontaneous contraction = 100%) induced by 1, 10, 100 nM, 1 and 10 μM iloprost were 95 ± 2.6%, 88 ± 6.5% 220 ± 86%, 383 ± 100% and 448 ± 103% ($n=4$) and those induced by 1, 10, 100 nM, 1 and 10 μM cicaprost were 102 ± 5.6%, 94 ± 2.7% 103 ± 29%, 370 ± 124% and 430 ± 180% ($n=4$), respectively. On the other hand, the effects of cicaprost and iloprost in the circular muscle were different; i.e., iloprost (100 nM–10 μM) increased the amplitude of the spontaneous contraction (control = 100%) in a concentration-dependent manner (100 nM, 102 ± 1.8%; 1 μM, 112 ± 2.9%; 10 μM, 118 ± 3.6%, $n=7$) but cicaprost inhibited the spontaneous contractions ($IC_{50}=5.9\pm2.3$ μM, $n=7$) and the maximum inhibition (88 ± 11%, $n=7$) was obtained at 10 μM.

3.5. Effects of excitatory EP receptor agonists

Since iloprost can act on not only IP receptors but also EP_1 and EP_3 receptors (Kiriyama et al., 1997), the contraction by iloprost suggest the presence of excitatory EP receptors. Therefore, the effects of EP_1 (ONO-DI-004) and EP_3 (ONO-AE-248) receptor selective agonists (Suzawa et al., 2000) were examined. ONO-DI-004 (100 nM–10 μM) increased the contractile activity of the longitudinal muscle up to 337 ± 56% ($n=5$) of the control. However, this agonist was almost ineffective in the circular muscle even at 10 μM. In contrast, ONO-AE-248 stimulated the contractile activity of both longitudinal and circular muscles but the magnitude of the responses of the longitudinal muscle was significantly higher than that of the circular muscle (Table 1).

The effects of two agonists were also compared in Kumagai solution. ONO-DI-004 (300 nM–10 μM) caused contraction of the longitudinal muscle, but the response did not reach a plateau even at 10 μM. On the other hand, the circular muscle was less effective to this agonist. Relative contractions (% to 50 mM high-K^+ response) induced by 1, 3 and 10 μM ONO-DI-004 were 25 ± 11%, 86 ± 20%, 121 ± 28% ($n=4$) in the longitudinal muscle, and 8 ± 3.5%, 8 ± 1.7% and 8 ± 2.2% ($n=4$) in the circular muscle, respectively. ONO-AE-248 also caused the muscle layer-related contraction (longitudinal muscle>circular muscle). Relative contractions induced by 1, 3 and 10 μM ONO-AE-248 were 31 ± 8%, 94 ± 23% and 130 ± 5% ($n=4$) in the longitudinal muscle, and 3 ± 0.8%, 4 ± 1.1% and 24 ± 11.5% ($n=4$) in the circular muscle, respectively.

3.6. Effects of inhibitory EP receptor agonists

The effects of EP_2 and EP_4 receptor agonists were tested to investigate the presence of inhibitory EP receptors in the porcine uterus. ONO-AE1-259, an EP_2 receptor agonist, reduced the amplitude of the spontaneous contractions and eventually abolished them in both muscle layers (3–10 μM). The IC_{50} value of ONO-AE1-259 was 390 ± 240 nM ($n=6$) in the longitudinal muscle, and this value was not significantly different from that in the circular muscle (520 ± 140 nM, $n=6$). On the other hand, ONO-AE1-329 (1 nM–10 μM), an EP_4 receptor agonist, did not change the spontaneous contraction in either muscle layer (Table 2).

4. Discussion

In the present study, the functional prostanoid receptors in the porcine uterus were pharmacologically characterized by the rank order of the responsiveness of naturally occurring prostaglandins (D_2, E_2, $F_{2\alpha}$, I_2) and by the actions of receptor selective synthetic agonists. The results indicate the existence of contractile (FP, EP_1, EP_3) and relaxatory (DP, IP, EP_2) prostanoid receptors in the non-pregnant porcine uterus. In addition, there are muscle layer-related differences in the responsiveness of prostanoid receptor agonists, and these differences suggest a heterogeneous distribution of prostanoid receptors between muscle layers.

Prostaglandins caused contractile responses in the longitudinal muscle, and prostaglandin $F_{2\alpha}$ showed the strongest effect. The magnitude of contractions induced by cloprostenol, a selective agonist for the FP receptor was similar to that induced by prostaglandin $F_{2\alpha}$. These results suggest the presence of FP receptors in the porcine uterus, as has been demonstrated in the uteri of humans (Senior et al., 1992), rabbits (Chen et al., 1998), rats (Goureau et al., 1992) and sheep (Crankshaw and Gaspar, 1995). Therefore, the FP receptor is thought to be one of widespread prostanoid receptors mediating the contractile action of prostaglandins in the uterus. In contrast to their actions in the longitudinal muscle, prostaglandin $F_{2\alpha}$ and cloprostenol induced only small contractions in the circular muscle, suggesting a muscle layer-related heterogeneous distribution of FP receptors (longitudinal muscle>circular muscle).

The rank order of the excitatory responses to prostaglandins in the longitudinal muscle (prostaglandin $F_{2\alpha} \geqq$ prostaglandin E_2>prostaglandin D_2>prostaglandin I_2) was not completely consistent with that for FP receptors (prostaglandin $F_{2\alpha}$>prostaglandin D_2>prostaglandin E_2) (Kiriyama et al., 1997; Narumiya et al., 1999). Prostaglandin E_2 showed a strong effect and this finding suggests the presence of contractile EP receptors. In the present study, a selective

EP_3 receptor agonist (ONO-AE-248) increased myometrial contractility of both muscle layers and the excitatory response was marked in the longitudinal muscle. An EP_1 receptor agonist (ONO-DI-004) also caused excitatory responses in the longitudinal muscle but had no effect in the circular muscle. These findings indicate the presence of EP_1 and EP_3 receptors in the porcine uterus. On the basis of results of contraction studies, Senior et al. (1991, 1993) and Goureau et al. (1992) have already suggested that EP_3 receptors are the dominant EP receptors in the human and rat myometrium. A recent molecular study also has demonstrated the expression of EP_3 receptors in the uterus and an increase in the expression level of the receptors during pregnancy, but the EP_1 receptor-coding gene was not detected (Ma et al., 1999). Although both EP_1 and EP_3 receptors were found in the porcine uterus, the fact that the K_i value of prostaglandin E_2 for the EP_1 receptor (20 nM) was higher than that for the EP_3 receptor (1 nM) (Kiriyama et al., 1997) suggest that the EP_3 receptor is a functionally important excitatory EP receptor for endogenous prostaglandin E_2. Comparison of the responses to EP_1 and EP_3 receptor agonists between longitudinal and circular muscles showed the muscle-layer dependent difference in the magnitude of the contraction. These results suggest a heterogeneous distribution of EP_1 and EP_3 receptors in muscle layers (longitudinal muscle>circular muscle), similar to that for the FP receptors.

The bell-shaped concentration–response curves for prostaglandin E_2 in the porcine uterus suggest the presence of inhibitory EP receptors. Subtypes of inhibitory EP receptors have been investigated in several animals by functional and molecular biological studies, and the presence of the EP_2 receptor has been demonstrated in the human (Senior et al., 1991, 1992; Hillock and Crankshaw, 1999; Popat and Crankshaw, 2001), baboon (Garcia-Villar et al., 1995), rat (Brodt-Eppley and Myatt, 1998; Dong and Yallampalli, 2000) and sheep uterus (Ma et al., 1999). In the present study, ONO-AE1-259, but not ONO-AE1-329 (Suzawa et al., 2000) caused inhibition of the spontaneous contraction, and there was no difference between the effects of ONO-AE1-259 in the longitudinal and circular muscles. These findings suggest that EP_2 receptors are distributed homogeneously in both muscle layers and that they mediate the inhibitory response to prostaglandin E_2. Although the EP_4 receptor has been reported to be expressed in the sheep uterus (Ma et al., 1999), existence of the EP_4 receptor was not found in the porcine uterus as was also the case in the human uterus (Senior et al., 1991, 1992; Hillock and Crankshaw, 1999; Popat and Crankshaw, 2001). Since the K_i value of prostaglandin E_2 for EP_2 receptor was about 12-times higher than that for the contractile EP_3 receptor (Kiriyama et al., 1997), a high concentration of prostaglandin E_2 was required to cause inhibitory response in the porcine uterus.

The presence of DP receptors in the uterus, based on findings of a relaxatory response to BW-245C (a DP receptor agonist) has so far been demonstrated only in humans (Senior et al., 1992; Fernandes and Crankshaw, 1995). However, in the present study, BW-245C inhibited the spontaneous contraction of both uterine smooth muscle layers at nanomolar concentrations, indicating the presence of DP receptors in the porcine uterus. There was a muscle layer-related difference in the inhibitory response to BW-245C (circular muscle>longitudinal muscle), which was quite different from those of FP, EP_1 and EP_3 receptor agonists. These muscle layer-dependent distributions of excitatory and inhibitory prostanoid receptors are supposed to reflect the different function of longitudinal and circular muscles in uterine motility, such as moving of luminal contents by contraction (longitudinal muscle) and retention of luminal contents by relaxation (circular muscle). Prostaglandin D_2, an endogenous DP receptor agonist, also inhibited the spontaneous contraction in the circular muscle but caused contraction in the longitudinal muscle in spite of the presence of DP receptors. Since prostaglandin D_2 can act on not only DP receptors ($K_i = 21$ nM) but also FP receptors ($K_i = 47$ nM) (Kiriyama et al., 1997), the different actions of prostaglandin D_2 in the longitudinal and circular muscles might be due to heterogeneous distributions of DP and FP receptors (DP, circular muscle>longitudinal muscle; FP, longitudinal muscle>circular muscle). In the longitudinal muscle, prostaglandin D_2 acts on a dominant FP receptor and causes contraction, while prostaglandin D_2 inhibits the contractility through activation of a dominant DP receptor in the circular muscle.

There have been very few reports on the relaxant actions of prostaglandin I_2 on the uterus (Senior et al., 1992). Prostaglandin I_2 exhibited a weak contraction in the porcine uterine longitudinal muscle at a high concentration. Generally, activation of IP receptors produces muscle relaxation (Narumiya et al., 1999), and, therefore, contraction of the longitudinal muscle caused by prostaglandin I_2 is thought to occur through activation of other prostanoid receptors (EP_1, EP_3 and FP receptors) (Coleman, 1987; Okada et al., 2000). Prostaglandin I_2 caused a weak inhibition of the contractility in the circular muscle, but a high concentration was needed. Therefore, it was thought to be difficult to examine the function of the IP receptor using prostaglandin I_2. Two selective synthetic IP receptor agonists, iloprost and cicaprost, were tested in the present study. Although iloprost caused a weak inhibition in the longitudinal muscle at low concentrations, predominant actions induced by iloprost were contraction of the myometrium. Iloprost also acts on EP_1 and EP_3 receptors at similar concentrations (K_i, 11 nM for IP, 21 nM for EP_1 and 27 nM for EP_3, Kiriyama et al., 1997), and IP receptor-mediated inhibition might be masked by the excitatory responses through EP_1 and EP_3 receptors. On the other hand, cicaprost showed different mechanical actions in the longitudinal muscle (contraction) and the circular muscle (relaxation). Cicaprost acts on both IP ($K_i = 11$ nM) and EP_3 receptors ($K_i = 170$ nM) (Kiriyama et al., 1997). Therefore, cicaprost caused weak inhibition (IP) at

low concentrations and marked contraction (EP_3) at higher concentrations in the longitudinal muscle. However, in the circular muscle, owing to the low density of EP_3 receptors suggested by the present results, the action of cicaprost on IP receptor was not masked and was exhibited as an inhibition of myometrial contractility. These results might suggest the presence of IP receptors in the porcine uterus. The possible heterogeneous density of IP receptors (circular muscle>longitudinal muscle) could also explain the different mechanical actions of cicaprost in the longitudinal and circular muscles.

In conclusion, the present functional study has indicated that the non-pregnant porcine uterus contains a heterogeneous population of prostanoid receptors, such as contractile (FP, EP_1, EP_3) and relaxatory (DP, IP, EP_2) prostanoid receptors. The muscle layer-related difference in the responsiveness of prostanoid receptor agonists suggest that there are unequal distributions of prostanoid receptors in the longitudinal and circular muscles (FP, EP_1 and EP_3, longitudinal muscle>circular muscle; DP, circular muscle>longitudinal muscle). Owing to the heterogeneous distribution of contractile and relaxatory prostanoid receptors, naturally occurring prostaglandins cause excitation of longitudinal muscle motility and inhibition of circular muscle motility in the porcine uterus.

Acknowledgements

This work was partly supported by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (13660304) and from Gakujitsu-Frontier Cooperative Research in Rakuno Gakuen University (2001-5). We wish to thank Ono Pharmaceutical for supplying ONO-DI-004, ONO-AE1-259, ONO-AE-248 and ONO-AE1-329, Schering for supplying iloprost and cicaprost, and GlaxoWellcome for supplying BW-245C.

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