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Iloprost: Intracellular Ca^{2+}-dependent Contractile Effect on Isolated Smooth Muscle Cells from Guinea-pig Ileum

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Abstract

Prostaglandin E_2 (PGE_2) and iloprost induced a concentration-dependent contraction of smooth muscle cells isolated from the circular layer of guinea-pig ileum. PGE_2- and iloprost-induced contractions were inhibited by the selective EP_1-receptor antagonist, SC19220 (1-acetyl-2-(8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl)-hydrazine), indicating the involvement of the EP_1 subtype of the PGE_2 receptor. When cells were incubated for 10 min in the presence of strontium (4 mM L^{-1}), an inhibitor of the release of Ca^{2+} from intracellular store, the contractile effect of PGE_2 and iloprost was inhibited. In contrast, incubation of cells in Ca^{2+}-free medium, Ca^{2+}-free medium plus EGTA, or in the presence of nifedipine, an organic Ca^{2+}-channel blocker, did not alter the PGE_2- and iloprost-induced contraction. These observations suggest that the myogenic effect of PGE_2 and iloprost on intestinal smooth muscle is dependent on the release of intracellular calcium.

The actions of prostaglandins (PG) in smooth muscle are variable, depending on the type of PG, the concentration, the organ, the species, and even the muscle layer studied (Sanders 1981; Gardiner 1986; Eglen & Whiting 1988; Botella et al 1993). Prostaglandin E_2 (PGE_2) receptors are pharmacologically subdivided into three subtypes, EP_1, EP_2 and EP_3, and these receptor subtypes are suggested to be different in their signal transduction (Coleman et al 1990).

Iloprost, a potent agonist at prostacyclin (IP) and EP_1 receptors (Schillinger et al 1986; Sheldrick et al 1988), produces opposite effects in smooth muscle, depending on the organ and species studied (Vermue et al 1987; Siegel et al 1989). In the gastrointestinal tract, iloprost contracts smooth muscle strips (Coleman et al 1985) and isolated smooth muscle cells (Botella et al 1993) from guinea-pig ileum via an EP_1-receptor. Recently, in a human erythroleukaemia cell line, iloprost has been reported to increase cytosolic Ca^{2+} concentration (Schwaner et al 1992). However, the intracellular pathway involved in iloprost-induced contraction of smooth muscle cells is yet unclear.

The aim of this study was to determine the influence of extracellular and intracellular calcium on PGE_2- and iloprost-induced contraction of isolated smooth muscle cells from guinea-pig ileum. The effects observed were compared with those of galanin, a contracting agent known to induce a contraction of smooth muscle cells by triggering an influx of extracellular Ca^{2+} (Botella et al 1992a) and of cholecystokinin octapeptide (CCK8), which induces cell contraction by releasing intracellular Ca^{2+} (Bitar et al 1986).

Materials and Methods

Materials

Stock solutions of prostanoids were prepared in ethanol and stored at −20°C. Iloprost was obtained from Schering (Berlin, Germany). SC19220 (1-acetyl-2-(8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl)-hydrazine) was a gift from Searle (Skokie, IL, USA). Pronase was purchased from Boehringer Mannheim Ltd (Meylan, France). Benzylpenicillin and streptomycin G were from Specia (Paris, France). Collagenases (Type IV, V), PGE_2, galanin, cholecystokinin octapeptide (CCK8) and all other reagents were obtained from Sigma (St Louis, MO, USA).

Method

Cell dispersion was achieved as previously described from small muscle strips from the circular muscle layer of guinea-pig ileum (Botella et al 1992b). After removal of serosa, longitudinal muscle layer and mucosa-submucosa layers from ileum, the strips were incubated for two periods of 15 min at 31°C in the medium (in mM: 132 NaCl, 5·4 KCl, 5 Na_2HPO_4, 1 NaH_2PO_4, 1·2 $MgSO_4$, 1 $CaCl_2$, 25 HEPES (*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulphonic acid)), 0·2% glucose (w/v), 0·2% bovine serum albumin (w/v); pH 7·4, bubbled with 95% O_2–5% CO_2 and supplemented with antibiotics, benzylpenicillin (100 int. units mL^{-1}) and streptomycin (50 μg mL^{-1}), containing collagenase 0·1 mg mL^{-1} type IV and 0·1 mg mL^{-1} type V. The strips were then transferred into fresh enzyme-free medium and left to stand for 10 min to allow the muscle cells to disperse spontaneously under very slow mechanical agitation. For contraction experiments, 250 μL cell suspension was added to 250 μL solution containing the agent to be tested, and incubated for 30 s at 31°C. The reaction was interrupted by addition of glutaraldehyde to a final concentration of 2·5%. In control experiments, 250 μL of the same medium was substituted for the tested agent.

To test the effect of Sr^{2+}, half the tissue was rinsed and then incubated in normal Ca^{2+}-containing buffer, while the other half was rinsed and then incubated in Ca^{2+}-free

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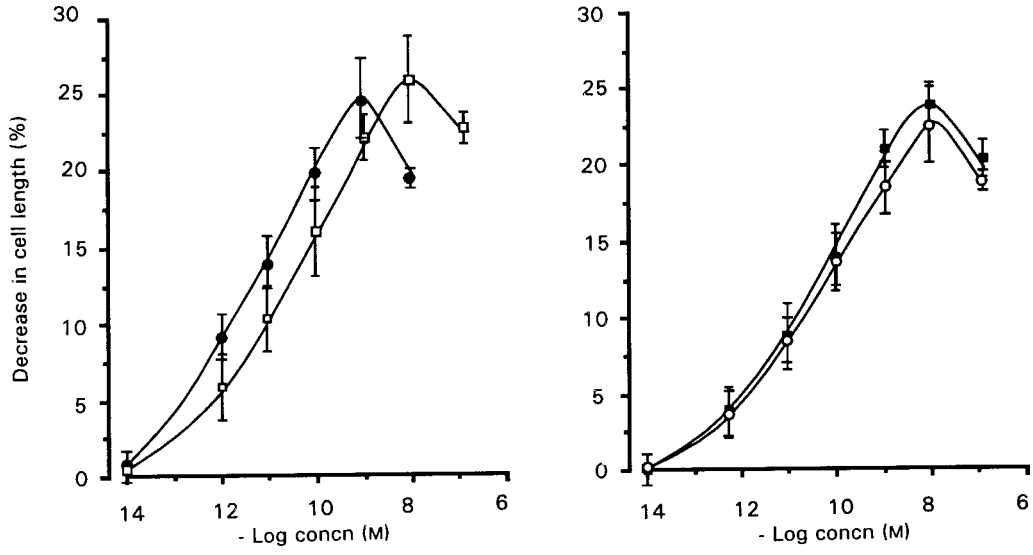

FIG. 1. Cell contraction induced by PGE_2 (■), iloprost (○), CCK8 (●) and galanin (□) in isolated circular smooth muscle cells from guinea-pig ileum. Cells were incubated for 30 s in the presence of various concentrations of agents at 31°C. Cell contraction induced by PGE_2, iloprost, CCK8 and galanin is expressed as the percentage of decrease in cell length from control. Values are means of five experiments in different animals.

medium containing 4 mM Sr^{2+}, for 10–20 min until cells spontaneously disassociated (Biancani et al 1987).

For experiments in Ca^{2+}-free medium, cells were first dispersed as described above. Thereafter, muscle strips were washed and incubated for 30 min to allow spontaneous dispersion in enzyme- and Ca^{2+}-free medium, with or without 2 mM EGTA (ethylene glycol-bis (b-aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid).

To measure cell length, a sample of cells fixed with glutaraldehyde was placed on a Malassez slide and the length of the first 50 cells randomly encountered in successive microscopic fields was measured. The contractile response was defined as the decrease in the average cell length of a population of muscle cells exposed to a tested agent in comparison with controls. Statistical evaluation was carried out using Student's *t*-test and the normality of the cell samples was assessed by the normal law test.

Results

Effect of PGE_2, iloprost, CCK8 and galanin

PGE_2, iloprost, CCK8 and galanin induced a contraction of isolated cells in a concentration-dependent manner. The maximal contraction was observed at 10 nM for PGE_2, iloprost, and galanin, and at 1 nM for CCK8, and corresponded to a 23·1 ± 2·1, 22·6 ± 2·2, 25·7 ± 3·5 and 24·0 ± 2·4% decrease in cell length from control, respectively. The EC50 value (concentration of an agonist inducing a contraction corresponding to 50% of its maximal effect) was 50 pM for PGE_2, 20 pM for iloprost, 80 pM for galanin and 8 pM for CCK8 (Fig. 1).

To demonstrate the direct action of PGE_2, iloprost, CCK8 and galanin on smooth muscle, the effect of tetrodotoxin (10 μM) was evaluated on the contraction these agents induced in isolated smooth muscle cells. Tetrodotoxin failed to inhibit cell contraction induced by PGE_2 (10 nM), iloprost (10 nM), CCK8 (1 nM) or galanin (10 nM) (data not shown).

Effect of SC19220 on PGE_2-, iloprost- and galanin-induced contraction

SC19220 inhibited cell contraction induced by PGE_2 (10 nM) and iloprost (10 nM) in a concentration-dependent manner. Concentrations inducing a half-maximal inhibition were 100 pM and 8 pM in the presence of PGE_2 and iloprost, respectively. The PGE_2- and iloprost-induced contractions were abolished at 1 μM and 10 nM of SC19220, respectively. By contrast, SC19220 failed to inhibit the contraction induced by 10 nM galanin or by 1 nM CCK8 at concentrations ranging from 10 fM to 1 μM (Fig. 2).

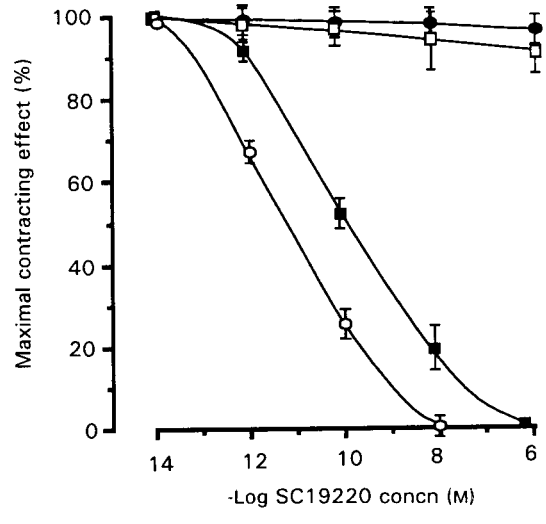

FIG. 2. Inhibition by the specific antagonist of the EP_1 receptor SC19220 of cell contraction induced by PGE_2 (■), iloprost (○), CCK8 (●) and galanin (□) in isolated cells from guinea-pig ileum. Cells were preincubated for 1 min at 31°C in presence of various concentrations of SC19220, the contracting agent (10 nM) was then added for 30 s. Results are expressed as the percentage of cell contraction observed in the absence of antagonist, taken as 100%. Points are means of five separate experiments.

Table 1. Influence of extra- and intracellular calcium on the contraction induced by PGE_2, iloprost, CCK8 and galanin on isolated smooth muscle cells from guinea-pig ileum.

	Decrease in cell length (%)				
	Control	Ca^{2+}-free	EGTA	Nifedipine	Sr^{2+}
Galanin (10 nM)	25·7 ± 3·5	5·6 ± 2·5*	0·4 ± 0·2*	0·3 ± 0·2*	24·5 ± 3·1
PGE_2 (10 nM)	23·1 ± 2·1	23·0 ± 2·2	23·5 ± 2·5	24·0 ± 2·5	2·5 ± 1·8*
Iloprost (10 nM)	22·6 ± 2·2	22·0 ± 2·5	22·0 ± 2·0	22·5 ± 2·5	2·9 ± 1·7*
CCK8 (1 nM)	24·0 ± 2·4	24·2 ± 2·3	23·5 ± 2·4	23·8 ± 2·7	2·1 ± 1·0*

*$P < 0·001$ compared with the corresponding control value. Values are means of five separate experiments.

Effect of extra- and intracellular calcium on the contractile response induced by PGE_2, iloprost, CCK8 and galanin

The incubation of cells in Ca^{2+}-free medium caused a significant decrease in the galanin-induced contraction (5·6% of the resting cells from control). By contrast, removing extracellular Ca^{2+} did not significantly impair the contraction caused by PGE_2 (10 nM), iloprost (10 nM) or CCK8 (1 nM) (Table 1). When cells were incubated in Ca^{2+}-free medium with added 2 mM EGTA, the contraction induced by galanin (10 nM) was abolished, while the PGE_2-, iloprost- and CCK8-induced contractions were unchanged. When cells were incubated in a 1 mM Ca^{2+} medium but in the presence of 1 μM nifedipine, the galanin-induced contraction was nearly abolished (0·3 ± 0·2% of the resting cells from control), while nifedipine had no effect on the contraction induced by PGE_2 (10 nM), iloprost (10 nM) and CCK8 (1 nM) (Table 1).

In contrast, when cells were preincubated in the presence of 4 mM strontium for 10–20 min, the contraction induced by PGE_2 (10 nM), iloprost (10 nM) and CCK8 (1 nM) was abolished, while the galanin-induced contraction was unchanged (Table 1).

Discussion

Numerous studies have demonstrated that iloprost, a potent agonist at IP- and at EP_1-receptors (Schillinger et al 1986; Sheldrick et al 1988), may produce either a contraction (Vermue et al 1987), no effect (Vermue et al 1987), or a relaxation (Siegel et al 1989) in various smooth muscle preparations. Iloprost usually causes relaxation of smooth muscle from cerebral arteries (Whalley et al 1989), while in canine and human basilar arteries it induces a biphasic response (Parsons & Whalley 1989). The present experiments confirm a previous study showing that PGE_2 and iloprost induce contraction of isolated smooth muscle cells from guinea-pig ileum (Botella et al 1993). The selective EP_1-receptor antagonist, SC19220 (Sanner 1972), inhibits these contractile effects, indicating that the receptor involved is of the EP_1-receptor subtype.

The intracellular pathway triggered by iloprost in smooth muscle is as yet unclear. Indeed, iloprost activates adenylate cyclase and increases intracellular cyclic AMP (Mene & Dunn 1988), but it also modulates calcium entry into smooth muscle from rabbit isolated vascular segments by voltage-dependent calcium channels (Demirel & Turker 1989). In our study, the contractile effects of PGE_2, iloprost and CCK8 were not affected by nifedipine, an organic Ca^{2+}-channel blocker, nor by incubating cells in Ca^{2+}-free medium or in Ca^{2+}-free medium plus EGTA. By contrast, in the same experimental conditions, the galanin-induced cell contraction was abolished or markedly reduced, as previously shown in smooth muscle cells from pig ileum (Botella et al 1992a). These observations indicate that the myogenic effect of PGE_2 and iloprost is independent of extracellular calcium entry. In contrast, when we substituted Ca^{2+} with Sr^{2+} in the medium, to inhibit the release of Ca^{2+} from intracellular stores (Biancani et al 1987), PGE_2-, iloprost- and CCK8-induced contractions were abolished. This observation indicates that the release of Ca^{2+} from intracellular stores participates in the mechanism underlying the contractile response to PGE_2 and iloprost, as it does for CCK8 as previously demonstrated (Bitar et al 1986). In a previous study, the intracellular pathway triggered by PGE_2 in human platelets involved an increase of intracellular IP_3 and the release of Ca^{2+} from intracellular stores (Allison et al 1986).

In summary, our results indicate that the concentration-dependent contraction induced by PGE_2 and iloprost in isolated smooth muscle cells from guinea-pig ileum is mediated through an EP_1 receptor and is dependent on the release of calcium from intracellular stores.

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