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DISTRIBUTION OF RECEPTORS MEDIATING PHOSPHOINOSITIDE HYDROLYSIS IN CULTURED HUMAN UMBILICAL ARTERY SMOOTH MUSCLE AND ENDOTHELIAL CELLS

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Abstract—Cultures of human umbilical artery smooth muscle and endothelial cells have been established and the effect of a range of calcium-mobilizing receptor agonists on inositol phospholipid hydrolysis has been compared in the two cell types. In human umbilical artery endothelial cells, histamine (EC_{50} 20 μM), ATP (EC_{50} 6.7 μM), sodium fluoride (20 mM) and thrombin (1 U/mL) produced marked increases in [^{3}H]inositol phosphate accumulation. In contrast, bradykinin (1 μM), 5-hydroxytryptamine (5-HT) (0.1 mM) and carbachol (1 mM) produced only a small (<1% of the response to 1 mM histamine) effect on [^{3}H]inositol phosphate accumulation in these cells. In human umbilical artery smooth muscle cells, histamine (EC_{50} 16 μM), bradykinin (EC_{50} 4.5 nM), 5-HT (EC_{50} 0.7 μM) and carbachol (EC_{50} 21 μM) produced substantial effects (>20% of the response to 1 mM histamine) on inositol phospholipid hydrolysis while ATP (1 mM) and thrombin (1 U/mL) were much less effective. The response to histamine in both smooth muscle and endothelial cells was antagonized by 50 nM mepyramine (apparent K_d = 5.6 and 2.9 nM in the two cell types, respectively). The response to 5-HT in smooth muscle cells was antagonized by 50 nM ketanserin (apparent K_d = 4.5 nM). In human umbilical artery smooth muscle cells the inositol phosphate response to carbachol was antagonized by 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP; pK_d = 9.3), atropine (pK_d = 9.7), pirenzepine (pK_d = 6.7) and methoctramine (pK_d = 6.9). These data are consistent with the involvement of an M_3-muscarinic receptor in this response. These studies suggest that receptors mediating inositol phospholipid hydrolysis are differentially distributed betwen human umbilical artery endothelial and smooth muscle cells.

Key words: smooth muscle cells; endothelial cells; inositol phospholipid hydrolysis; human umbilical cord

A range of vasoactive agents can induce smooth muscle contraction in segments of human umbilical artery [1]. The most potent smooth muscle spasmogens in this tissue include 5-HT†, bradykinin and histamine [1, 2]. These three agonists have also been shown to produce large and rapid changes in cyclic GMP accumulation in segments of human umbilical artery [3, 4] although the relative involvement of endothelial and smooth muscle cells in this response remains to be established.

The use of cell culture techniques has provided important information on the signal transduction pathways operating in human umbilical vein endothelial cells [5–7]. In these cells both histamine and thrombin have been shown to stimulate inositol phospholipid hydrolysis [8] leading to inositol-1,4,5-trisphosphate formation and intracellular calcium mobilization [6–8]. A clear relationship has been established between the mobilization of intracellular calcium induced by ATP, thrombin and histamine and the release of the arachidonic acid metabolite prostacyclin from human umbilical vein endothelial cells [6, 9]. In addition, there is an agonist-induced influx of calcium ions into human umbilical vein endothelial cells [7, 10] which may be more important for the release of endothelium-derived relaxant factor (nitric oxide; [11]).

At the present time there is very little information available concerning the intracellular calcium signalling systems operating in smooth muscles cells derived from human umbilical cord. The aim of the present study was to establish primary cultures of both human umbilical artery endothelial and smooth muscle cells for this purpose. We report here a comparison of the effects of a range of calcium-mobilizing receptor agonists on inositol phospholipid hydrolysis in these two cell populations.

MATERIALS AND METHODS

Chemicals. Anti-alpha smooth muscle actin monoclonal antibody and FITC-conjugated rabbit anti-mouse antibody were purchased from Dako Ltd (High Wycombe, U.K.). Endothelial cell growth factor was purchased from Advanced Protein Products Ltd (Brierley Hill). [^{3}H]Myo-inositol (20 Ci/mmol) was obtained from New England Nuclear products (Herts, U.K.). Histamine dihydrochloride, mepyramine maleate, atropine, carbachol chloride,

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† Abbreviations: 5-HT, 5-hydroxytryptamine; MEM, minimal essential medium; DMEM, Dulbecco's modified Eagle's medium; 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine.

thrombin, angiotensin II, bradykinin acetate, des-^{9}Arg-bradykinin, ATP-disodium salt and 5-HT were purchased from Sigma (Poole, U.K.). Ketanserin tartrate, pirenzepine dihydrochloride, methoctramine tetrahydrochloride and 4-DAMP methiodide (4-diphenylacetoxy-*N*-methylpiperidine methiodide) were obtained from Research Biochemical International (Herts, U.K.).

Isolation of human umbilical artery endothelial cells. Lengths of umbilical cord (10–20 cm) were cut from the placental portion as soon as practically possible after delivery. The cords were placed in sterile 0.9% saline and transported to the laboratory immediately. Both ends of one of the two arteries were cannulated using cut down vessel dilators (French type, JCD 4.0, Cook Group Co.) and Nipro 3-way taps into which 10 mL syringes were fitted. Red cells were rinsed from the artery with sterile isotonic saline and the artery was filled with medium 199 containing 0.5 mg/mL collagenase (type II, Sigma) at 37°. The umbilical cord was then incubated for a further 5 min in a saline bath at 37°. Sheets of endothelial cells were removed from the artery by flushing with 10 mL of medium 199. An equal volume of foetal calf serum was then added and the cell suspension centrifuged at 200 *g* for 5 min. The cell pellet was resuspended in 3 mL of medium 199 containing 10% human serum, 10% newborn calf serum, 2 mM glutamine and 25–50 mg/mL endothelial cell growth factor and added to a 25 cm^2 flask. Cells were incubated at 37° in humidified air/CO_2 (95:5) and penicillin G (200 U/mL) and streptomycin (200 μg/mL) were added for the first week of culture.

Flasks were confluent in 5–7 days and passaged using trypsin/EDTA solution (Gibco) into a 75 cm^2 flask. Thereafter, cells were passaged at a split ratio of 1:3. All flasks and 24-well plates were coated with 1% gelatin (Sigma). Cells were used for experimental purposes between passage 3 and 7.

Isolation of human umbilical artery smooth muscle cells. Human artery smooth muscle cells were grown from explant cultures essentially as described previously [12]. Briefly, explants (1 mm^2) of human umbilical artery were incubated in 75 cm^2 flasks in D-valine MEM (Gibco) containing 10% foetal calf serum, 2 mM glutamine, penicillin G (200 U/mL) and streptomycin (200 μg/mL) at 37° in humidified air/CO_2 (95:5). Medium was replenished every 3–4 days and antibiotic supplements were removed after 9–10 days. Explants were removed from the flasks when colonies of smooth muscle cells were established. At passage 2, medium was changed to DMEM (Northumbria Biologicals Ltd) containing 10% foetal calf serum and 2 mM glutamine. Cells were used for experiments between passage 3 and 8.

Histological identification of smooth muscle and endothelial cells. Smooth muscle or endothelial cells were plated into 8-well glass chamber slides (which had been coated with 1% gelatin in the case of endothelial cells). At or near confluence, cells were washed (3 × 5 min in PBS) and then fixed for 15 min at −20° with ice-cold methanol. Cells were then rinsed (3 × 5 min PBS) and incubated with PBS containing 10% horse serum for 45 min at room temperature. For endothelial cells, 100 μL of rabbit anti-human factor VIII related antigen (1:80 in 10% horse serum in PBS) was added to duplicate wells for 60 min. The wells were then washed once for 15 min with PBS containing 10% horse serum and then three times with PBS. Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (100 μL) (1:20 in 10% horse serum in PBS) was then added for 60 min. For smooth muscle cells the first antibody was the monoclonal anti-smooth muscle alpha actin antibody (1:40 in 10% horse serum in PBS) and the second antibody was fluoroscein isothiocyanate-conjugated rabbit anti-mouse antibody (1:40 in 10% horse serum in PBS). Cells were viewed under a Zeiss epifluorescent photomicroscope III using the 487710 filter set.

[^{3}H]*Inositol phosphate accumulation.* For measurement of the accumulation of total [^{3}H]inositol phosphates, endothelial cells were grown to confluency in 24-well plates in medium 199 containing 10% human serum, 10% newborn calf serum, 2 mM glutamine and 25–50 mg/mL endothelial cell growth factor. Monolayers of endothelial cells were then loaded with [^{3}H]myo-inositol (1 μCi/well) for 24 hr in medium 199 containing 10% dialysed human serum, 10% dialysed newborn calf serum and 2 mM glutamine. Human umbilical artery smooth muscle cells were loaded in 24-well cluster dishes with [^{3}H]-myo-inositol (1 μCi/well) for 24 hr in inositol-free DMEM (Flow) medium containing 10% dialysed foetal calf serum and 2 mM glutamine. Prelabelled cells were then washed twice with 1 mL/well Hanks–HEPES buffer (pH 7.4) and then incubated at 37° for 20 min in 290 μL Hanks–HEPES buffer containing 20 mM LiCl. Where appropriate antagonists were added at the same time as the LiCl. Agonists were added in 10 μL of Hanks–HEPES medium and the incubation continued for 5–45 min. Incubations were stopped by the removal of agonists and the addition of 1 mL methanol/0.12 M HCl (1:1 v/v) Cells were left overnight at −20° before neutralization with 25 mM Tris:0.5 M NaOH; H_2O (11:1.2:34). Total [^{3}H]inositol phosphates were finally separated from free [^{3}H]myo-inositol by anion exchange chromatography [13].

Data analysis. Concentration–response curves were fitted to a logistic equation using the non-linear programme GraphPad (ISI). The equation fitted was:

$$\text{Response} = \frac{E_{MAX} \times A^n}{(EC_{50})^n + A^n}$$

where E_{MAX} is the maximal response (relative to 1 mM histamine), A is the agonist concentration and n is the Hill coefficient.

Apparent antagonist dissociation constants (K_d) were determined, assuming competitive antagonism, by one of two methods: (a) from shifts in the agonist concentration–response curves using the relationship: $K_d = D/(K_2/K_1 - 1)$ where D is the concentration of antagonist, K_1 is the concentration of agonist producing half maximal response and K_2 is the concentration of agonist producing the same response in the presence of antagonist; (b) using a modification of the null method described by

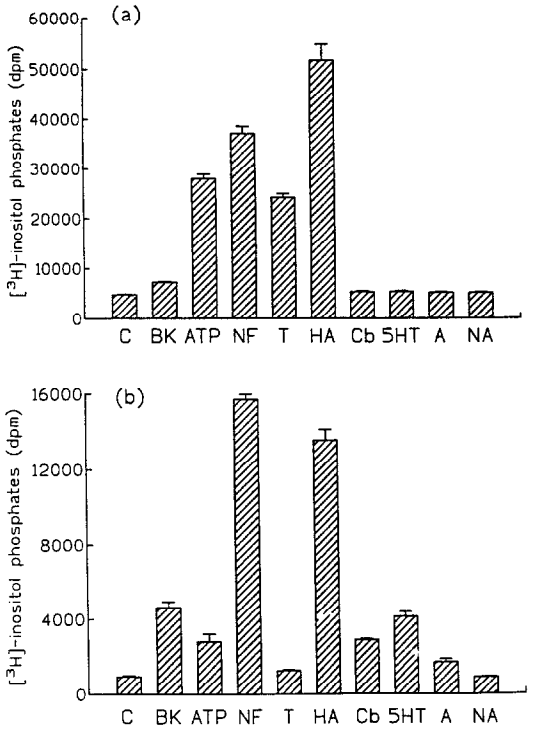


Fig. 1. Agonist-stimulated inositol phospholipid hydrolysis in human umbilical artery (a) endothelial cells and (b) smooth muscle cells. Responses to 1 μM bradykinin (BK), 1 mM ATP, 20 mM NaF (NF), 1 U/mL thrombin (T), 1 mM histamine (HA), 1 mM carbachol (CB), 0.1 mM 5-hydroxytryptamine (5-HT), 10 μM angiotensin (A) and 1 mM noradrenaline (NA). Values represent means ± SE mean of triplicate determinations in a single experiment. Similar results were obtained in two further experiments.

Lazareno and Roberts [14]. Briefly, a concentration–response curve to an agonist was generated and a concentration (C; usually 100 μM) of agonist was chosen which gave a response greater than 50% of the maximum agonist response. The concentration of antagonist (IC_{50}) required to reduce the response of this concentration (C) of agonist by 50% was then determined. The agonist concentration–response curve was fitted to a logistic equation as described above and a concentration of agonist identified which yielded a response equivalent to 50% of that produced by concentration C (in the absence of antagonist). The apparent K_d was then determined from the relationship:

$$C/C' = IC_{50}/K_d + 1$$

pK_d values were calculated as $-\log K_d$.

Statistical analysis was performed by paired or unpaired *t*-tests. N in the text refers to the number of separate individual experiments. All experiments were performed on cells from at least three different donors.

RESULTS

The morphology of the human umbilical artery endothelial cells was very similar to that of human umbilical vein endothelial cells with polygonal shaped cells forming a monolayer of cobblestone appearance on reaching confluence. The endothelial cells all stained positively for human factor VIII related antigen. Smooth muscle cells grew out from the explants of human umbilical artery after approximately 10 days. On reaching confluency the smooth muscle cell monolayer exhibited a "hills and valleys" appearance. The smooth muscle cells all contained smooth muscle alpha actin lying in strands along the length of the cells.

Agonist-induced [^{3}H]*inositol phosphate accumulation in human umbilical artery endothelial cells*

A large stimulation of [^{3}H]inositol phosphate accumulation was obtained with histamine (1 mM) in both human umbilical artery endothelial and smooth muscle cells (Fig. 1, Table 1). The mean stimulation produced by histamine was very similar in smooth muscle (14.1 ± 1.1-fold over basal levels of 1273 ± 62 dpm, N = 25) and endothelial (11.5 ± 2.2-fold over basal levels of 5492 ± 450 dpm, N = 22) cells. ATP (1 mM; EC_{50} = 6.7 ± 2.3 × 10^{-6} M; N = 3) and thrombin (1 U/mL) were much more effective in endothelial cells than smooth muscle cells, whereas the converse was true for 5-HT (0.1 mM), carbachol (1 mM) and bradykinin (1 μM) (Fig. 1, Table 1). A response of similar magnitude to histamine (1 mM) was obtained with the G-protein activator sodium fluoride (20 mM) in both cell types (Fig. 1, Table 1).

In umbilical artery endothelial cells, histamine produced a rapid stimulation of [^{3}H]inositol phosphate accumulation over the first 5 min which then increased linearly at a slower rate between 5 and 45 min (Fig. 2b). The selective H_1-receptor antagonist mepyramine (50 nM) produced a parallel shift in the concentration–response curve to histamine yielding an apparent K_d of 5.6 ± 1.6 × 10^{-9} M (N = 3; Fig. 2a). The EC_{50} values obtained for histamine at incubation times of 20 min (2.9 ± 0.7 × 10^{-5} M; N = 4; Fig. 3) and 45 min (2.0 ± 0.6 × 10^{-5} M; N = 4) were very similar.

Agonist-induced [^{3}H]*inositol phosphate accumulation in human umbilical artery smooth muscle cells*

Histamine (1 mM; N = 3), carbachol (1 mM; N = 3) and 5-HT (0.1 mM; N = 3) produced [^{3}H]inositol phosphate responses in smooth muscle cells with similar temporal characteristics to those observed with histamine in endothelial cells (Fig. 2b). The concentration–response curves for a range of agonists are shown in Fig. 3 and the respective EC_{50} values are given in Table 2. It is notable that EC_{50} values obtained for histamine and ATP are very similar to those obtained in human umbilical artery endothelial cells (Table 2). The B1-receptor agonist Des-Arg bradykinin (10^{-8}–10^{-5} M) was without significant effect upon [^{3}H]inositol phosphate accumulation in umbilical artery smooth muscle cells. The responses to histamine and 5-HT were, respectively, antagonized by the H_1-receptor antagonist mepyramine (K_d = 2.9 ± 0.2 nM; N = 3) and the 5-HT_2-receptor antagonist ketanserin (K_d 4.5 ± 1.8 nM; N = 3).

Table 1. A comparison of the effects of different receptor agonists on inositol phospholipid hydrolysis in human umbilical artery smooth muscle and endothelial cells

	[^{3}H]Inositol phosphate accumulation (% of response to 1 mM histamine)	
	Smooth muscle cells	Endothelial cells
Histamine (1 mM)	100*	100*
NaF (20 mM)	100.9 ± 3.9* (9)	85.1 ± 4.6* (12)
Carbachol (1 mM)	27.4 ± 7.7* (9)	0.7 ± 0.2* (9)
5-hydroxytryptamine (0.1 mM)	20.8 ± 5.2* (9)	0.6 ± 0.2* (9)
Bradykinin (1 μM)	22.3 ± 3.8* (10)	4.7 ± 1.0* (9)
Bradykinin (1 μM; 10 min)†	41.7 ± 5.8* (3)	
ATP (1 mM)	7.6 ± 1.3* (7)	54.4 ± 3.8* (11)
Thrombin (1 U/mL)	9.5 ± 4.4 (7)	42.0 ± 5.0* (11)
Noradrenaline (1 mM)	2.2 ± 1.0 (6)	0.4 ± 0.2 (9)
Angiotensin II (10 μM)	3.4 ± 0.8* (5)	0.6 ± 0.2* (9)

Data (mean ± SEM) are expressed as a percentage of the response to 1 mM histamine (after subtraction of basal levels) which was measured in every experiment. The number of separate experiments are given in parentheses. * $P < 0.05$ compared to basal levels (paired t-test). The mean stimulation (fold over basal levels) produced by 1 mM histamine in each cell type was 14.1 ± 1.1 (N = 25; smooth muscle cells) and 11.5 ± 2.2 (N = 22; endothelial cells). Mean basal levels were 1273 ± 62 dpm (smooth muscle cells, N = 25) and 5492 ± 450 dpm (endothelial cells, N = 22). Agonist incubation times were 45 min unless otherwise stated.

† Incubation time of 10 min and data expressed with respect to the response to 1 mM histamine obtained after 10 min in the same experiments.

Characteristics of the response to carbachol in human umbilical artery smooth muscle cells

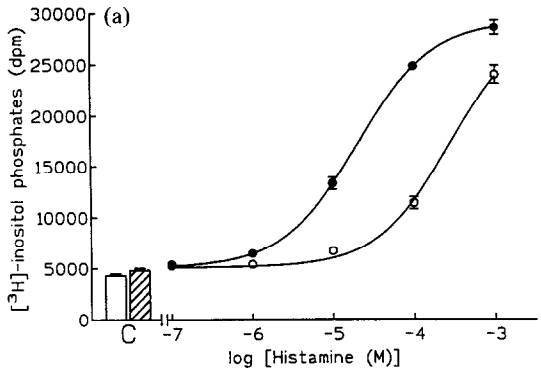
The pK_d values ($-\log K_d$) for a series of antagonists, which can discriminate between muscarinic receptor subtypes were determined from inhibition of the [^{3}H]inositol phosphate response to 0.1 mM or 1 mM carbachol (Table 3). The antagonists used were pirenzepine, methoctramine, 4-DAMP (Fig. 4) and the non-selective antagonist atropine. Similar pK_d values were obtained at both carbachol concentrations for both atropine and 4-DAMP.

DISCUSSION

This study has shown that endothelial cells and smooth muscle cells cultured from human umbilical artery differentially express a number of receptors coupled to [^{3}H]inositol phosphate accumulation. Histamine H_1-receptor stimulation produced the largest [^{3}H]inositol phosphate response in both cell types and histamine appears to have a similar potency in smooth muscle and endothelial cells (EC_{50} values of 20 and 16 μM, respectively). Similar EC_{50} values were also obtained for ATP in the two cell types although a much smaller response was produced in the smooth muscle cells (compared to the response to this purine in endothelial cells). Thrombin was the only other agent to produce a marked stimulation of inositol phospholipid hydrolysis in the endothelial cells, although it was without significant effect in umbilical arterial smooth muscle cells. The umbilical artery endothelial cells therefore appear to have very similar characteristics to those previously reported for endothelial cells derived from human umbilical vein which possess receptors for histamine, ATP and thrombin coupled to inositol phospholipid hydrolysis, calcium mobilization and prostacyclin formation [5–7, 9].

In marked contrast to the selectivity of ATP and thrombin for endothelial cells, the responses to carbachol, 5-HT and bradykinin were significantly larger in the smooth muscle cells relative to the endothelial cells. It was notable that noradrenaline and angiotensin II produced weak or marginal effects on inositol phospholipid hydrolysis in human umbilical arterial smooth muscle cells. However, these findings are consistent with the low efficacy (with respect to histamine, bradykinin and 5-HT) of these two spasmogens reported on strips of human umbilical artery (Altura *et al.* [1]). Preliminary pharmacological analysis of the receptors involved in the responses to bradykinin, 5-HT and histamine are consistent with the involvement of B2-, 5-HT_2- and H_1-receptors, respectively. Thus, the B1-selective agonist Des-Arg-bradykinin [15] was without significant effect at concentrations up to 10 μM. Furthermore, the K_d values obtained for mepyramine (2.9 nM) and ketanserin (4.5 nM) are in keeping with those reported for histamine H_1- [16] and 5-HT_2-receptors [17], respectively.

A more detailed characterization of the muscarinic receptor response has been undertaken in the umbilical artery smooth muscle cells. These studies indicated that the M_1- and M_3-antagonist 4-DAMP had a high affinity for the muscarinic receptor mediating inositol phospholipid hydrolysis in umbilical arterial smooth muscles yielding very similar pK_d values of 9.3 and 9.5 at the two concentrations (0.1 and 1 mM) of carbachol used for competition studies. A similar high affinity was obtained with the non-subtype selective antagonist atropine (pK_d = 9.7 and 9.5 at 0.1 and 1 mM carbachol). The M_1-



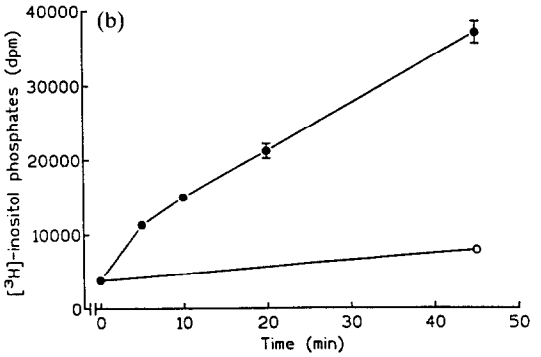


Fig. 2. Histamine-stimulated inositol phosphate accumulation in human umbilical artery endothelial cells. (a) Concentration–response curve obtained in the absence (●) or presence (○) of 50 nM mepyramine. The histograms show the basal (C) responses obtained in the absence (open bar) or presence (cross-hatched bar) of mepyramine (50 nM). (b) Time course of the response to 1 mM histamine (●). The open symbols show the basal accumulation of inositol phosphates. Values represent means ± SE mean of triplicate determinations in a single experiment. Similar results were obtained in two further experiments (both a and b).

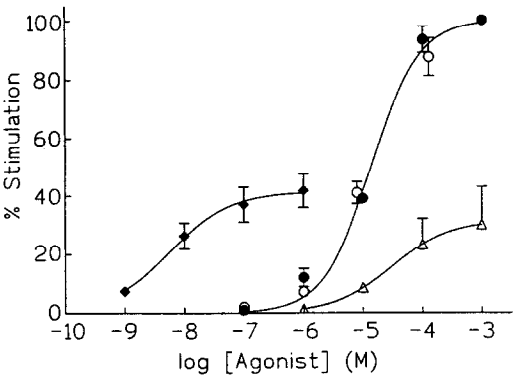


Fig. 3. Concentration–response curves for agonist-stimulated inositol phospholipid hydrolysis in human umbilical artery smooth muscle cells. (◆) Bradykinin; (●; ○) histamine; (△) carbachol. Data are expressed as a percentage of the response to 1 mM histamine which was measured in every experiment. Agonist incubation times were 10 min (◆); 20 min (○) and 45 min (●, △). Values represent means ± SEM of triplicate determinations in three to five separate experiments.

preferring antagonist pirenzepine ($pK_d = 6.7$) had a lower affinity than that expected for an M_1-mediated effect ($pK_d = 7.8$–8.3; [18, 19]) but was similar to the value obtained on M_3-receptors ($pK_d = 6.7$–7.2; [18, 19]). Similarly, the pK_d value obtained for the M_2-preferring methoctramine (6.9) was similar to those reported for M_3-receptors (6.9, 6.7) but much lower than those obtained at the M_2-receptor (7.8, 7.6; [18, 19]). Taken together, the data obtained in human umbilical arterial cells are consistent with the involvement of M_3-receptors in the inositol phosphate response to carbachol.

The inositol phosphate responses to 5-HT, histamine, bradykinin and carbachol obtained in human umbilical arterial smooth muscle cells are consistent with the contractile response to these agents which have been observed in segments of human umbilical artery [1]. A 5-HT_2-receptor-

Table 2. Agonist EC_{50} (M) values obtained from concentration–response curves determined in umbilical artery smooth muscle and endothelial cells

	Endothelial cells (M)	Smooth muscle cells (M)
Bradykinin*	ND	$4.5 \pm 1.0 \times 10^{-9}$ (3)
Histamine	$2.0 \pm 0.6 \times 10^{-5}$ (4)	$1.6 \pm 0.1 \times 10^{-5}$ (5)
5-HT	ND	$0.7 \pm 0.1 \times 10^{-6}$ (4)
ATP	$6.7 \pm 2.3 \times 10^{-6}$ (3)	$5.5 \pm 1.0 \times 10^{-6}$ (3)
Carbachol	ND	$2.1 \pm 0.5 \times 10^{-5}$ (8)

ND = not determined due to small size of inositol phosphate response. Values represent means ± SEM of EC_{50} (M) determinations obtained after 45 min agonist incubation. The number of determinations is given in parenthesis.

* Concentration–responses curves measured after 10 min.

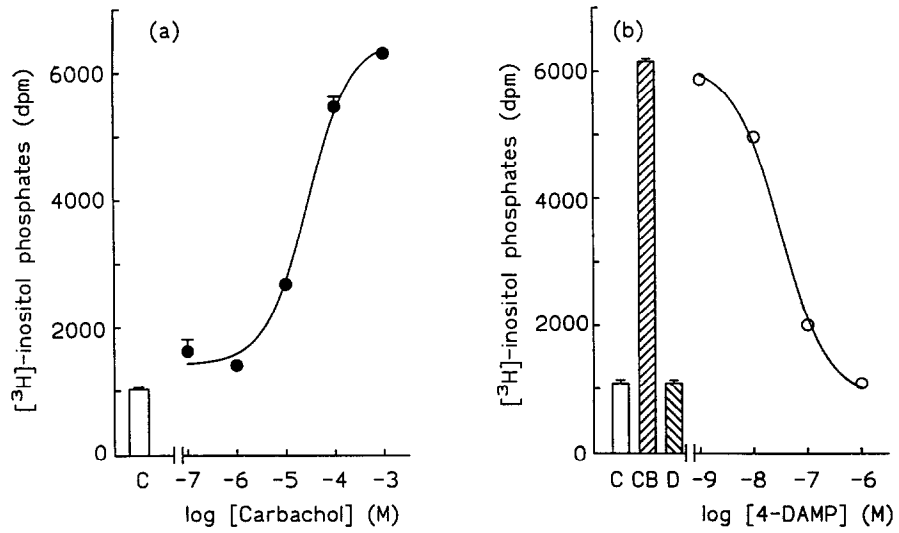


Fig. 4. Muscarinic receptor-mediated inositol phosphate accumulation in human umbilical artery smooth muscle cells. (a) Concentration–response curve to carbachol. (b) Antagonism of the response to 1 mM carbachol by 4-DAMP. Histograms show the basal accumulation in the absence (open bars; C) or presence (bar D) of 1 μM 4-DAMP. The bar marked CB in (b) shows the response to 1 mM carbachol in the absence of 4-DAMP. Values represent mean ± SEM of triplicate determinations in a single experiment (a and b were obtained in the same experiment). Similar data were obtained in two further experiments.

Table 3. pK_d values determined for muscarinic receptor antagonists from antagonism of carbachol-induced inositol phospholipid hydrolysis in cultured human umbilical artery smooth muscle cells

	Carbachol concentration 0.1 mM	1 mM
4-DAMP	9.25 ± 0.08 (3)	9.51 ± 0.14 (3)
Atropine	9.74 ± 0.13 (3)	9.47 ± 0.28 (3)
Pirenzepine	6.67 ± 0.20 (4)	
Methoctramine	6.90 ± 0.08 (3)	

Values represent means ± SEM. The number of determinations is given in parenthesis. pK_d values (−log K_d) were determined using a modification of the null method described by Lazareno and Roberts [14] as described in Materials and Methods. Antagonist inhibition curves were determined in the presence of either 0.1 or 1 mM carbachol.

mediated contractile response to 5-HT has also been reported by Templeton *et al.* [20]. Cyclic GMP accumulation has also been observed in segments of human umbilical artery in response to bradykinin, 5-HT, histamine H_1- and muscarinic receptor stimulation [3, 4]. In light of the differential effect of stimulants of these receptors on inositol phospholipid hydrolysis in smooth muscle cells (compared to endothelial cells) derived from this tissue, it is likely that the cyclic GMP responses are occurring in smooth muscle.

In conclusion, the present study has established that smooth muscle and endothelial cells derived from human umbilical artery can be maintained in culture and that they exhibit a differential distribution of receptors coupled to inositol phospholipid hydrolysis. Thus, endothelial cells maintain good responses to thrombin, histamine and ATP in culture while smooth muscle cells respond much better to 5-HT, bradykinin and muscarinic M_3-receptor stimulation. These cells should therefore make an ideal model system with which to investigate the interactions (in terms of intracellular signalling) between smooth muscle and endothelial cells in co-culture.

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