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Histamine stimulates inositol phosphate accumulation via the H_1-receptor in cultured human endothelial cells

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SUMMARY The effects of histamine on [^{3}H]inositol phosphate ([^{3}H]IP) accumulation was examined in the presence of lithium in [^{3}H]inositol-prelabelled human umbilical vein endothelial cells. Histamine stimulated total [^{3}H]IP formation in a dose-dependent manner with a half-maximal value (EC_{50}) of around 1-2 x 10^{-6}M. Mepyramine, but not cimetidine, completely abolished the histamine response indicating that activation of phosphoinositide hydrolysis is mediated via H_1-receptors. These data are the first to suggest that activation of inositol lipid hydrolysis is the underlying transmembrane signalling mechanism histamine H_1-receptors employ in mediating various endothelial cell functions.

It is now generally accepted that receptor-mediated phosphoinositide (PPI) hydrolysis is a common transmembrane signalling mechanism utilized by various calcium-mobilizing agonists to activate their target cells [1,2]. This involves a guanine nucleotide-binding protein (G-protein)-coupled phosphoinositidase C (PIC)-activated hydrolysis of the membrane inositol lipid, phosphatidylinositol-(4,5)-bisphosphate (PIP_2) to yield at least two second messengers, 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). While DAG is known to activate protein kinase C, IP_3 has been shown to release intracellular sequestered calcium from a non-mitochondrial pool, presumably endoplasmic reticulum.

It is known that many inflammatory mediators including histamine are able to increase vascular

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permeability of endothelial cells to macromolecules after cell separation in the postcapillary venules [3]. Recently, Rotrosen and Gallin (1986) have shown that histamine receptor occupancy concomitantly increases cytosolic calcium levels, reduces F-actin content and promotes albumin diffusion in cultured endothelial cells [4]. These investigators suggested that regulation of endothelial shape change and vessel wall permeability by histamine is a function of intracellular calcium concentrations. However, the underlying transduction mechanism mediating these histamine receptor-activated events is still unknown.

In the present study, we have investigated the effects of histamine on PPI hydrolysis in cultured human umbilical vein endothelial cells (HUVEC). Accumulation of total [^{3}H]inositol phosphate (inositol mono-, bis-, tris- and tetrakis-phosphates) in the presence of 10 mM LiCl in [^{3}H]inositol-prelabelled HUVEC was used as an index of PPI turnover.

MATERIALS AND METHODS

Human umbilical vein endothelial cells (HUVEC) were obtained in culture by collagenase (0.2% collagenase CLS II, Cooper Biomedical, U.K.) treatment of umbilical vein and subsequent culture [11] in Medium 199 (Gibco) supplemented with 15% foetal calf serum (Gibco), 90 µg/ml heparin (from porcine intestinal mucosa, Grade II, Sigma), 20 µg/ml endothelial cell growth supplement (Sigma). Cells were used between 2-5 passages.

The identity of the endothelial cells was confirmed by (1) the morphology of confluent cultures (polygonal monolayer) and (2) immunological marker (Factor VIII-related antigen).

For routine biochemical studies, cells were trypsinised (trypsin-EDTA, Gibco) and adjusted to a density of 40,000 viable cells/ml growth medium. Aliquots of 0.5 ml cell suspension were seeded in Nunc 24-well mutidishes coated with 1% gelatin. After overnight incubation, the growth medium was removed and fresh medium containing 4 µCi/ml [^{3}H]inositol (16.5 Ci/mmol, NEN) was added to each well.

After prelabelling with [^{3}H]inositol for 48 h, cells were washed thoroughly with Krebs-Ringer Bicarbonate (KRB) buffer supplemented with glucose and were then challenged with various concentrations of agonists including histamine in KRB buffer containing 10 mM LiCl at 37 ^{o}C for 1 h (final incubation volume, 0.4 ml). For inhibition studies, antagonists were added 10 min prior to the

addition of histamine. Reaction was terminated by the addition of three volumes of ice-cold chloroform:methanol (1:2) solution. Total water-soluble [^{3}H]inositol phosphate ([^{3}H]IP) was separated and quantitated by anion-exchange chromatography using the Biorad AG 1X-8 resin (formate form) as described previously [12].

Histamine hydrochloride, mepyramine and cimetidine were purchased from Sigma. All other drugs and peptides were obtained from either Calbiochem, Bachem, Sigma or Boehringer Mannheim. Porcine interleukin 1 and hyaluronic acid fragment were gifts from Dr.J.Saklatvala of Strangeways Laboratories, Cambridge and Dr.D.West of Christie Hospital, Manchester, respectively. Myo-[2-^{3}H]inositol was obtained from New England Nuclear. All other chemicals were purchased from Fisons.

Difference between values was only considered to be significant if $P < 0.05$ (two-tailed unpaired Student's t test).

RESULTS AND DISCUSSION

Fig. 1. shows that histamine stimulated total inositol phosphate accumulation in the presence of lithium in a dose-dependent manner. The EC_{50} value of the histamine response is $1.51 \pm 0.22 \times 10^{-6}$M (six separate determinations). Maximal stimulation of PPI hydrolysis by histamine is around 10^{-4}M. These values are in accord with the half-maximal and maximal concentrations of 5×10^{-6}M and 10^{-4}M, respectively, of histamine-induced

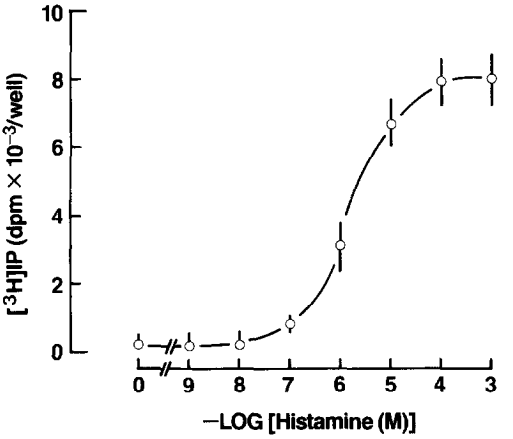

Fig. 1. Dose-dependent stimulation of total [^{3}H]inositol phosphate accumulation by histamine in cultured endothelial cells. Data presented are means ± S.E. of a representative experiment in triplicate determinations. Similar results have been obtained in three independent experiments using endothelial cells cultured from three different umbilical veins.

Table 1. Effects of mepyramine and/or cimetidine on histamine stimulation of [^{3}H]inositol phosphate formation in [^{3}H]inositol-labelled HUVEC

	[^{3}H]IP (dpm/well)		
		Histamine	
	Control	10^{-7}M	10^{-5}M
Basal	283 ± 17	711 ± 43	6288 ± 314
Mepyramine (10^{-6}M)	230 ± 12	417 ± 91*	328 ± 55***
Cimetidine (10^{-6}M)	272 ± 25	741 ± 15	6162 ± 924
Mepyramine + Cimetidine	256 ± 15	403 ± 32**	397 ± 16***

[^{3}H]Inositol-prelabelled endothelial cells were exposed to histamine (10^{-7} or 10^{-5}M) in the absence or presence of mepyramine and/or cimetidine for 1 h. Total inositol phosphate was extracted, separated and quantitated as described in the text. Results presented are means ± S.E. of three determinations in a representative experiment. Similar results were obtained in three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs corresponding histamine stimulation in control cells.

elevation of intracellular calcium levels in cultured endothelial cells [4].

Pharmacological analyses using specific histamine receptor antagonists revealed that histamine-H_1, rather than H_2, receptors are responsible for the activation of PIC. Hence, mepyramine (10^{-6}M), but not cimetidine (10^{-6}M), significantly inhibited histamine stimulation of [^{3}H]inositol phosphate production in [^{3}H]inositol-labelled endothelial cells (Table 1). The Ki (inhibition conatant) value of mepyramine in antagonising the histamine response is 1.2 nM (mean of three determinations, value was calculated from the relative shift of histamine

dose-response curves in the presence or absence of mepyramine using the equation: dose-ratio = [A]/Ki + 1 where [A] is the concentration of mepyramine). These results are consistant with those obtained by Rotrosen and Gallin (1986) that histamine H_1-receptors regulate both shape change and vessel wall permeability in cultured endothelial cells [4].

A role for receptor-activated inositol phospholipid hydrolysis in mediating various endothelial cell functions has also been implicated for other stimuli in cultured endothelial cells from a variety of species. Thus, bradykinin (B_2) receptor activation in porcine aortic [5] and bovine aortic and cerebral microvacular [6] endothelial cells is linked to a rapid increase in inositol phosphate formation. The mitogen thrombin has been shown to stimulate PPI turnover in both pig aortic [7] and human umbilical vein endothelial cells [8]. The present report, hence, suggests the inclusion of histamine receptors in the accumulating list of receptor systems which utilize an enhanced PPI turnover as their signal transduction mechanism in endothelial cells.

We have also tested a variety of compounds on their ability to stimulate PPI hydrolysis in HUVEC. Table 2 shows a list of compounds which had no significant effect on [^{3}H]IP formation in [^{3}H]inositol-prelabelled HUVEC. For all of the compounds tested, we measured total [^{3}H]IP production in the presence of lithium (still the most reliable means of assessing receptor activation in terms of PPI turnover [13]) in a one-hour incubation. For interleukin 1 and a degradation product of hyaluronic acid (HA3), we have also measured longer time-points of 6 and 24 hours. Bradykinin (10^{-11} to 10^{-5}M), however, significantly stimulated [^{3}H]IP accumulation in a dose-related fashion with nanomolar affinity (manuscript in preparation).

Regarding the fact that histamine stimulates prostaglandin (PG) synthesis in cultured endothelial cells [9,10], it is of interest to determine whether receptor-stimulated PPI breakdown is directly or

Table 2. Agents which are inactive in stimulating [^{3}H]IP formation in [^{3}H]inositol-labelled endothelial cells

Agonist	Concentrations
Substance P	10^{-8} - 10^{-6}M
Bombesin	10^{-8} - 10^{-6}M
Epidermal Growth Factor	25 x 10^{-8}M
Cholecystokinin (CCK-8)	10^{-5}M
Angiotensin II	10^{-5}M
Neuropeptide Y	2 x 10^{-6}M
Vasopressin	10^{-5}M
Vasointestinal Peptide	10^{-5}M
Noradrenaline	25 x 10^{-5}M
Acetylcholine	25 x 10^{-4}M
Interleukin 1	0.01 - 10 ng/ml
Hyaluronic acid (HA3)	0.10 - 10 µg/ml

indirectly related to PGs release induced by histamine. For example, arachidonic acid (AA), the precursor of various eicosanoids, can be synthesized from DAG (by DAG-lipases) liberated from PPI hydrolysis induced by histamine. On the other hand, histamine-stimulated formation of (1,4,5)IP_3 would also increase intracellular calcium levels which could in turn stimulate the calcium-dependent phospholipase A_2 to release AA from various phosphlipids. Utilization of DAG-lipase inhibitors as probes would undoubtedly yields useful information concerning the nature of the mechanism underlying all these processes.

To the best of our knowledge, this report is the first to present evidence for an involvement of PPI hydrolysis underlying histamine H_1-receptor activation which may be casually related to various histamine-mediated responses, such as increases in cytosolic calcium levels and vessel permeability, in human umbilical vein endothelial cells.

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Note added in proof During the preparation of this manuscript, a paper has been published by Resink et al. (BBRC, 144:438-446) describing a similar activation of inositol lipid hydrolysis induced by histamine in HUVEC. However, the article has not provided any information on the pharmacological specificity of the histamine receptor involved.

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