Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 438-446

HISTAMINE-INDUCED PHOSPHOINOSITIDE METABOLISM IN CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. ASSOCIATION WITH THROMBOXANE AND PROSTACYCLIN RELEASE

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Received February 27, 1987

SUMMARY: Histamine stimulation of cultured human umbilical vein endothelial cells induced dose- and time-dependent increases in glycerophosphoinositol (GroPIns), inositol-1-phosphate (InsP), inositolbisphosphate ($InsP_2$) and inositoltrisphosphate ($InsP_3$) in addition to release of thromboxane A_2 and prostacyclin. Increases in $InsP_2$ and $InsP_3$ were immediate while increases in GroPIns and InsP occurred only after 1 min. Thromboxane A_2 and prostacyclin release paralleled GroPIns and InsP production. The data indicate that, in endothelial cells, histamine evokes early hydrolysis of polyphosphoinositides, and that subsequent mobilization of arachidonic acid for thromboxane and prostacyclin synthesis involves both deacylation and phosphodiesteratic cleavage of phosphatidylinositol.

The release of histamine into the bloodstream evokes a characteristic vascular response of systemic vasodilation. It has been demonstrated that the most immediate effects of histamine infusion reflect histamine's interaction with an H_1-type receptor (1). Human endothelial cells possess a histamine-stimulated pathway for the synthesis of prostacyclin, which is mediated via an H_1-receptor and can account for some of the effects of histamine on the vascular system (2,3). Paradoxically, endothelial cells also release small quantities of thromboxane A_2, a potent platelet activator and vasoconstrictor, in response to the same hormones which induce relaxation (4,5). However, its production is small in comparison to that of prostacyclin and is probably of little physiological significance (6).

The H_1-receptor mediated action of histamine on endothelial cells is not linked to adenylate cyclase (7), and available evidence rather implicates involvement of the Ca^{2+}-second messenger system. Recently, it has been reported that activation of H_1-receptors, notably in CNS, results in breakdown of phosphoinositides (8-10). It was also shown that histamine increases cytosolic free calcium in cultured endothelial cells (11). The mechanism of

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prostacyclin and thromboxane A_2 release from endothelial cells involves both calcium influx (4) and intracellular calcium mobilization (12). In addition, cellular prostacyclin and thromboxane synthesis is thought to be initiated by liberation of arachidonic acid from membrane phospholipids (13), a step involving two Ca^{2+}-dependent enzymes: phospholipase C and phospholipase A_2 (for reviews, 14,15). In numerous secretory cell types these agonist-induced Ca^{2+}-linked events have been demonstrated to be directly associated with phosphoinositide metabolism (16-18). This study investigates the role of phosphoinositide breakdown in activation of human umbilical vein endothelial cells by histamine.

METHODS: Human umbilical vein endothelial cells were isolated, cultured and characterized as previously described (19). Cells were cultured in medium 199 supplemented with 20% (v/v) heat inactivated human serum, 200 µg/ml of endothelial cell growth factor from human brain, 100 µg/ml heparin, 100 U/ml lyncomycine and 100 µg/ml fungizon. Cultures used in our experiments were from 2nd to 5th passages and did not contain contaminating smooth muscle cells or fibroblasts. On the second day after seeding (6-well dishes) cells were incubated for 48 hours in the presence of myo-[2-^{3}H]inositol (5 µCi/ml). The medium containing [^{3}H]inositol was then removed and confluent cell layers (~10^6 cells/well) washed twice with 2 ml of serum free medium 199. One ml serum free medium 199 containing 50 mM LiCl was added to each well and cells were incubated at 37°C with various compounds (or vehicle) at the concentrations and for time periods given in legends to figures. Thereafter 0.5 ml of cell medium overlay was removed for thromboxane B_2 (TxB_2) and 6-keto-prostaglandin F_{1a} (6-keto-PGF_{1a}) content, and incubation of cells terminated by addition of 1 ml boiling 1% (w/v) SDS/30 mM EDTA. Dishes were heated for 5 min at 96°C and then 3 ml distilled water was added to each well. Total lysates were applied to columns containing 0.5 ml Dowex 1 (x 4; 200-400 mesh; formate form). Columns were first washed with 14 ml water to wash out myo-[^{3}H]inositol. Subsequently the inositol phosphate esters were eluted by stepwise addition of solutions containing increasing levels of formate (20). Details of solutions used are provided in the legend to Figure 1. Radioactivity in eluates was measured by liquid scintillation counting. Prostacyclin and thromboxane A_2 were assayed serologically as their stable hydrolysis products 6-keto-PGF_{1a} and TxB_2, respectively, using ^{125}I-RIA kits (Institute of Isotopes, Budapest, Hungary). Radioimmunoassays were performed on 100 µl aliquots of cell medium overlay according to the manufacturer's instructions.

All materials for cell culture were obtained from Flow Laboratories, U.K. and GIBCO, U.K. Myo-[2-^{3}H]inositol (16.3 Ci/mmol) was from Amersham International, U.K. Histamine was from Sigma Chem. Co., U.S.A., pyrilamine maleate from Ayerst Laboratories, U.S.A., and cimetidine was a kind gift from Orion Pharmaceuticals, Finland. All other chemicals were purchased from Sigma Chem. Co., U.S.A. or Fluka AG, Switzerland and were of the highest purity available.

RESULTS: Anion exchange chromatography of the lysate from human prelabelled umbilical vein endothelial cells contained five distinct ^{3}H-containing peaks (Fig. 1). On the basis of previous studies in other tissues (20), the peaks correspond to: (A) inositol; (B) glycerophosphoinositol (GroPIns); (C) inositol-1-phosphate (InsP); (D) inositolbisphosphate ($InsP_2$); (E) inositol-trisphosphate ($InsP_3$). Treatment of endothelial cells with histamine induced

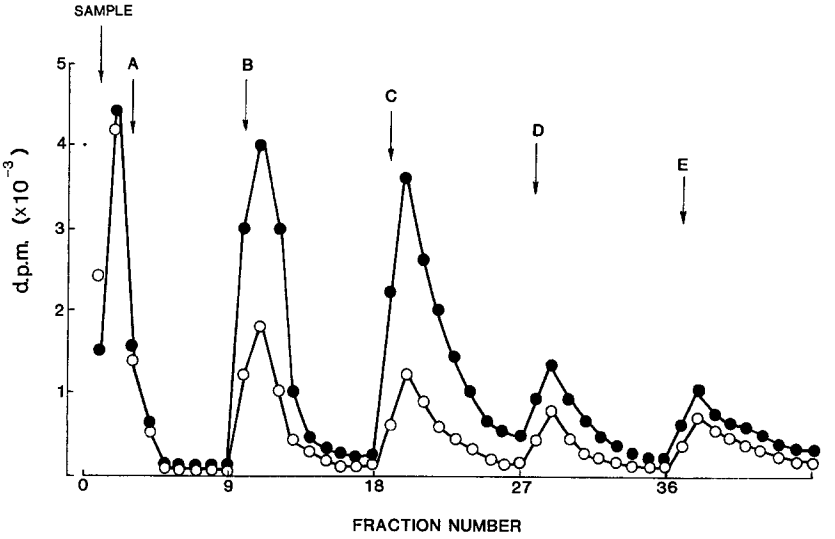

Figure 1. Anion-exchange elution profiles of lysates from myo-[2-^{3}H]inositol prelabelled umbilical vein endothelial cells: Umbilical vein endothelial cells were prelabelled with myo-[2-^{3}H]inositol and pretreated with 50 mM LiCl as described in Methods. Cells were further incubated for 15 min at 37°C in the absence (○) or presence (●) of 10^{-5} M histamine. Incubations were stopped as described in Materials and Methods. Cell lysates were applied to Dowex-1 anion exchange columns and eluted with: A, distilled water; B, 60 mM ammonium formate; C, 200 mM ammonium formate/0.1 M formic acid; D, 400 mM ammonium formate/0.1 M formic acid; E, 1.0 M ammonium formate/0.1 M formic acid. Fractions were collected in 1.0 ml aliquots except for the sample flow-through and water wash which were collected in 2.0 ml aliquots.

large increases in GroPIns, InsP, $InsP_2$ and $InsP_3$ without any alteration in inositol (Fig. 1).

Histamine-induced increases in inositol phosphates were time-dependent (Fig. 2A). Increases in $InsP_2$ and $InsP_3$ were immediate and reached maximal plateau levels (∼3-fold above control) after 2 min exposure of cells to histamine. Increases in GroPIns and InsP were measureable only after 1 min of histamine treatment and thereafter increased linearly until maximal levels (∼ 4-5-fold above control) were obtained (∼20 min). Extrapolation of the linear portion of the increases in both GroInsP and InsP indicated lag periods of 30-40 sec. Increased levels of both TxB_2 and 6-keto-PGF_{1a} were detected in medium overlay after 1-2 min exposure of cells to histamine and linear extrapolation yielded lag periods of ∼20 sec (Fig. 2B). Maximum levels of prostanoids were reached after 20 min exposure of cells to histamine. 6-keto-PGF_{1a} was always produced in quantities 4-7 times more than those of TxB_2.

Histamine-induced increases in levels of inositol phosphates and prostanoids were dose-dependent (Fig. 3). Half-maximally effective concentrations (EC_{50}) of histamine were 1.44 ± 0.56 μM, 1.53 ± 0.35 μM, 1.16 ± 0.36 μM and 1.45 ± 0.46 μM (mean ± SEM, n=3) for GroPIns, InsP, $InsP_2$ and $InsP_3$, respec-

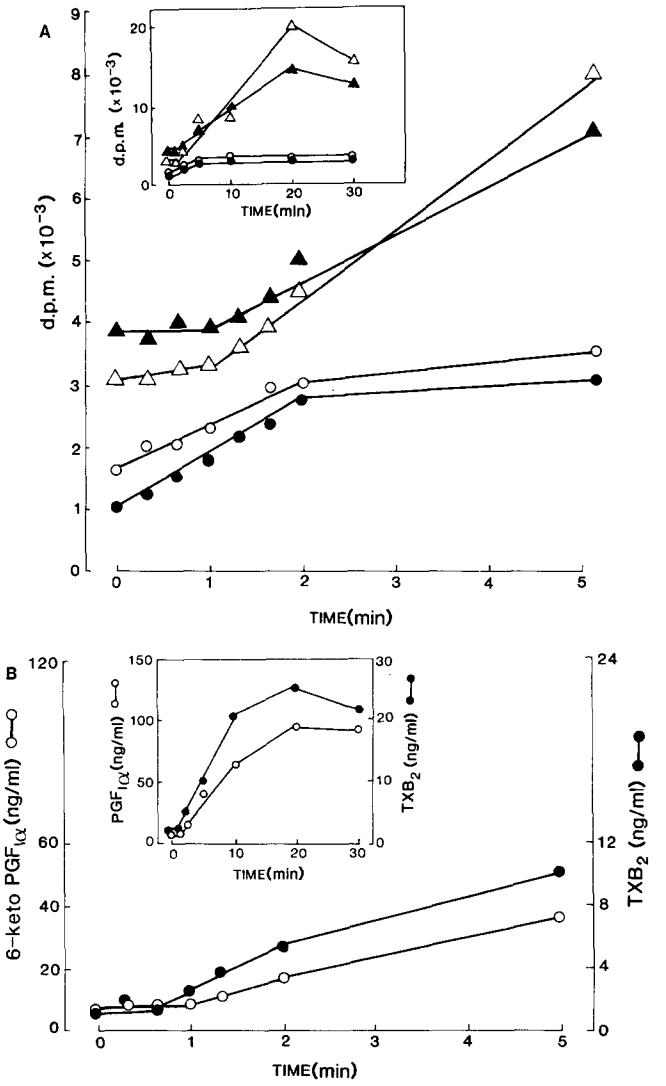

Figure 2. Kinetics of the effect of histamine on umbilical vein endothelial cells: Myo-[2-^{3}H]inositol-prelabelled endothelial cells were exposed to 10^{-5} M histamine for the indicated time periods. Panel A: [^{3}H]inositol phosphates in cell lysates were separated and radioactivity determined in total eluate (7 ml) volumes. ▲ , GroPIns; △ , InsP; ○, $InsP_2$; ●, $InsP_3$. Panel B: Prior to cell lysis medium overlay was removed for serological determination of TxB_2 (●) and 6-keto-PGF_{1a} (○) release. Values are means of duplicate incubations from a single representative experiment. Comparable time courses were obtained from three other separate experiments. Details of all methods are given in Methods and legend to Figure 1.

tively (Fig. 3A). EC_{50} values were 4.16 ± 1.37 μM and 5.16 ± 0.92 μM (mean ± SEM, n=3) for TxB_2 and 6-keto-PGF_{1a}, resepctively (Fig. 3B).

Experimental values from the kinetics of TxB_2/6-keto-PGF_{1a} and GroPIns/InsP production were normalized to the maximal response. The percentage production of either GroPIns or InsP was then plotted against percentage release of 6-keto-PGF_{1a} (Fig. 4A) or TxB_2 (Fig. 4B). Regression analysis of

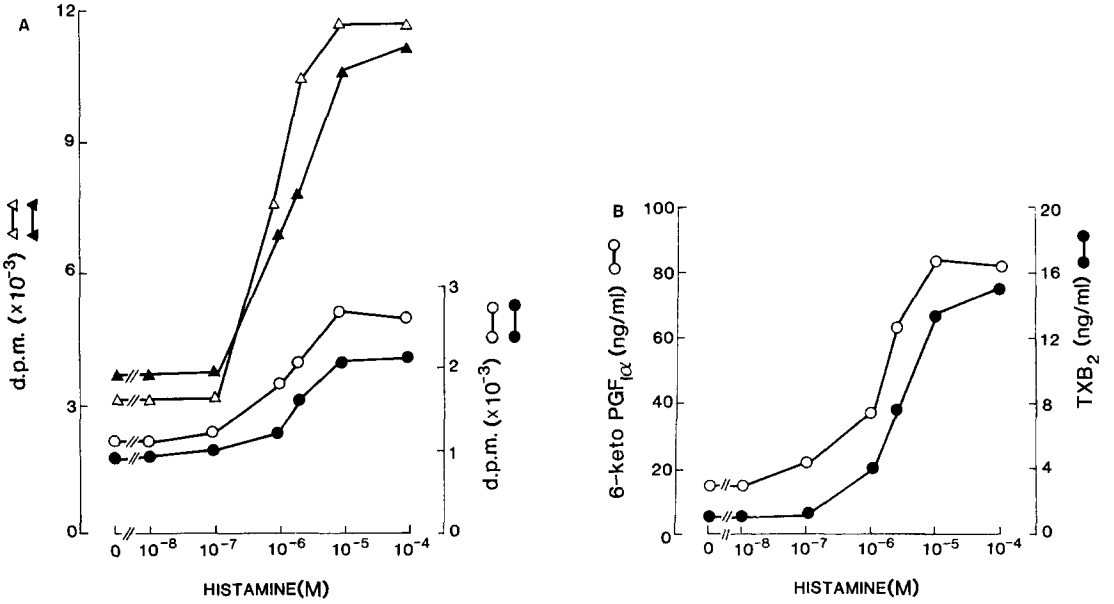

Figure 3. Dose-dependent effects of histamine on human umbilical vein endothelial cells: Myo-[^{3}H]inositol-prelabelled endothelial cells were incubated for 10 min with the indicated concentrations of histamine. Panel A: [^{3}H]inositol phosphates in cell lysates; ▲ , GroPIns; △ , InsP; o, $InsP_2$; ●, $InsP_3$. Note that the ordinate for GroPIns and InsP is ranged from 0 to 12 and that for $InsP_2$ and $InsP_3$ from 0 to 3. Cells were incubated for 10 min with varying concentrations of histamine. Panel B: TxB_2 (●) and 6-keto-PGF_{1a} (o) released into medium overlay. Values are means of duplicate incubations from a single representative experiment. Comparable profiles were obtained in two other separate experiments. EC_{50} values for histamine (given in the text as means ± SEM) were determined following independent analysis of each experiment.

the normalized plots revealed direct correlations between 6-keto-PGF_{1a} release and both GroPIns (r=0.7492) and InsP (r=0.8684) formation, and between TxB_2 release and both GroPIns (r=0.8292) and InsP (r=0.9493) formation. The effects of histamine (10^{-5})M on both inositol phosphate production and prostanoid release were completely inhibited by the H_1-receptor antagonist pyrilamine (10^{-5}M), whereas cimetidine, an H_2-receptor antagonist, at the same concentration was ineffective (data not shown).

DISCUSSION: The participation of phosphatidylinositol turnover in receptor-agonist mediated secretion has been demonstrated for diverse secretory systems such as neutrophils (21) exocrine pancreas (20), parotid (20) and platelets (23). Our results show that phosphatidylinositol turnover is also associated with histamine evoked secretion response in endothelial cells.

The immediate and parallel increase in appearance of both $InsP_2$ and $InsP_3$ is consistent with the hypothesis (15,24-27) that the first event in hormonal stimulation of phosphoinositide breakdown is polyphosphoinositide

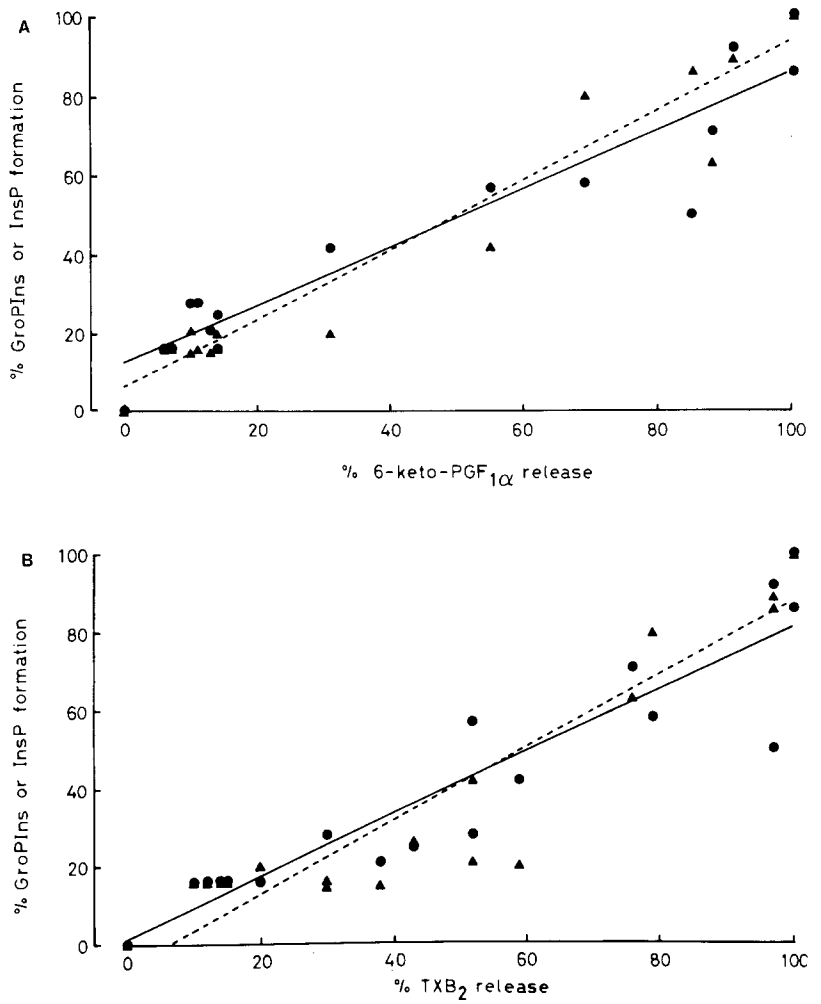

Figure 4. Correlations between 6-keto-$PGF_{1\alpha}$ release and GroPIns/InsP formation (Panel A) and between TxB_2 release and GroPIns/InsP formation (Panel B): Data from two separate kinetic experiments were independently normalized. The response for each parameter after 30 min exposure of cells to histamine (10^{-5} M) was arbitrarily taken as 100%. Correlation coefficients (given in text) were determined using linear regression analysis.—●—, GroPIns;-- ▲--, InsP.

hydrolysis via the phosphodiesterase (phospholipase C) pathway. Formation of InsP is thought to be a consequence of degradation of the former products and not of the hormone-sensitive phospholipase C (25-27). Stability of maximally stimulated levels of $InsP_2$ and $InsP_3$ on prolonged exposure (up to 30 min) of cells to histamine suggests that these compounds are not rapidly degraded by phosphomonoesterase. Another explanation of this phenomenon might be that they have reached a new steady-state in which their rates of breakdown and synthesis are balanced. However, our results indicate that the apparent rates of formation of $InsP_2$ and $InsP_3$ were lower than that of InsP. Thus, InsP was apparently formed directly via phospholipase C mediated hydrolysis of phos-

phatidylinositol rather than via phosphomonoesterase(s) action of $InsP_2$ and $InsP_3$.

Subsequent to $InsP_3$ production, $InsP_3$-induced mobilization of Ca^{2+} from intracellular pools (25-27) may activate the secondary Ca^{2+}-dependent breakdown of inositol phospholipids (28). Isolated phosphatidylinositol specific phospholipases C, in the presence of EGTA, cleave only PtdIns4P and PtdIns $(4,5)P_2$, whereas in the presence of Ca^{2+} all three phosphoinositides are hydrolyzed nearly equally (29). Furthermore, in hepatocytes neither EGTA treatment, nor the addition of A23187 in the presence of external Ca^{2+} activated breakdown of PtdIns$(4,5)P_2$ or altered the effect of vasopressin on phosphoinositide hydrolysis (30). The temporal effects of histamine on inositol phosphates are thus compatible with evidence that phospholipase C degradation of polyphosphoinositides occurs at resting free Ca^{2+} concentrations, whereas that of phosphoinositide depends on prior mobilization of Ca^{2+} (24,28,29). The delayed appearance of glycerophospholipid may also be explained by the Ca^{2+}-mobilization requirements for phospholipase A_2-mediated deacylation of phosphatidylinositol (14). Our results from kinetic experiments thus suggest a sequential association between receptor-linked and calcium-controlled pathways for breakdown of inositol phospholipids. This is consistent with proposed models for receptor stimulus transduction processes in cells responsive to hormones which exert their effects via phosphatidylinositol turnover (25-27).

Hormone-stimulated prostaglandin release from bovine pulmonary artery endothelial cells has been shown to be inhibited by TMB-8, an inhibitor of intracellular Ca^{2+}-mobilization (12). This, together with the Ca^{2+}-mobilizing properties of $InsP_3$ implies an association between phosphatidylinositol turnover and prostacyclin and thromboxane release. The time course for thromboxane and prostacyclin secretion coincides with that for GroPIns and InsP formation, such that, in umbilical vein endothelial cells, phosphatidylinositol catabolism may provide arachidonic acid for synthesis of its active metabolites thromboxane and prostacyclin. However, we cannot exclude that some arachidonic acid may also be derived from other phospholipids such as phosphatidylcholine (14,15).

Phospholipase A_2 has been assumed to play an essential role in the synthesis of biologically active metabolites of arachidonic acid (15), and there are reports on stimulated deacylation of phosphatidylinositol in tissues during the liberation of arachidonic acid (16,21). Arachidonic acid and lysophosphatidylinositol are immediate products of phospholipase A_2-mediated deacylation of phosphatidylinositol while GroPIns is the product of subsequent phospholipase B action on lysophosphatidylinositol (14). The findings with respect to GroPIns are quite comparable with an earlier study

on phospholipase A_2 activation in bradykinin-stimulated pig aortic endothelial cells (31) in which a rapid but transient increase (maximum at 1 min) in lysophosphatidylinositol was demonstrated. Our observation of a correlation between GroInsP production and thromboxane/prostacyclin release provides support for the participation of phospholipase A_2 in the arachidonic acid metabolism of endothelial cells. However, the simultaneous and parallel generation of InsP, which also correlated with thromboxane/prostacyclin production, indicate that the alternative phospholipase C - diacylglycerol lipase pathway for arachidonic acid production (15) is also activated in histamine-stimulated endothelial cells. The relative importance of each pathway in governing the availability of arachidonic acid is difficult to assess and apparently depends on cell type. For example, in platelets the phospholipase C - diacylglycerol pathway predominates (23), while in mouse BALB/3T3 cells only the deacylation pathway is operative (21). Apparently both routes function in human umbilical vein endothelial cells.

In conclusion, our results demonstrate that both deacylation- and phosphodiesteratic-mediated phosphoinositide breakdown are intimately associated with the arachidonic acid cascade in histamine-stimulated cultured human umbilical vein endothelial cells.

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