5-HT\textsubscript{1A} and Histamine H\textsubscript{1} Receptors in HeLa Cells Stimulate Phosphoinositide Hydrolysis and Phosphate Uptake via Distinct G Protein Pools*

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Regulation of phosphate uptake was studied in a HeLa cell line after transfection with DNA encoding the human 5-HT\textsubscript{1A} receptor. In these cells, 5-HT stimulates sodium-dependent phosphate uptake via protein kinase C activation. Endogenous histamine H\textsubscript{1} receptors (739 ± 20 fmol/mg protein) were identified with \[^3\text{H}\]pyrilamine. Histamine (i) stimulated phosphoinositide hydrolysis (EC\textsubscript{50} = 8.6 ± 4.1 μM), (ii) activated protein kinase C (2.4-fold increase in activity), and (iii) increased phosphate uptake (EC\textsubscript{50} = 3.2 ± 1.8 μM) by increasing maximal transport (V\text{max}\text{histamine} = 0.2 ± 0.3 versus V\text{max}\text{histamine} = 9.1 ± 0.4) without changing the affinity of the transport process for phosphate. Prolonged treatment with 16 μM phorbol 12-myristate 13-acetate completely blocked protein kinase C activation and markedly attenuated the stimulation of phosphate uptake induced by histamine, establishing that 5-HT and histamine stimulate phosphate uptake through the common pathway of protein kinase C activation. The linkages of the histamine H\textsubscript{1} and 5-HT\textsubscript{1A} receptors to G protein pools were assessed in two ways. (i) The stimulation of phosphoinositide hydrolysis, protein kinase C activity, and phosphate uptake associated with histamine were insensitive to pertussis toxin, whereas those associated with 5-HT were very sensitive to pertussis toxin. (ii) The stimulation of phosphoinositide hydrolysis, protein kinase C activity, and phosphate uptake induced by histamine and 5-HT were additive. These findings suggest that distinct receptor types can stimulate phosphoinositide hydrolysis, protein kinase C, and phosphate uptake in an additive fashion through distinct pools of G proteins in a single cell type.

Sodium-dependent phosphate uptake, which is a component of many mammalian cell systems (1–9) is subject to dynamic regulation by various hormones, receptors, second messengers and kinases and thus serves as a useful example of modifiable cellular transport. The regulation of phosphate uptake has been studied primarily in cells of renal (9–21) or bone (6) origin. We have previously examined the regulation of phosphate uptake in the HeLa cell line, originally derived from a human cervical carcinoma (22, 23). In these cells, activation of protein kinase A (PKA) and protein kinase C (PKC) cause inhibition (22, 23) and stimulation (22) of phosphate uptake, respectively. This opposite regulation by PKC and PKA differs from the two predominant renal model systems of phosphate uptake. In the opossum kidney (OK) cell line, activation of both kinases causes inhibition of phosphate uptake (13, 15, 20, 24). In the porcine kidney (LLC-PK\textsubscript{1}) cell line, both kinases appear to mediate the stimulation of phosphate uptake (16, 25). We have utilized the HeLa cell line, because it represents a unique model for the regulation of sodium-dependent phosphate uptake in that the effects of the two kinases can be more easily distinguished.

HeLa cells contain an endogenous histamine H\textsubscript{1} receptor (26). We have previously demonstrated that recombinant human 5-HT\textsubscript{1A} receptors stably expressed in these cells stimulate phosphate uptake primarily through activation of PKC (22). Because histamine H\textsubscript{1} receptors are classically linked to the stimulation of phosphoinositide hydrolysis through a pertussis toxin-sensitive G protein pool, it was of interest to examine whether histamine would stimulate sodium-dependent phosphate uptake in these cells and to compare the mechanism to that of 5-HT-mediated stimulation of phosphate uptake. We therefore predicted (i) that the histamine H\textsubscript{1} receptor would also stimulate sodium-dependent phosphate uptake in these cells and if so (ii) that these effects would be mediated through a distinct pool of G proteins than that used by the 5-HT\textsubscript{1A} receptor.

EXPERIMENTAL PROCEDURES

Materials—Cell culture materials were purchased from GIBCO. \[^3\text{P}\]PO\textsubscript{4}, \[^3\text{H}\]pyridilamine, \[^3\text{H}\]NAD, \[^3\text{H}\]inositol, and \[^3\text{H}\]cyclic AMP (31.5 Ci/mmol) were from Du Pont-New England Nuclear. Protein kinase C DNA probes were from American Type Culture Collection. Other reagents were from Sigma or Bio-Rad.

Cell Culture—A clonal HeLa cell line expressing 2.8 ± 0.6 pmol 5-HT\textsubscript{1A} receptors was used.

The abbreviations used are: PKA, protein kinase A; PKC, protein kinase C (calcium- and phospholipid-dependent kinase); OK cells, opossum kidney cells; 5-HT, serotonin; DME, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; SD, sodium dodecyl sulfate; PDBu, phorbol 12,13-dibutyrate; DiC\textsubscript{8}, sn-1,2-diacylphosphatic; 8-BR-CAMP, 8-bromo-cyclic AMP; G protein, guanine nucleotide binding protein; G\textsubscript{S}, guanine nucleotide binding proteins which interact with phospholipase C; MARCKS, myristoylated alanine-rich C kinase substrate; Heps, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
recombinant human 5-HT\textsubscript{A} receptors/mg protein (22, 27) was grown in monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 \mu g/ml). Cells were seeded in plastic multiwell culture dishes (Costar, Cambridge, MA) and incubated at 37 °C in a humidified atmosphere with 95% air and 5% CO\textsubscript{2}. Subconfluent monolayers (70–90%) were used for assays of \textsuperscript{32}P\textsubscript{O}\textsubscript{4} transport and phosphoinositide hydrolysis. This cell line, obtained by transfection and single cell dilution cloning (22, 27), has been maintained in culture for nearly 2 years with continuous expression of \alpha\textsubscript{1H}IA protein. Typically, >75–90% of transfected cells in a single microscopic field respond to exposure to 5-HT with an increase in cytosolic calcium as measured by single cell calcium imaging techniques.

Phosphate Uptake—Cells were grown on six-well dishes. Before the uptake studies were performed, the medium was replaced with Earle's solution (143 mM Na\textsuperscript{+}, 5.4 mM K\textsuperscript{+}, 0.8 mM Mg\textsuperscript{2+}, 1.8 mM Ca\textsuperscript{2+}, 125 mM Cl\textsuperscript{−}, 15 mM Hepes, 5 mM glucose, pH 7.4) and allowed to equilibrate at 37 °C for 30 min. Unless otherwise indicated, 10 min prior to measurement of phosphate uptake, the Earle's solution was replaced with Earle's solution with or without the various drugs or hormones and incubated at 37 °C. Following that, the Earle's solution was washed with transport media (either Earle's solution or sodium-free choline-substituted Earle's solution) containing \textsuperscript{32}P\textsubscript{O}\textsubscript{4} and various concentrations of total phosphate (0.02–2 mM for kinetic studies; 0.1 mM for kinetic studies of phosphate uptake) followed by incubation for 15 min. At these time points were multiplied by three so that comparisons with other uptake rates (expressed as nanomoles/mg protein/3 min) could be made. All other uptakes were for 3 min.

Formation of Inositol Phosphates—Cells were grown in six-well dishes and equilibrated for 24 h in regular medium supplemented with 5 \mu Ci/ml of myo-[\textsuperscript{3}H]inositol (14.6 Ci/mmol). After washing with phosphate-buffered saline (PBS), cells were incubated for 30 min in PBS containing 20 mM LiCl (37 °C). The medium was then replaced with phosphate-free medium supplemented with various concentrations of the drugs. After 15 min the reaction was terminated by aspiration with phosphate-buffered saline (PBS), cells were incubated for 30 min with \textsuperscript{32}P\textsubscript{O}\textsubscript{4}, and then twice for 1 h at 65 °C. The filters were then exposed to Kodak X-AR film at -70 °C for 3–6 days using intensifying screens.

Statistics—Analysis of kinetic data was by nonlinear regression using simple weighting of individual values (ENZFITTER, Elsevier–J. P. Middleton, unpublished observation.)
RESULTS

Histamine Stimulates Phosphate Uptake in HeLa Cells—Incubation of HeLa cells with various concentrations of histamine for 10 min resulted in a dose-dependent (EC50 = 3.2 ± 1.8 μM) increase in sodium-dependent phosphate uptake at a fixed phosphate concentration of 0.2 mM (Fig. 1A). The maximal increase averaged 38.3 ± 4.9%. Kinetic analysis demonstrated that histamine (100 μM) increased maximal transport without an apparent increase in the affinity of the transporter for phosphate (Vmax(basal) = 6.2 ± 0.3 versus Vmax(histamine) = 9.1 ± 0.4; KMichaelis(basal) = 0.34 ± 0.03 versus KMichaelis(histamine) = 0.31 ± 0.05) (Fig. 1B). The magnitude of this stimulation of sodium-dependent phosphate uptake is similar to that observed with 5-HT in these cells (22). The stimulation of phosphate uptake by histamine was rapid, reaching maximal values after 10–15 min of incubation and returning toward base line within 60 min (Fig. 2). The time course for the stimulation of phosphate uptake correlates very well with those previously described in these cells for 5-HT, phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dibutyrate (PDBu), and sn-1,2-diacylglycerol (DiCS) (22) and is consistent with the involvement of a kinase in this process.

Histamine Stimulates Phosphate Uptake via Histamine H1 Receptors—HeLa cells have been reported to have histamine H1 receptors which regulate potassium channels (26) and intracellular Ca2+ levels (41). Three series of studies were performed to establish that the histamine-induced stimulation of phosphate uptake is mediated through the histamine H1 receptor and not through the transfected 5-HT1A receptor or other endogenous receptor subtype. These experiments were necessary to rule out an interaction with 5-HT1A receptors because of their high density (~2.8 fmol/mg protein) in these cells and the high doses of histamine utilized in these experiments. First, histamine (100 μM) increased phosphate uptake 39.8 ± 4.3% in nontransfected HeLa cells which do not express 5-HT1A receptors, whereas the histamine H1 antagonist cimetidine (Cimet) (5-HT1A receptor antagonist) did not. Similarly, the 5-HT1A receptor antagonist pindolol (100 μM) and siperone (100 μM) did not reduce the histamine-induced stimulation of phosphate uptake (n = three separate experiments performed in duplicate for each drug, not shown). Experiments were performed three to five separate times in duplicate at a fixed phosphate concentration of 0.2 mM in HeLa cells.

H1 receptor and not through the transfected 5-HT1A receptor or other endogenous receptor subtype. These experiments were necessary to rule out an interaction with 5-HT1A receptors because of their high density (~2.8 ± 0.6 pmol/mg protein) in these cells and the high doses of histamine utilized in these experiments. First, histamine (100 μM) increased phosphate uptake 39.8 ± 4.3% in nontransfected HeLa cells which do not express 5-HT receptors linked to adenyl cyclase or phosphoinositide hydrolysis (22, 27) (n = three experiments performed in triplicate, p < 0.01). Second, a series of antagonists were utilized to characterize the pharmacology of the effect of histamine in HeLa cells. As shown in Fig. 3, the histamine H1 receptor antagonists chlorpheniramine (Chlor), pyrilamine (Pyril), and doxepin (Dox) or the histamine H2 receptor antagonist cimetidine (Cimet). The 5-HT1A receptor antagonist pindolol (100 μM) and siperone (100 μM) did not reduce the histamine-induced stimulation of phosphate uptake, whereas the histamine H2 receptor antagonist cimetidine did not. Similarly, the 5-HT1A receptor antagonists pindolol and siperone did not (not shown). Similar results were obtained in assays of phosphoinositide hydrolysis (not shown).

Third, the existence of endogenous histamine H1 receptors was confirmed by binding studies performed with [3H]pyrilamine (Fig. 4). Saturation analysis (Fig. 4A) revealed that [3H]pyrilamine binds to a single class of binding sites (740 ± 20 fmol/mg protein) with a Kd of 164 ± 6 nM. This affinity is somewhat lower than that reported in brain tissue (42, 43) but very similar to that reported in DDT1-MF-2 cells (36) for the histamine H1 receptor. The order of potency of various ligands to inhibit [3H]pyrilamine binding corresponds to that
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Fig. 4. Pharmacological characteristics of the \([^{3}H]\)pyrilamine binding matches that of the histamine H\(_{1}\) receptor. Experiments were performed as described under "Experimental Procedures." A, saturation analysis revealed 740 ± 20 fmol of \([^{3}H]\)pyrilamine binding sites/mg protein and Scatchard analysis (inset) fits best to a single line, suggesting a single class of binding sites with a K\(_{D}\) of 0.2 nM. Depicted is a representative experiment (n = 3, experiments performed in duplicate). B, the rank order of potency of the various ligands for the binding site is that expected for the histamine H\(_{1}\) receptor. Binding was inhibited more potently by histamine (\(\Delta\), K = 5.6 ± 2.2 nM) and the histamine H\(_{1}\) receptor antagonists chlorpheniramine (□), 3.3 ± 1.4 nM, pyrilamine (○), 1.2 ± 0.2 nM, and doxepin (■, 450 nM) than by the histamine H\(_{2}\) receptor antagonist cimetidine (△) or the 5-HT\(_{1A}\) receptor antagonist pindolol (●), which were essentially inert.

expected for the histamine H\(_{1}\) receptor (Fig. 4B).

Histamine Stimulates Phosphate Uptake in the Presence of 8-Br-cAMP—We have shown previously that agents which stimulate or mimic cAMP accumulation in HeLa cells inhibit phosphate uptake (23). Histamine (up to 1 nM) does not lower basal or forskolin-stimulated whole cell cAMP or membrane adenyl cyclase in these cells over a 30 min period (n = 3 for both assays, not shown). To further establish that the histamine effect on phosphate uptake occurs independent of any potential effect on adenyl cyclase, we examined the effects of histamine on phosphate uptake in the presence of 8-Br-cAMP. In this regard, 100 μM 8-Br-cAMP decreased phosphate uptake from 2.1 ± 0.3 nmol/mg protein/3 min to 1.7 ± 0.2 nmol/mg protein/3 min at a phosphate concentration of 0.2 mM (p < 0.01, n = 3). Despite the continued presence of 8-Br-cAMP, 100 μM histamine increased phosphate uptake to 2.4 ± 0.2 nmol/mg protein/3 min (p < 0.05 versus basal, p < 0.01 versus 8-Br-cAMP alone, n = 3). This 41.1 ± 30.0% stimulation in the presence of 8-Br-cAMP is similar to the degree of stimulation in cells not treated with 8-Br-cAMP (38.3 ± 4.9). These findings indicate that histamine stimulates phosphate uptake by a mechanism other than inhibition of adenyl cyclase.

PKC Mediates the Histamine H\(_{1}\) Receptor-induced Stimulation of Phosphate Uptake—We have demonstrated previously that agents which activate PKC stimulate phosphate uptake in HeLa cells and that the 5-HT\(_{1}\)-induced stimulation of phosphate uptake is primarily mediated through PKC (22, 23). Because the histamine H\(_{1}\) receptor is classically linked to hydrolysis of phosphoinositides, diacylglycerol-induced activation of PKC seemed the most likely pathway for the stimulation of phosphate uptake. Data supportive of this hypothesis are as follows. There is close concordance of the EC\(_{50}\) values for the stimulation of phosphate uptake (3.2 ± 1.8 μM) and total inositol phosphates (8.6 ± 4.1 μM). Second, 100 μM histamine activates PKC in these cells as measured by the intact cell phosphorylation of the MARCKS protein (formerly known as the 80–87-kDa protein) (Fig. 5). This assay showed that histamine stimulated phosphorylation of the MARCKS protein by 2.4 ± 0.2-fold (n = 3, p < 0.05). Third, depletion of PKC by overnight treatment with 16 μM PMA attenuates the histamine-induced stimulation of phosphate uptake (Fig. 6). For these studies, effective down-regulation of PKC was confirmed by lack of effect of 1.6 μM PMA on phosphate uptake or phosphorylation of the MARCKS protein (Fig. 6 and not shown). Although the effects of prolonged high-dose PMA treatment are likely very complex, collectively, these results support the hypothesis that histamine stimulates
phosphate uptake via activation of PKC. We cannot exclude an effect of PMA on other elements of the signal transduction system which could lead to a blunted responsiveness. In this regard, the actions of histamine are similar to those of 5-HT, which activates PKC and stimulates phosphate uptake in these cells (22).

Histamine and 5-HT Responses Have Differential Sensitivity to Pertussis Toxin—The 5-HT1A receptor activates phosphoinositide hydrolysis and stimulates phosphate uptake in HeLa cells through a pertussis toxin-sensitive G protein (IC50 ≈ 10 ng/ml for 4 h) (22, 27). In the current study, treatment with various doses of pertussis toxin (up to 1 pg/ml for 24 h) had no effect on histamine (100 μM)-induced phosphate uptake (Fig. 7A). Such treatment also had no effect on the histamine-induced stimulation of phosphoinositide hydrolysis as measured by accumulation of total inositol phosphates at 15 min (Fig. 7B), strongly suggesting that the effects of histamine and 5-HT are mediated through functionally distinct pools of G proteins in HeLa cells. Confirmation that pre-treatment with pertussis toxin (100 ng/ml for 4 h) was adequate to eliminate all pertussis toxin substrate available for ADP-ribosylation is presented in Fig. 8C.

Histamine and 5-HT Have Additive Effects on Phosphoinositide Hydrolysis and Phosphate Uptake—To further confirm that the histamine H1 receptor and the 5-HT1A receptor couple to distinct G proteins in HeLa cells, we examined the additivity of the histamine and 5-HT-induced stimulations of phosphoinositide hydrolysis and phosphate uptake. As shown in Fig. 8A, the stimulatory effects of the two agonists on phosphoinositide hydrolysis were completely additive. Maximal stimulation was 88 ± 7% for histamine alone and 202 ± 12% for histamine in the presence of 10 μM 5-HT. Maximal stimulation for 5-HT alone was 106 ± 10% and for 5-HT in the presence of 1 mM histamine was 206 ± 4%. Half-maximal stimulatory doses for phosphoinositide hydrolysis were as follows: histamine alone (8.6 ± 4.1 μM) or +100 μM 5-HT (12.0 ± 3.2 μM), 5-HT alone (290 ± 70 nM) or +1 mM histamine (260 ± 50 nM). As shown in Fig. 8B, a similar additivity was shown for phosphate uptake. Phosphate uptake was stimulated 29 ± 6.2% by histamine alone and 50.1 ± 8.9% in the presence of 100 μM 5-HT. Maximal stimulation was 32.6 ± 6.6% by 5-HT alone and 51.0 ± 4.5% in the presence of 1 mM histamine. Half-maximal stimulatory doses for phosphate uptake were as follows: histamine alone (3.2 ± 1.8 μM) or +100 μM 5-HT (2.0 ± 2.2 μM), 5-HT alone (990 ± 200 nM) or +1 mM histamine (760 ± 140 nM). The observations that the stimulatory effects of histamine and 5-HT on phosphoinositide hydrolysis and phosphate uptake are additive in HeLa cells support the notion that the histamine H1 and 5-HT1A receptors couple to distinct pools of G proteins in these cells.

Histamine and 5-HT Have Additive Effects on MARCKS Protein Phosphorylation—If the stimulation of phosphate transport by both agonists is due to activation of protein kinase C, one would expect that phosphorylation of the MARCKS protein would be additive. Data presented in Fig.
Protein kinase C by either agonist reaches a maximal effect, performed for each condition. The Bonferroni correction was performed as in Fig. 5. The counts incorporated (over background) were as follows: A, 5-HT, 1028 cpm; 1 mM histamine, 621 ± 43 cpm; 10 μM histamine, 793 ± 59 cpm; 1 μM histamine, 871 ± 86 cpm; 1 μM 5-HT, 682 ± 49 cpm; 10 μM 5-HT, 1014 ± 32 cpm; 100 μM 5-HT, 1028 ± 43 cpm. B, basal, 514 ± 36 cpm; 1 μM histamine alone, 871 ± 86 cpm; 1 μM histamine + 1 μM 5-HT, 1099 ± 50 cpm; 1 μM histamine + 100 μM 5-HT, 1424 ± 71 cpm; 100 μM 5-HT alone, 1028 ± 43 cpm. Values depicted represent the means ± S.E. of three to five separate experiments performed for each condition. The Bonferroni correction was used for statistical comparisons within each panel.

FIG. 9. Additivity of the effects of histamine and 5-HT on phosphorylation of the MARCKS protein. Experiments were performed as in Fig. 5 and under “Experimental Procedures.” Counts incorporated (over background) were as follows: A, basal, 514 ± 36 cpm; 10 μM histamine, 621 ± 43 cpm; 100 μM histamine, 793 ± 59 cpm; 1 mM histamine, 671 ± 96 cpm; 1 μM 5-HT, 692 ± 49 cpm; 10 μM 5-HT, 1014 ± 32 cpm; 100 μM 5-HT, 1028 ± 43 cpm. B, basal, 514 ± 36 cpm; 1 μM histamine alone, 871 ± 86 cpm; 1 μM histamine + 1 μM 5-HT, 1099 ± 50 cpm; 1 μM histamine + 100 μM 5-HT, 1424 ± 71 cpm; 100 μM 5-HT alone, 1028 ± 43 cpm. Values depicted represent the means ± S.E. of three to five separate experiments performed for each condition. The Bonferroni correction was used for statistical comparisons within each panel.

FIG. 10. Northern blot analysis of HeLa cell mRNA. Experiments were performed as under “Experimental Procedures,” using 30 μg total RNA/lane. Films were exposed for 1 week at −70 °C. The strips were probed with subtype-specific DNA probes as follows: lane A, PKC-α; lane B, PKC-β; lane C, PKC-γ. Under the washing conditions used for these blots (0.2 x SSC, 0.1% SDS, 65 °C), we have observed no cross-reactivity between the various PKC subtypes in other human cell lines.

9 support this notion. In A, a plateau of stimulation is demonstrated for both agonists, suggesting that activation of protein kinase C by either agonist reaches a maximal effect, as would be expected from the dose-response curves for phosphoinositide hydrolysis presented in Fig. 8. B of Fig. 9 demonstrates that the phosphorylation of the MARCKS protein induced by 5-HT and histamine are also additive, even when maximal doses of both agents are utilized. The stimulation (over basal) induced by 1 mM histamine was 69 ± 17%, by 10 μM 5-HT was 100 ± 8%, and for both agents together was 177 ± 14%. These values compare with the previously published 170 ± 40% increase induced by 1.6 μM PMA (22).

Northern Blot Analysis of Protein Kinase C Subtypes Present in HeLa Cells—Fig. 10 demonstrates that RNA derived from HeLa cells cross-hybridizes with human DNA probes for three subtypes of PKC (α, β, γ). Each transcript is approximately 3.0-3.4 kilobases in size, consistent with previously published data (38, 44). It is unlikely that these bands represent cross-hybridization of one probe with mRNA for another PKC subtype, because under the same stringent washing conditions (0.2 x SSC, 0.1% SDS, 65 °C), we have observed no detectable cross-hybridization of these probes with mRNA for other PKC subtypes from other human cell lines. Although PKC-γ is generally regarded as brain-specific (45), it has recently been shown by Western and Northern blotting techniques to be present in the Jurkat human leukemic T cell line (44). The same authors also presented Northern blot data demonstrating the presence of PKC-γ mRNA transcripts in two other human cell lines, the MOLT-4 T cell leukemia and Raji B lymphoblastoid cell lines (44). The current data suggest that there are multiple subtypes of PKC expressed in HeLa cells and that PKC-γ may be expressed in multiple forms of human malignancies, as well as in brain.

DISCUSSION

The regulation of sodium-dependent phosphate uptake has been the subject of considerable recent interest. Previous studies have demonstrated that these regulatory processes are remarkably complex. Of particular importance are the respective roles of PKA and PKC and of the receptors and second messengers which activate them. For example, distinct hormones may exert similar effects on phosphate transport in the same cell. Specificity at this level may be conferred by the complement of receptor subtypes present in a given cell type. Specificity or diversity of these effects may be conferred at the level of the G proteins, second messengers, kinases, or even at the level of the sodium-dependent transporter itself, depending on what potential sites of kinase interaction and/or dynamic phosphorylation are present.

Several examples have been described in which a given hormone may activate two distinct second messenger cascades which regulate phosphate transport synergistically. For example, in OK cells, parathyroid hormone activates phosphoinositide hydrolysis and increases cAMP accumulation; both events may inhibit phosphate transport in these cells, although the relative importance of each individual pathway is still unresolved (15, 24). In LLC-PK1 cells, calcitonin both activates phosphoinositide hydrolysis and increases cAMP accumulation, leading to a stimulation of phosphate uptake (16). In this case, diacylglycerol-induced activation of PKC appears to be the more important pathway (16). In both cases, it is unclear whether multiple or single subtypes of receptors for parathyroid hormone or calcitonin are present or if single receptor subtypes are linked to the distinct second messenger cascades through the same or different G proteins. In this respect, distinct G proteins may exert opposing effects on

3 L. M. Obeid, unpublished observation.
phosphate transport in the same cell. In OK cells, α2-adrenergic receptors, which activate a “G-like” G protein, inhibit the parathyroid hormone-induced decrease in phosphate uptake, which is presumably mediated through G, (18). In the current study, we explored the possibility that two different hormones could alter phosphate transport additively via the same or different G proteins in HeLa cells.

The finding that endogenous histamine H1 receptors present in HeLa cells stimulate phosphate uptake via PKC activation supports the hypothesis that receptors classically linked to phosphoinositide hydrolysis will stimulate phosphate uptake in these cells. The close concordance of the maximal degree of stimulation of both phosphoinositide hydrolysis and phosphate uptake induced by both histamine and 5-HT further verifies the close linkage between these two processes in HeLa cells (22). It is now known that there are several distinct G proteins which can activate phosphoinositide hydrolysis (so-called G, proteins). That the histamine effects are mediated through a different G, protein pool than those linked to 5-HT in HeLa cells is supported by the additivity of phosphoinositide hydrolysis PKC activation, and phosphate uptake and the differential sensitivity to pertussis toxin. The additive effect on phosphate uptake seemed to plateau at high doses (Fig. 8), implying saturability of a process distal to phosphoinositide hydrolysis.

At least in the case of the 5-HT1A receptor, which is expressed at very high density in these cells (2.8 ± 0.6 pmol/mg protein), one might predict that under conditions of maximal stimulation by agonist (100 μM 5-HT), the G protein pool utilized by the 5-HT1A receptor should be fully activated (46). Therefore, receptors which utilize the same G proteins would not further stimulate phosphoinositide hydrolysis, whereas receptors which use distinct G proteins would augment the 5-HT1A receptor-induced stimulation of phosphoinositide hydrolysis. This prediction is reasonable if the pertussis toxin-sensitive G, protein is not present in great excess to its linked receptors. It also assumes that phospholipase(s) C are present in excess of the various G protein pools capable of coupling to phosphoinositide hydrolysis. Recent studies using cells that express various levels of recombinant muscarinic acetylcholine receptors (46) or α1-adrenergic receptors (47) suggest one aspect of this model. In both cases, increasing the numbers of receptors expressed per cell increased activation of phosphoinositide hydrolysis to a maximal point, but eventually the degree of stimulation of phosphoinositide hydrolysis reached a plateau. The observations that the stimulatory effects of histamine and 5-HT on phosphoinositide hydrolysis, PKC activity, and phosphate uptake are additive in HeLa cells, coupled with the differential sensitivity of both processes to pertussis toxin, support the notion that the histamine H1 and 5-HT1A receptors couple to distinct pools of G proteins in these cells.

These studies further illustrate that distinct pools of G, can exist in a single cell type (45, 48) and have important ramifications regarding the multiplicity of control mechanisms for the physiological transport of phosphate and, perhaps, other solutes. These G, proteins can be distinguished by their sensitivity to pertussis toxin (46, 49), which uncouples certain G proteins from receptors by catalyzing the ADP-ribosylation of the G protein α-subunits (50–52). The presence of distinct G, proteins in a single cell which can couple selectively to different receptors may be a mechanism by which common cellular responses such as phospholipase C activation can be compartmentalized. Such an arrangement also provides a mechanism by which cellular effectors can be further activated under conditions of maximal stimulation of a single receptor (or G protein) subtype.

The differential sensitivity of the 5-HT1A and histamine H1 receptors to pertussis toxin is consistent with that observed for these receptors in other cell lines or tissues. The cellular effects of 5-HT1A receptor activation are typically sensitive to pertussis toxin (53), whereas most of those associated with the histamine H1 receptor are not (41, 54). The differential sensitivity of the G, proteins linked to phosphoinositide hydrolysis and stimulation of phosphate uptake in HeLa cells most likely relates to structural differences between the G, proteins. In that regard, the α-subunit of a G protein which exhibits a high degree of primary amino acid sequence identity with the Gβ-like G proteins has been described (55, 56). This α-subunit lacks a carboxyl terminus cysteine residue which is the pertussis toxin-catalyzed ADP-ribosylation acceptor site (57). This region may also be involved in the coupling of receptors or effector enzymes to G protein (57).

It is not clear from the current studies whether the differential pertussis toxin sensitivity of G, proteins expressed in HeLa cells is due to differences in the primary amino acid sequences of the α-subunits or to different post-translational modifications of the G proteins which cause subtle structural differences which influence parameters essential for coupling of G, proteins to receptors or the phospholipase C effector enzymes and sensitivity to pertussis toxin. Moreover, it is not known whether these distinct G, proteins couple to the same or different phospholipase(s) C. The existence of at least five subtypes of phospholipase C has been documented (58). A similar heterogeneity of PKC subtypes (at least seven) has also been described (59). Our data support the presence of mRNA for at least three subtypes of PKC (α, β, γ) in HeLa cells (Fig. 10). Therefore, there remains the possibility that the compartmentalization of the signal transduction pathways linked to histamine and 5-HT in HeLa cells extends beyond the G, proteins and may include isoforms of PKC. However, we have not directly addressed this hypothesis in the current studies.

A central concern in cellular signaling is the mechanism by which specificity of responses can be conferred by a limited repertoire of machinery necessary for signal transduction. In the case of sodium-dependent phosphate transport, both additive (15, 16, 24) and opposing (22, 27) regulation of PKA and PKC have been described. At the level of the G protein, opposite regulation by receptors putatively linked to G, and G, has been described (18). The current studies document that additive regulation of phosphate transport can occur via distinct pools of G proteins, thus adding to the complexity of interactions between components of the cellular machinery which modulate the transport of phosphate. Thus, the regulation of phosphate transport can be augmented or antagonized at multiple points in the signal transduction cascade, including interactions with distinct pools of G proteins.

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