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Cyclic AMP Potentiates Receptor-Stimulated Phosphoinositide Hydrolysis in Human Neuroepithelioma Cells

STEPHEN K. FISHER, EDWARD L. McEWEN, SIMON C. LOVELL, and RACHEL E. LANDON

Neuroscience Laboratory (S.K.F., E.L.M., S.C.L., R.E.L.) and Department of Pharmacology (S.K.F.), University of Michigan, Ann Arbor, Michigan 48104

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SUMMARY

A stimulatory role for cAMP in the regulation of receptor-activated phosphoinositide hydrolysis has been examined in human SK-N-MCIXC and SK-N-MCIIE neuroepithelioma cells. The addition of optimal concentrations of oxotremorine-M, norepinephrine, endothelin-1, and ATP enhanced the release of inositol phosphates by 2–9-fold after activation of muscarinic, α_1-adrenergic, endothelin, and P_2 nucleotide receptors, respectively. All combinations of these agonists elicited a release of inositol phosphates that was at least additive. However, the combined presence of oxotremorine-M and norepinephrine resulted in a phosphoinositide hydrolysis that was 30% greater than additive. This potentiation of inositol lipid hydrolysis resulted from an increased activity of the muscarinic receptor after the addition or norepinephrine and persisted after α_1-adrenergic receptor blockade. The enhancement of muscarinic receptor-stimulated inositol phosphate release could be quantitatively mimicked by inclusion of the β-adrenergic agonist isoproterenol ($EC_{50} \sim 0.1$ μM), but not by α_1- or α_2-adrenergic agonists. Potentiation of oxotremorine-M-stimulated inositol lipid hydrolysis observed in the presence of either norepinephrine or isoproterenol was reduced in the absence of added Ca^{2+}. Addition of either norepinephrine or isoproterenol to SK-N-MCIXC cells also resulted in a 16-fold increase in cAMP concentration. Although the cell-permeant 8-chloro-4-phenylthio-cAMP had a small inhibitory effect on basal inositol phosphate release, its inclusion resulted in a 19–31% enhancement of muscarinic, endothelin, ATP, and α_1-adrenergic receptor-stimulated phosphoinositide hydrolysis. We conclude 1) that, in SK-N-MCIXC cells, the addition of β-adrenergic agonists selectively enhances muscarinic receptor-stimulated phosphoinositide hydrolysis through a cAMP-dependent process and 2) that the ability of exogenously added cAMP to enhance the activation of all four inositol lipid-linked receptors indicates that the effects of cAMP on inositol lipid hydrolysis are compartmentalized in these cells.

Receptor activation of phosphoinositide-specific phospholipase C, with the attendant formation of inositol 1,4,5-trisphosphate and diacylglycerol, and modulation of adenylyl cyclase activity represent two of the major mechanisms for signal transduction in eukaryotic cells. Evidence has accumulated that cross-talk exists between these two pathways, such that each may regulate the activity of the other. For example, in some tissues, the activation of protein kinase C, either directly by administration of phorbol esters or indirectly after receptor activation, can either increase or decrease adenylyl cyclase activity (1–7). An increase in the cellular concentration of cAMP, and the presumed activation of protein kinase A, frequently leads to a marked inhibition of stimulated phosphoinositide hydrolysis (8–14). In keeping with the latter observation, receptors that are negatively coupled to adenylyl cyclase activity have been reported to enhance receptor-stimulated inositol phosphate formation (15, 16). The possibility that an increased cAMP concentration may also facilitate the hydrolysis of inositol lipids has been raised but remains controversial. Thus, whereas either the addition of a cell-permeant analog of cAMP (17) or receptor activation of adenylyl cyclase (18, 19) has been reported to increase receptor stimulation of inositol phosphate and diacylglycerol production in hepatocytes, other studies have failed to detect such changes (20–22).

In the present study, we have utilized human SK-N-MCIXC cells to determine whether cAMP plays a stimulatory role in the regulation of receptor-mediated phosphoinositide hydrolysis. When these cells are exposed to either NE or the muscarinic agonist Oxo-M, each agent elicits an increased phosphoinositide hydrolysis mediated by α_1-adrenergic receptors and mAChRs, respectively. In the presence of both NE and Oxo-M, the resulting phosphoinositide hydrolysis is consistently greater than additive and persists after the blockade of α_1-adrenergic receptors but not of mAChRs. By means of selective adrenergic agonists, we demonstrate that the potentiation of

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ABBREVIATIONS: NE, norepinephrine; Oxo-M, oxotremorine-M; mAChR, muscarinic acetylcholine receptor; ET-1, endothelin-1; 8CPT-cAMP, 8-chloro-4-phenylthio-cAMP; G_p, guanine nucleotide-binding protein that regulates phospholipase C; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

inositol phosphate formation occurs in the presence of β-adrenergic agonists, the addition of which also elicits an increased cAMP production. Only mAChR-stimulated inositol lipid hydrolysis is enhanced, whereas that mediated by either endothelin or P_2 nucleotide receptors is unaffected. Although the addition of a cell-permeant analog of cAMP results in a small inhibition of the basal release of inositol phosphates, a marked increase in mAChR-, α_1-adrenergic receptor-, endothelin receptor-, and P_2 nucleotide receptor-stimulated phosphoinositide hydrolysis occurs in its presence. These results not only support the concept that an agonist-mediated rise in cAMP concentration can facilitate the activity of a phosphoinositide-linked receptor but also indicate that a compartmentation of these effects occurs in neuroepithelioma cells.

Materials and Methods

SK-N-MCIXC and SK-N-MCIIE neuroepithelioma cells were obtained from Dr. June Biedler, Sloan Kettering Institute (New York). *myo*-[2-^{3}H]Inositol (15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). (−)-NE, atropine, ATP, 8CPT-cAMP, phenylephrine, DL-propranolol, and (−)-isoproterenol were obtained from Sigma Chemical Co. (St. Louis, MO). (−)-Epinephrine bitartrate, 6-fluoronorepinephrine, prazosin, Oxo-M, and phenoxybenzamine were purchased from Research Biochemicals, Inc. (Natick, MA). ET-1 was obtained from Peninsula Laboratories (Belmont, CA). Bromoxidine (UK 14304) was a generous gift from Dr. R. Neubig, University of Michigan. Tissue culture supplies were obtained from Corning Glass Works (Corning, NY). Powdered Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from GIBCO (Grand Island, NY). Dowex-1 (100–200 mesh, ×8 in the formate form) was obtained from Bio-Rad (Rockville Center, NY).

Cell culture conditions. Human SK-N-MCIXC (passages 16–37) and SK-N-MCIIE cells (passages 16–25) (each subcloned twice from the parent SK-N-MC line; see Ref. 23) were grown in tissue culture flasks (75 cm^2/250 ml), in 20 ml of Dulbecco's modified Eagle's medium supplemented with 10% (by volume) fetal calf serum (medium I). Cells were grown for 7–20 days at 37° in an atmosphere consisting of 10% CO_2 and 90% humidified air. Subculture was achieved by aspirating medium I and isolating the cells after incubation in a modified Puck's D_1 solution containing 0.5 mM EDTA and 0.05% trypsin (medium II) (24). After centrifugation of the cell suspension for 1 min at 300 × *g*, the supernatant was removed and the cells were resuspended in 10 ml of medium I, which was then inoculated into flasks (one confluent flask into five to seven flasks) on day 0. The culture medium was changed on day 4 and on alternate days thereafter, by the addition of 10 ml of fresh medium I and removal of 10 ml of medium.

Measurement of phosphoinositide turnover. SK-N-MCIXC or SK-N-MCIIE cells were allowed to prelabel for 3 days in Dulbecco's modified Eagle's medium/10% fetal bovine serum containing 10 μCi/ml [^{3}H]inositol. Isotopic equilibrium labeling of inositol lipids in both cell lines was attained within a 48-hr labeling period. Cells were detached in medium II and washed twice in buffer A (142 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 3.6 mM $NaHCO_3$, 1 mM $MgCl_2$, 5.6 mM D-glucose, 30 mM sodium HEPES buffer, pH 7.4). Cells were then resuspended in buffer A and incubated at 37° in the presence of agonists and/or antagonists (final volume, 0.5 ml). For experiments with phenoxybenzamine, cells were first preincubated with a 10 μM concentration of the alkylating agent for 15 min at 37° and were then extensively washed with buffer A before assay. The accumulation of ^{3}H-inositol phosphates was monitored in the presence of Li^+, as previously described (25). Unless stated otherwise, a 30-min incubation period was routinely used. In some experiments, the individual inositol phosphate isomers were separated and quantitated by high performance liquid chromatography (26). As previously observed for the parent SK-N-MC cell line (24), the majority (>85%) of radiolabel was recovered in the inositol 1-monophosphate/inositol 3-monophosphate, inositol 4-monophosphate, and inositol bisphosphate fractions. The remainder of the radioactivity was recovered in inositol 1,4,5-trisphosphate, inositol 1,3,4-trisphosphate, and inositol tetrakisphosphate fractions. No obvious differences in inositol phosphate isomer profile were observed for NE, Oxo-M, ET-1, or ATP, whether added individually or in combination. Protein was determined by the method of Geiger and Bessman (27).

Determination of intracellular cAMP concentrations. SK-N-MCIXC cells (1–2 mg of protein/ml) were incubated for 30 min at 37° in the presence of either buffer A, 1 mM NE, or 10 μM (−)-isoproterenol in the presence of 1 mM 3-isobutyl-1-methylxanthine. Reactions were terminated and cAMP was extracted and quantitated as described previously (8).

Data analysis. Values quoted are means ± standard errors for the number (n) of separate experiments performed. Student's two-tailed t tests were used to evaluate the statistical differences of the means of paired or unpaired sets of data. The release of inositol phosphates (fold stimulation) was calculated from the equation inositol phosphate release = $A - Z/B - Z$, where A and B are the values obtained for inositol phosphate release in the presence of the agonist(s) (A) or absence of the agonist (B) after a 30-min incubation period and Z is inositol phosphate release obtained at zero time. The extent of potentiation is calculated from the equation:

$$\text{Potentiation} = (Y - B)/[(A_P - B) + (A_Q - B)]\%$$

where A_P and A_Q are the values obtained for inositol phosphate release elicited by agonists P and Q when added separately and Y is the inositol phosphate value obtained in the presence of the two agonists when added together. Dose-response curves were analyzed by the GraphPad InPlot curve-fitting program.

Results

Agonist-stimulated phosphoinositide hydrolysis in SK-N-MCIXC and SK-N-MCIIE neuroepithelioma cells. The addition of either Oxo-M, NE, ET-1, or ATP resulted in an increased release of inositol phosphates that proceeded approximately linearly with time for at least 30 min. The most effective agonists tested were Oxo-M and ET-1, both of which elicited a 6–9-fold stimulation, whereas 2–3-fold increases were observed for ATP and NE. Combinations of agonists resulted in releases of inositol phosphates that were at least equal to the sum of the individual responses. However, when Oxo-M and NE were present together, a greater than additive stimulation of phosphoinositide hydrolysis occurred (Figs. 1 and 2). Although consistently observed, in individual experiments the extent of this potentiation of inositol phosphate release varied between 14 and 64% [means, 28 ± 2% (n = 39) and 28 ± 2% (n = 7) for the SK-N-MCIXC and SK-N-MCIIE cell lines, respectively]. Because the magnitude of inositol phosphate release was greater in SK-N-MCIXC neuroepithelioma, all subsequent experiments were conducted with this clonal cell line. The potentiation of phosphoinositide hydrolysis observed in SK-N-MCIXC cells in the presence of Oxo-M and NE was detected at all time points examined (51 ± 9, 35 ± 2, and 27 ± 6% at 5, 15, and 30 min of incubation, respectively; n = 3; see Fig. 3). Although potentiation of inositol phosphate release was greater at shorter time intervals when expressed as a percentage, in absolute terms it was more readily detectable after a 30-min incubation, the time point routinely chosen for subsequent studies. As observed for the parent SK-N-MC cell line (24), omission of Ca^{2+} resulted in 72 ± 2 and 40 ± 5% reductions of Oxo-M- and NE-stimulated release of inositol phosphates, re-

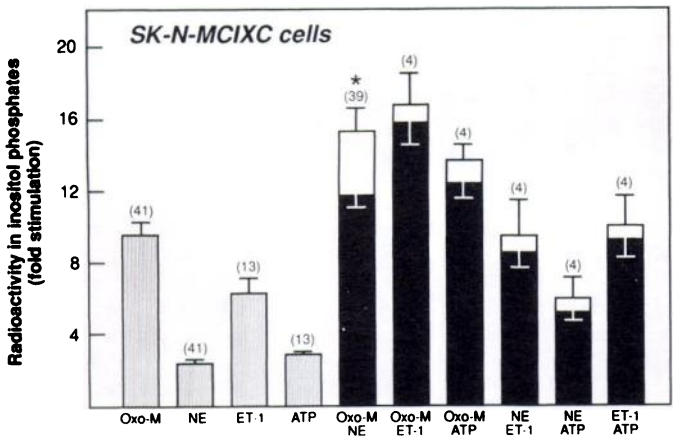

Fig. 1. Agonist-stimulated inositol phosphate formation in SK-N-MCIXC neuroepithelioma. Prelabeled cells (approximately 1–2 mg of protein) were incubated for 30 min at 37° with 1 mM concentrations of Oxo-M, NE, or ATP or 0.5 μM ET-1, added singly or in combination, as indicated. Reactions were terminated by the addition of an equal volume of 20% trichloroacetic acid, and labeled inositol phosphates present in neutralized extracts were quantitated by anion-exchange chromatography. Results are expressed as release of inositol phosphates, relative to control incubations, for the number of separate experiments indicated. □, Agonists added singly; □, agonists added in combination; ■, theoretical values if agonist responses were additive. The standard error values for the latter were calculated as $\sqrt{x^2 + y^2}$, where x and y represent the standard errors for the two individual agonist responses. *, Different from additivity, $p < 0.001$ (matched-pair analysis).

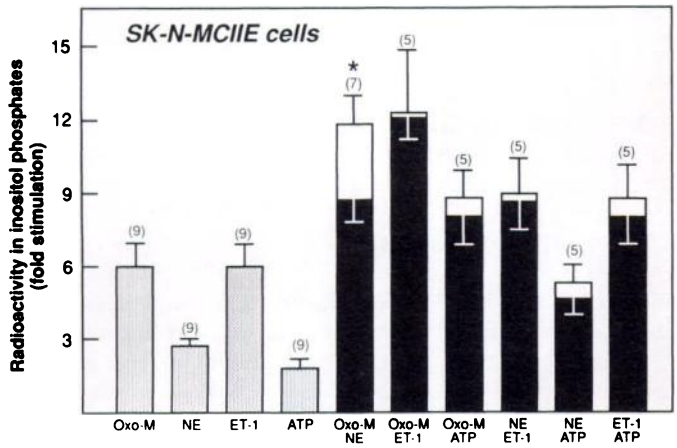

Fig. 2. Agonist-stimulated inositol phosphate formation in SK-N-MCIIE neuroepithelioma. See legend to Fig. 1 for experimental details.

spectively, in SK-N-MCIXC cells ($n = 4$). Moreover, in the absence of added Ca^{2+}, inositol phosphate release elicited by a combination of Oxo-M and NE was additive rather than potentiative (potentiation, 9 ± 4% and 28 ± 1% in the absence and presence of added Ca^{2+}, respectively; $p < 0.001$; $n = 4$). Potentiation of inositol phosphate release involves an increased maximum release of inositol phosphates without a change in agonist potencies. When dose-response curves were constructed for NE and Oxo-M, added separately, the respective EC_{50} values were 3 ± 2 and 35 ± 2 μM ($n = 3$ or 4). In the presence of a 1 mM concentration of Oxo-M, the EC_{50} value for NE was unchanged (2 ± 1 μM). Similarly, the presence of 1 mM NE did not alter the EC_{50} value for Oxo-M (34 ± 5 μM). At all ligand concentrations examined, a greater than additive release of inositol phosphates was observed (Figs. 4 and 5).

Pharmacological characteristics of enhanced phosphoinositide hydrolysis. The pharmacological identities of receptors involved in the potentiation of inositol lipid hydrolysis were probed with specific antagonists and agonists. Inclusion of a 10 μM concentration of the muscarinic antagonist atropine inhibited stimulated phosphoinositide hydrolysis elicited by Oxo-M by 99 ± 1% ($n = 3$). When atropine was added to incubations containing both Oxo-M and NE, stimulated inositol phosphate release was no different from that obtained for NE alone. In contrast, although inclusion of the α_1 antagonist prazosin inhibited NE-stimulated phosphoinositide hydrolysis by 90 ± 3% ($n = 5$), a 39 ± 10% increase in Oxo-M-stimulated inositol phosphate formation was still observed in the presence of the antagonist when incubations contained both Oxo-M and NE ($n = 8$). Further indication of the involvement of an adrenergic receptor other than the α_1 subtype in the enhanced inositol lipid hydrolysis was obtained from experiments in which α_1-adrenergic receptors were initially inactivated by treatment with the alkylator phenoxybenzamine (28). Although a 98 ± 2% inhibition of NE-stimulated inositol phosphate formation occurred under these conditions, the addition of NE still elicited a 49 ± 1% increase in the Oxo-M response ($n = 3$). When both prazosin and atropine were present, inositol phosphate release elicited by the combined addition of Oxo-M and NE was fully blocked (98 ± 1%; $n = 3$). Taken collectively, these results indicate that the enhancement of stimulated phosphoinositide hydrolysis in SK-N-MCIXC cells occurs through the mAChR and is mediated by an adrenergic receptor other than the α_1 subtype. Identification of the adrenergic receptor subtype involved in the enhanced mAChR

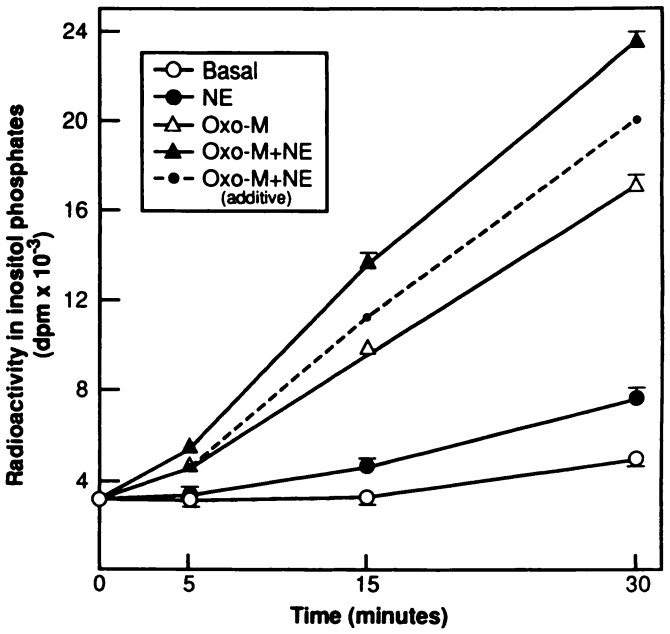

Fig. 3. Time course of basal, Oxo-M-stimulated, and NE-stimulated inositol phosphate formation. Prelabeled SK-N-MCIXC cells (approximately 1 mg of protein) were incubated in either the absence (basal) or the presence of NE (1 mM) and/or Oxo-M (1 mM), for the times indicated, at 37°. Reactions were terminated by the addition of an equal volume of 20% trichloroacetic acid, and a total inositol phosphate fraction present in neutralized extracts was quantitated by anion-exchange chromatography. Values shown are means ± standard errors of quadruplicate replicates from one of three experiments that gave similar results. In the experiment shown, the potentiation at 5, 15, and 30 min was 53, 31, and 22%, respectively. When error bars are not shown, the standard error fell within the symbol.

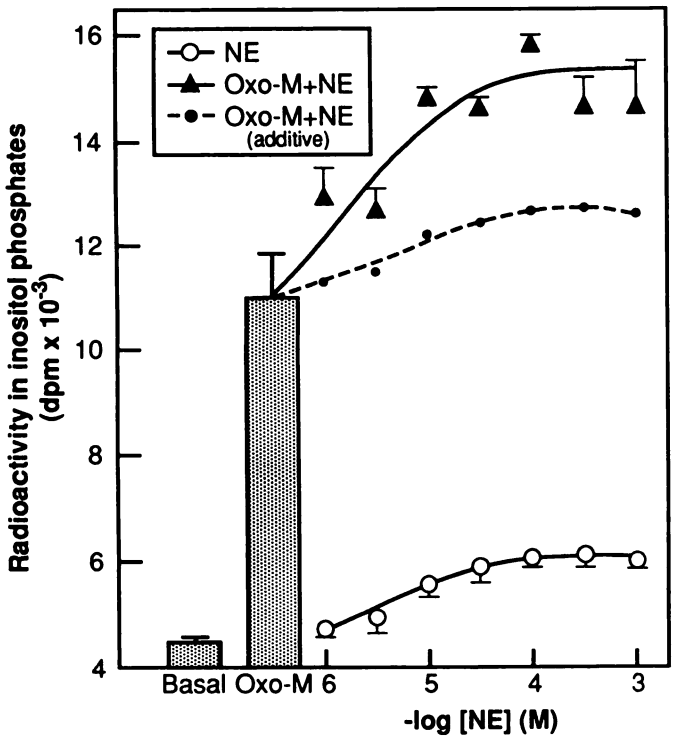

Fig. 4. Dose-response relationship for NE-stimulated inositol phosphate formation in the absence (○) or presence of (▲) of 1 mM Oxo-M. – – –, Theoretical additive response. Values shown are means ± standard errors for triplicate replicates from one of three experiments that gave similar results. In the experiment shown, the calculated EC_{50} values were 3 and 2 μM in the absence and presence of 1 mM Oxo-M, respectively. At 1 mM concentrations of Oxo-M and NE, a 25% potentiation of phosphoinositide hydrolysis was observed.

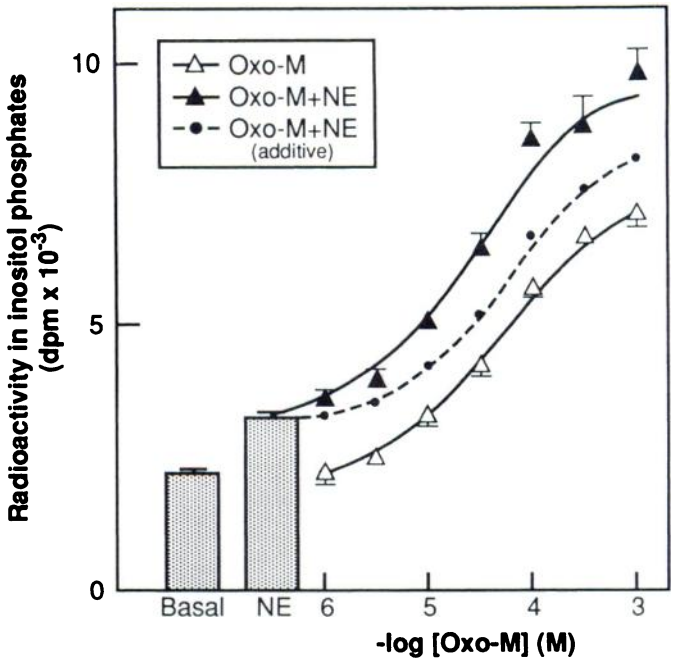

Fig. 5. Dose-response relationship for Oxo-M-stimulated inositol phosphate formation in the absence (△) or presence (▲) of 1 mM NE. – – –, Theoretical additive response. Values shown are the means ± standard errors for triplicate replicates from one of four experiments that gave similar results. In the experiment shown, the calculated EC_{50} values were 38 and 26 μM in the absence and presence of 1 mM NE, respectively. At 1 mM concentrations of Oxo-M and NE, a 23% potentiation of phosphoinositide hydrolysis was observed.

response was probed further with selective agonists (Fig. 6). The ability of Oxo-M to elicit an increased release of inositol phosphates was facilitated by the presence of either NE or epinephrine, both mixed α/β-adrenergic agonists. In contrast, the addition of either phenylephrine or 6-fluoronorepinephrine, two α_1-selective agonists, did not increase Oxo-M-stimulated inositol lipid hydrolysis. Similarly, the α_2-selective agonist bromoxidine had no effect on mAChR-stimulated inositol phosphate formation. However, inclusion of the β-selective agonist (−)-isoproterenol enhanced the ability of Oxo-M to promote inositol lipid hydrolysis by 25–30% but had no effect on inositol phosphate release when added alone (Fig. 6). As previously observed for NE, the isoproterenol potentiation of Oxo-M-stimulated inositol phosphate release was reduced in the absence of added Ca^{2+} (potentiation, 8 ± 3% and 27 ± 4% in the absence and presence of added Ca^{2+}, respectively; $n = 4$). When added to incubations containing optimal concentrations of Oxo-M and NE, isoproterenol did not elicit any further increase in inositol phosphate release, suggesting that both adrenergic ligands operate through a common mechanism. Half-maximal increases in Oxo-M-stimulated inositol phosphate release were obtained at approximately 0.1 μM isoproterenol (Fig. 7). In the presence of 10 μM (−)-isoproterenol, Oxo-M-stimulated inositol phosphate release was fully blocked by inclusion of 10 μM atropine. In contrast, inclusion of the β-adrenergic antagonist propranolol, at a concentration of 50 μM, resulted in a variable inhibition of (−)-isoproterenol-potentiated Oxo-M-stimulated inositol lipid hydrolysis (mean, 37 ± 12%; $n = 6$). Propranolol at this concentration had no effect on inositol phosphate release elicited by the addition of Oxo-M alone (101 ± 6% of that observed in the absence of propranolol; $n = 4$).

Role of cAMP in the enhanced phosphoinositide response. In the parent SK-N-MC cell line, agonist occupancy of β-adrenergic receptors results in a 6-fold increase in the activity of adenylyl cyclase (29, 30). In SK-N-MCIXC cells, the addition of either 1 mM NE or 10 μM isoproterenol resulted in a 16-fold increase in the mass of cAMP (basal, 102 ± 24; NE, 1604 ± 386; isoproterenol, 1635 ± 407 pmol/mg of protein; $n = 5$). To determine whether these increases in cAMP were mechanistically linked to the facilitation of mAChR-stimulated phosphoinositide hydrolysis, SK-N-MCIXC cells were preincubated for 10 min with 1 mM 8CPT-cAMP, a membrane-permeant analog of cAMP (31), before agonist addition. Although 8CPT-cAMP had a small inhibitory effect on basal inositol phosphate release, its inclusion led to a 31 ± 5% increase in the Oxo-M response. However, 8CPT-cAMP did not further potentiate inositol phosphate release elicited by a combination of Oxo-M and NE (data not shown). In contrast to the selective stimulation of mAChR-stimulated inositol phosphate formation observed after the addition of β-adrenergic agonists, preincubation of cells with 8CPT-cAMP also resulted in increases in ET-1 receptor-, ATP receptor-, and α_1-adrenergic receptor-stimulated phosphoinositide hydrolysis (Fig. 8).

Discussion

Although much recent attention has been focused on the individual components of the phosphoinositide signaling system, relatively little is known of the mechanism(s) that underlie the regulation of this ubiquitous transmembrane signaling sys-

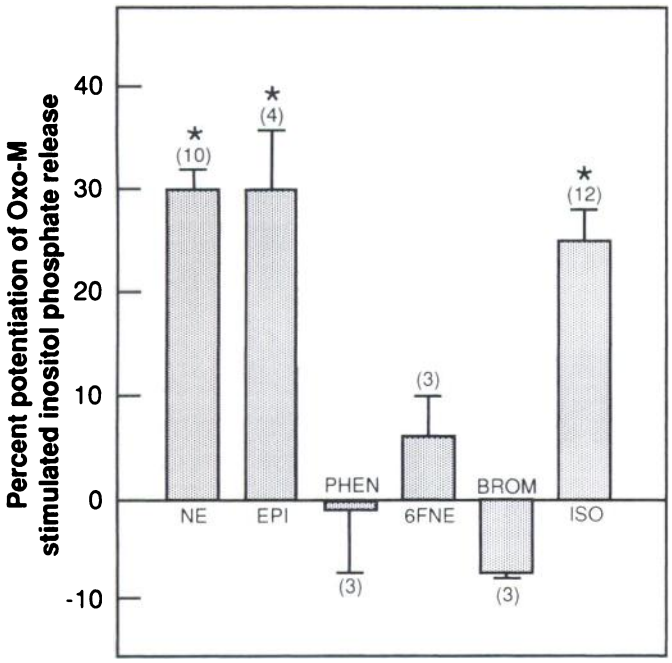

Fig. 6. Differential ability of adrenergic agonists to enhance Oxo-M-stimulated inositol phosphate formation. SK-N-MCIXC cells were incubated with 1 mM Oxo-M either in the absence or in the presence of 1 mM NE, 1 mM epinephrine (*EPI*), 100 μM phenylephrine (*PHEN*), 100 μM 6-fluoronorepinephrine (*6FNE*), 100 μM bromoxidine (*BROM*), or 10 μM (−)-isoproterenol (*ISO*). The addition of NE, epinephrine, phenylephrine, and 6-fluoronorepinephrine alone increased inositol phosphate release to 298 ± 20, 435 ± 50, 188 ± 13, and 240 ± 25% of control, respectively, whereas no stimulation of phosphoinositide hydrolysis occurred in the presence of either bromoxidine or (−)-isoproterenol, when added alone (107 ± 5 and 98 ± 6%, respectively). Results are expressed as potentiation of Oxo-M-stimulated inositol phosphate release in the presence of the adrenergic agonist, for the number of separate experiments indicated. See Materials and Methods for details of calculation. *, Different from additivity, $p < 0.001$ (matched-pair analysis).

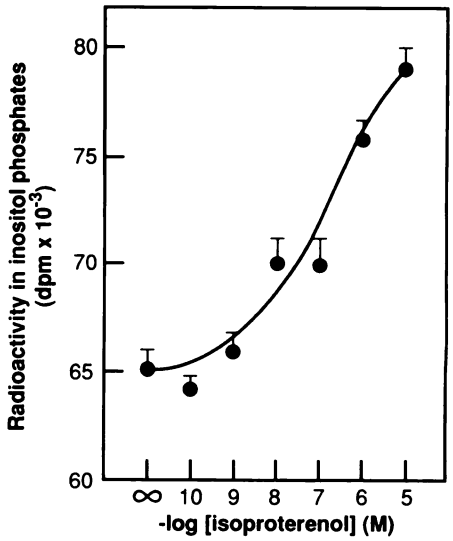

Fig. 7. Enhancement of Oxo-M-stimulated inositol phosphate formation by isoproterenol. Prelabeled cells were incubated with 1 mM Oxo-M in the absence or presence of isoproterenol at the concentrations indicated. Values shown are means ± standard errors for quadruplicate replicates. The basal release of inositol phosphates was 17,290 dpm. The calculated EC_{50} value for isoproterenol was 0.1 μM. No further increase in inositol phosphate release occurred at concentrations of isoproterenol greater than 10 μM. In the experiment shown, inclusion of 10 μM isoproterenol potentiated Oxo-M-stimulated inositol phosphate formation by 30%.

tem. However, it now appears likely that multiple mechanisms exist for both the homologous and heterologous regulation of stimulated inositol lipid hydrolysis (9, 15, 17, 32–35). In the present study, one of the principal conclusions is that, in human neuroepithelioma cells, a rise in cellular cAMP, generated either from agonist addition or after exogenous application, can serve to facilitate receptor-stimulated phosphoinositide hydrolysis. This is in marked contrast to the pronounced inhibitory effects of cAMP on inositol lipid hydrolysis encountered in many other preparations (8–14). The conclusion that cAMP can potentiate receptor-stimulated inositol lipid hydrolysis in SK-N-MCIXC cells is based upon three experimental observations. First, the stimulatory effect of NE on mAChR-stimulated inositol phosphate formation could be selectively mimicked by inclusion of the β-adrenergic agonist isoproterenol, at concentrations reported to activate adenylyl cyclase in the parent SK-N-MC cell line (30). Second, under standard assay conditions, the addition of either NE or (−)-isoproterenol elicited 16-fold increases in the mass of cAMP in SK-N-MCIXC cells. Third, preincubation of cells with 8CPT-cAMP resulted in an increase in mAChR-stimulated phosphoinositide hydrolysis that was quantitatively similar to that observed for either NE or (−)-isoproterenol. Although the magnitude of the potentiation determined in the present study was modest, potentiation was consistently observed and was similar in extent to that previously reported for glucagon enhancement of stimulated inositol phosphate and diacylglycerol formation in hepatocytes (18, 19). Combinations of either NE and (−)-isoproterenol or NE and 8CPT-cAMP did not result in any further increase in mAChR-stimulated inositol phosphate formation, indicating that these agents share a common mechanism and that a maximum potentiation can be achieved with each. Taken collectively, the results from this and previous studies (17–19) indicate that, in some tissue preparations, activation of adenylyl cyclase may enhance rather than inhibit the receptor activation of phospholipase C.

The selective ability of β-adrenergic agonists to enhance Oxo-M-stimulated inositol lipid hydrolysis suggests that activation of a β-adrenergic receptor underlies the observed potentiation. However, some caution is warranted in this regard, because inclusion of 50 μM DL-propranolol resulted in only a partial inhibition of the isoproterenol-mediated potentiation of the Oxo-M response. Propranolol is known to effectively block both β_1- and β_2-adrenergic receptors but is a relatively weak antagonist at "atypical" β-adrenergic receptors (36). The presence of these latter receptors on SK-N-MCIXC cells and their involvement, if any, in the potentiation remain to be determined.

A second conclusion to emerge from the present study is that the effects of cAMP on phosphoinositide hydrolysis in SK-N-MCIXC cells may be compartmentalized. Thus, only mAChR-stimulated inositol phosphate release was enhanced by NE in either SK-N-MCIXC or SK-N-MCIIE subclones, whereas that elicited by either ATP or ET-1 was unaffected (Figs. 1 and 2). In contrast, a generalized increase in cellular cAMP after 8CPT-cAMP administration resulted in an increased activity of all four phosphoinositide-linked receptors (Fig. 8). Previous indications for the compartmentation of cAMP effects on phosphoinositide hydrolysis have been reported. For example, in tracheal smooth muscle, cAMP inhibits histamine- but not

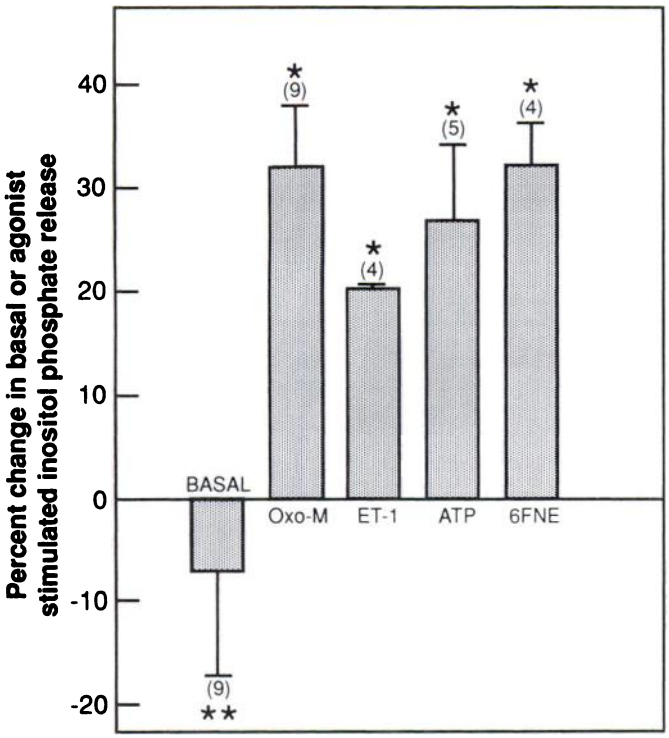

Fig. 8. Effect of 8CPT-cAMP on basal and agonist-stimulated inositol phosphate formation. Prelabeled cells were preincubated for 10 min in the absence or presence of 1 mM 8CPT-cAMP before the addition of either buffer (basal), Oxo-M (1 mM), ET-1 (0.5 μM), ATP (1 mM), or 6-fluoronorepinephrine (*6FNE*) (0.1 mM). Incubations were allowed to proceed for 30 min, and reactions were terminated by the addition of an equal volume of trichloroacetic acid. Results are expressed as percentage of change in either basal or agonist-stimulated inositol phosphate formation for the number of separate experiments indicated in parentheses. *, Different from agonist alone, $p < 0.001$ (matched-pair analysis). **, Different from basal alone, $p < 0.05$ (matched-pair analysis).

carbachol-stimulated inositol lipid hydrolysis (11, 37). In SK-N-SH neuroblastoma cells, only increases in cAMP that were generated independently of adenylyl cyclase-linked receptors resulted in an inhibition of mAChR-stimulated inositol phosphate formation (8). A possible alternative explanation for the results outlined above would be that cyclase- and phosphoinositide-linked receptors exist on different cell populations. This appears less likely in the present study, because a clonal cell line was used. Furthermore, although the release of inositol phosphates cannot be directly measured at the single-cell level, we have determined that the activation of all four phosphoinositide-linked receptors elicits an increase in the concentration of cytoplasmic Ca^{2+} in individual SK-N-MCIXC cells, when monitored by fura-2 microfluorimetry.[1] Thus, at present, it appears more likely that a discrete compartmentation of receptor-mediated increases in cAMP accounts for the differential effects of cAMP on stimulated phosphoinositide hydrolysis. However, direct demonstration of this must await the application of techniques that permit the visualization of localized changes in cAMP mass within single cells (38).

The mechanism whereby cAMP potentiates stimulated inositol lipid hydrolysis remains to be established. Three potential sites of interaction exist, namely, the receptor, G_p, or phospholipase C itself. However, because 8CPT-cAMP inhibits (rather than enhances) basal phospholipase C activity, the ability of the cAMP analog to potentiate receptor-stimulated phosphoinositide hydrolysis indicates an interaction of cAMP at the level of the receptor or G_p. Both the inhibitory and the stimulatory effects of cAMP on phosphoinositide hydrolysis have previously been attributed to a regulation of the G_p-phospholipase C interaction (8, 18, 39). A role for extracellular Ca^{2+} in potentiation is also indicated, because in its absence potentiation of Oxo-M-stimulated inositol phosphate release observed in the presence of NE, (−)-isoproterenol, or 8CPT-cAMP is reduced. Regardless of the mechanism involved, the present results point to similarities in the modes of regulation of adenylyl cyclase and phospholipase C signaling systems. Thus, just as the activation of protein kinase C can regulate adenylyl cyclase either positively or negatively, it appears that protein kinase A may either inhibit or facilitate the activation of phospholipase C.

In summary, in the present study we have demonstrated that an elevation in the concentration of cAMP results in an increased activity of phosphoinositide-linked receptors in human neuroepithelioma cells. Because exogenous but not endogenous cAMP potentiates the activity of all four inositol lipid-linked receptors, we conclude that the effects of cAMP on phosphoinositide hydrolysis may be compartmentalized in these cells.

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Send reprint requests to: Dr. Stephen K. Fisher, University of Michigan, Neuroscience Laboratory, 1103 E. Huron St., Ann Arbor, MI 48104–1687.