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Receptor-Coupled Phosphoinositide Hydrolysis in Human Retinal Pigment Epithelium

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Abstract: Carbachol and histamine stimulated phosphoinositide (PPI) hydrolysis in cultured human retinal pigment epithelium (RPE), as reflected by an accumulation of ^{3}H-inositol phosphates in the presence of 10 m*M* Li^+. Carbachol increased PPI hydrolysis to greater than 600% of basal with an EC_{50} of 60 μM; stimulation was linear up to 60 min. This activation likely occurred via the M_3 muscarinic cholinergic receptor based on the IC_{50} values for 4-diphenylacetoxy-*N*-methylpiperidine methiodide (0.47 n*M*), pirenzepine (280 n*M*), and 11-[[2-[(diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (1.4 μM). Carbachol-mediated PPI hydrolysis was decreased by 80% in the absence of extracellular Ca^{2+}. Histamine stimulated PPI turnover in a linear manner by 180% with an EC_{50} of 20 μM by the H_1 histaminergic receptor. Serotonin, glutamate, norepinephrine, and dopamine were inactive. In human RPE, the resting cytoplasmic Ca^{2+} concentration, as determined by fura-2 fluorescence, was 138 ± 24 n*M*. On the addition of carbachol, there was a 180% increase in peak intracellular Ca^{2+}; addition of histamine increased intracellular Ca^{2+} by 187%. These results suggest receptor-mediated, inositol lipid hydrolysis is coupled to intracellular Ca^{2+} flux in human RPE. **Key Words:** Human pigment retinal epithelium—M_3 muscarinic receptor—H_1 histaminergic receptor—Phosphoinositide hydrolysis—Ca^{2+} activation—Fura-2 AM. **Feldman E. L. et al.** Receptor-coupled phosphoinositide hydrolysis in human retinal pigment epithelium. *J. Neurochem.* **56,** 2094–2100 (1991).

In neural tissues, the interaction of a large number of neurotransmitters and growth factors with their membrane receptors results in phosphoinositide (PPI) hydrolysis (Fisher and Agranoff, 1986). In the eye, nearly all work on this second messenger system has been done on the retina (Cutcliffe and Osborne, 1987; Ghazi and Osborne, 1988), a well-organized structure of ganglion cells, photoreceptors, and supporting cells. Our interest has centered on PPI hydrolysis in one ocular cell type, nontransformed human retinal pigment epithelium (RPE). The RPE, located between the choriocapillaris and the photoreceptors, is responsible for the transport of ions, metabolites, and fluid across the subretinal space to the inner retina (Noell, 1963; Steinberg and Miller, 1979). In this capacity, the RPE maintains the ionic mileau of the subretinal space by active cellular absorption and secretion (Miller and Steinberg, 1977; Hughes et al., 1988, 1989). Disruption of RPE function leads to retinal detachment with fluid accumulation in the subretinal space (Zauberman, 1979; Marmor et al., 1980).

In exocrine cells, PPI hydrolysis plays an active role in cellular secretion by mobilizing intracellular Ca^{2+} stores (Tennes and Putney, 1986). This flux in cytosolic Ca^{2+} alters membrane permeability to ions, promoting exocrine cell fluid and protein transport (Tennes and Putney, 1986; Putney et al., 1989). Ion permeability and fluid transport are altered by chelation of intracellular Ca^{2+} in both exocrine cells (Tennes and Putney, 1986) and RPE (Keller et al., 1986). Agonists, some of which alter RPE ion transport (Edelman and Miller, 1990) and stimulate retinal PPI metabolism (Ghazi and Osborne, 1988; Osborne, 1990) are also coupled to exocrine cell secretion (Tennes and Putney, 1986). These similarities between exocrine cell and RPE function lead us to investigate the possibility of receptor-coupled PPI hydrolysis in cultured human RPE.

We report that carbachol and histamine stimulated both RPE PPI turnover and intracellular Ca^{2+} release by the M_3 (low affinity for pirenzepine) muscarinic acetylcholine receptor and the H_1 (high affinity for mepyramine) histaminergic receptor, respectively. We

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Abbreviations used: AF-DX 116, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one; 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine methiodide; MEM, minimum essential medium; PPI, phosphoinositide; RPE, retinal pigment epithelium; TCA, trichloroacetic acid.

speculate that PPI turnover in human RPE may play an important role in subretinal space fluid homeostasis.

MATERIALS AND METHODS

Chemicals

Minimum essential medium (MEM), Hanks' balanced salt solution, and trypsin-EDTA were purchased from Grand Island Biological (Grand Island, NY, U.S.A.). Bovine calf serum was obtained from Hyclone Labs (Logan, UT, U.S.A.). *myo*-[2-^{3}H]Inositol (15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). Carbamoylcholine (carbachol), atropine, histamine, pyrilamine, D-glucose, HEPES, serotonin, dopamine, and glutamate were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Pirenzepine was obtained from Boehringer Ingelheim (Ridgefield, CT, U.S.A.). Fura-2 AM was obtained from Molecular Probes (Eugene, OR, U.S.A.). Dowex-1 (100–200 mesh; X8 in the formate form) was obtained from BioRad (Richmond, CA, U.S.A.). Tissue-culture supplies were obtained from Costar (Cambridge, MA, U.S.A.) and Corning Glass Works (Corning, NY, U.S.A.). All other chemicals were of reagent grade and were purchased from Baker Chemical (Phillipsburg, NJ, U.S.A.) or Fisher Scientific (Fair Lawn, NJ, U.S.A.). 4-Diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) and 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AF-DX 116) were generous gifts from Dr. Stephen Fisher (The University of Michigan, Ann Arbor, MI, U.S.A.).

Cell culture

Human eyes were obtained postmortem from the Michigan Eye Bank, and the primary RPE cell cultures and lines were established using the modified method of DelMonte and Maumenee (1980). Briefly, the anterior segment, vitreous and neurosensory retina were surgically removed from the eyes and the RPE was gently vacuumed from Bruch's membrane. Cells were distributed evenly into sterile, uncoated, 35-mm culture dishes. Cell attachment and initiation of colony formation was monitored by phase-contrast microscopy.

Cells were periodically subcultured at a density of $3–5 \times 10^6$ cells per 75-cm^2 tissue-culture flask (Corning Glass Works) in MEM containing 20% calf serum. Cells were detached from the flasks by aspirating medium and incubating at 37°C for 30 min in 0.05% trypsin, 0.53 m*M* EDTA. Trypsinization was terminated by addition of MEM supplemented with 20% calf serum; the cell suspension was centrifuged at 430 *g* for 5 min and resuspended in medium. Cells were maintained at 37°C in a humidified 95% O_2/5% CO_2 atmosphere with fresh medium changes three times weekly. The experiments were performed on passage numbers 12–18 of RPE cell lines established from three separate patients.

Assay of PPI turnover

For these studies, 1×10^5 cells were seeded in 3.5-cm culture wells (Costar) in MEM supplemented with 5% calf serum. After 6–7 days in culture, cells were prelabeled for 24 h in serum-supplemented MEM containing 1 μCi/ml of *myo*-[2-^{3}H]inositol, which had been passed through a Dowex-1 (formate form) column to remove polar contaminants. Previous experiments had determined equilibrium labeling at 24 h. At the time of agonist stimulation, the medium was aspirated and the cells washed with 1 ml of buffer A (142 m*M* NaCl, 5.6 m*M* KCl, 2.2 m*M* $CaCl_2$, 3.6 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, and 30 m*M* Na^+ HEPES buffer, pH 7.4). Cells were incubated at 37°C in the presence or absence of various test factors in 1 ml of buffer A containing 5.6 m*M* D-glucose and 10 m*M* lithium chloride for the indicated concentrations and times. The reaction was terminated by aspiration of medium and addition of 1 ml of 10% trichloroacetic acid (TCA). Cells were scraped from each well with a rubber policeman and transferred to a test tube. The TCA extract was washed five times with H_2O-saturated diethyl ether and neutralized with $KHCO_3$. Radioactivity in total inositol phosphates or in the individual inositol phosphate species was determined as described by Berridge et al. (1983).

To determine the incorporation of *myo*-[2-^{3}H]inositol into phospholipids, TCA precipitates were extracted with 1.5 ml of chloroform/methanol (1:2; vol/vol) and 0.5 ml of H_2O (Fisher and Agranoff, 1980) and an aliquot of the lower organic phase was counted after addition of Universol scintillation fluid. When individual inositol lipids were separated on oxalate-impregnated TLC plates with the solvent system chloroform/methanol/acetone/acetic acid/water (90:15:15:12:8; by volume) (Jolles et al., 1981), 94% of the label was incorporated into phosphatidylinositol, 5% into phosphatidylinositol 4-phosphate, and 1% into phosphatidylinositol 4,5-bisphosphate.

In some experiments, after short-term carbachol stimulation, the ether-washed, water-soluble extracts were applied to a Whatman Partisil 10 SAX column for HPLC separation of individual inositol phosphates (Dean and Moyer, 1987). Greater than 90% of the recovered radioactivity was in inositol 1-phosphate; however, a five- to sixfold increase in both inositol 1,4-bisphosphate and inositol 1,3,4-trisphosphate indicated PPI breakdown. Inositol 1,4,5-trisphosphate did not accumulate, presumably secondary due to rapid metabolism, similar to reports in guinea pig brain (Fisher and Bartus, 1985), rat cortex (Heacock et al., 1987), and neuroblastoma cells (Fisher and Cioffi, 1990).

Measurement of intracellular Ca^{2+} concentrations

Cells were loaded with fura-2 AM after a modification of the method of Fisher et al. (1988). Briefly, after aspirating the medium, cells were rinsed, trypsinized as previously described for 15 min, resuspended in MEM containing 20% calf serum, and counted on a hemocytometer. Immediately before loading with fura-2 AM, the cells were resuspended at 1×10^7 cells/ml in buffer A containing 5.6 m*M* D-glucose and gassed for 30 min with 95% O_2/5% CO_2, allowing for recovery after trypsinization.

Cells were loaded at 5×10^6 cells/ml in 2 μ*M* fura-2 AM in buffer A at 37°C for 15 min with 95% O_2/5% CO_2 gassing. The loaded cells were diluted with buffer A to remove excess intracellular fura-2 AM, centrifuged, washed with buffer A two times, and resuspended at 1×10^7 cells/ml in buffer A at room temperature. This lower temperature retards leakage of the fura-2 AM from the dye-loaded cells and decreases compartmentalization of the fura-2 AM into cell organelles (Fisher et al., 1988).

The fluorescence measurements were taken on 1-ml aliquots of cells, stirred, and maintained at 37°C. A Shimadzu spectrophotofluorometer was used ($\lambda_{excitation}$ = 340 nm; $\lambda_{emission}$ = 490 nm) and was interfaced with a chart recorder, and quantitative fluorescence values were obtained from a printout. After measurement of the fluorescence (F) under both resting and drug-induced conditions, the cells were lysed with 50 μ*M* digitonin. Tris base (15 m*M*, pH > 8) was added, and the minimum fluorescence (F_{min}) was obtained after Ca^{2+}

TABLE 1. *Effect of agonists on ^{3}H-inositol phosphate formation in human RPE*

Agonist	Maximum concentration used (M)	% of basal value (n)
Carbachol[a]	10^{-2}	673 ± 64 (4)
Histamine[a]	10^{-3}	179 ± 10 (5)
Serotonin[b]	10^{-3}	90 ± 9 (2)
Glutamate[b]	10^{-4}	103 ± 2 (4)
Norepinephrine[b]	10^{-3}	103 ± 3 (2)
Dopamine[b]	10^{-3}	103 ± 2 (3)

Cultured human RPE were incubated with *myo*-[^{3}H]inositol for 24 h, rinsed, and stimulated with the appropriate agent. Data are expressed as the percentage of accumulation of ^{3}H-inositol phosphates, where 100% is defined as accumulation in the absence of agonist. Each value represents the average ± SEM of two to five separate experiments (n), where each determination was done in triplicate.

[a] Statistically significant difference from control values at $p < 0.01$.

[b] Nonsignificant values ($p > 0.05$), using the Student's t test.

chelation with 10 mM EGTA. The maximum fluorescence (F_{max}) was obtained by adding 10 mM $CaCl_2$ and autofluorescence (F_{auto}), by adding 20 mM $MnCl_2$. Intracellular Ca^{2+} concentrations were calculated from the equation $(F - F_{min})/(F_{max} - F)K_D$, where K_D is the affinity of fura-2 AM for Ca^{2+} at 37°C and is ~224 nM (Tsien et al., 1982; Grynkiewicz et al., 1985).

RESULTS

Agonist-stimulated PPI turnover

Table 1 presents the effects of multiple agonists, tested at the indicated concentrations, on PPI turnover in human RPE. Among these agents, only carbachol and histamine stimulated ^{3}H-inositol phosphate formation by 673 and 179%, respectively. Carbachol- and histamine-stimulated PPI hydrolysis remained linear up to 60 min (Fig. 1). Atropine (10^{-6} M) blocked the carbachol effect by >90%, whereas pyrilamine (10^{-7} M) completely eliminated the histamine effect (data not shown). The dose–response curve for carbachol-stimulated PPI hydrolysis indicates an EC_{50} of 60 μM, whereas that for histamine gives an EC_{50} of 20 μM (Fig. 2).

The effect of extracellular Ca^{2+} on PPI hydrolysis was determined. Stimulation of RPE by 1 mM carbachol in Ca^{2+}-free buffer A (no exogenous Ca^{2+} added) for 30 min resulted in a 70% decrease of inositol phosphate accumulation. Under these same conditions, further addition of 0.5 mM EGTA decreased inositol phosphate accumulation by 78% (Fig. 3).

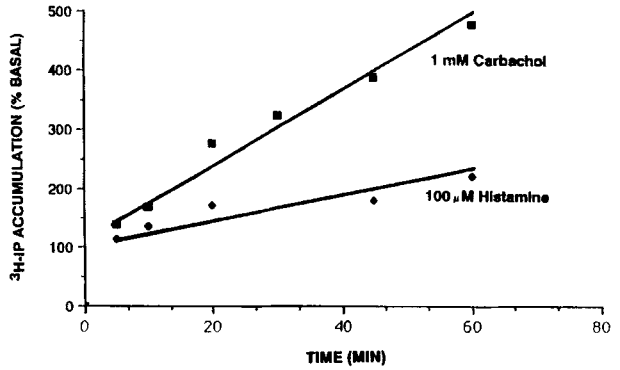
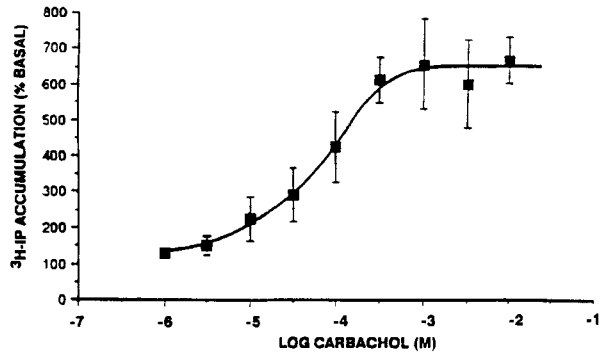


FIG. 1. Time course for agonist-stimulated PPI hydrolysis in cultured human RPE. Cultured human RPE were incubated with *myo*-[^{3}H]inositol for 24 h, rinsed, and stimulated with either 1 mM carbachol or 100 μM histamine for 0–60 min. Labeled inositol phosphates (^{3}H-IP) were extracted as described in Materials and Methods. Results are expressed as the percentage of accumulation of ^{3}H-IP, where 100% is defined as the accumulation in the absence of agonist (7,075 ± 386 cpm). Results shown are the mean of three replicates from one of three experiments that gave similar results.



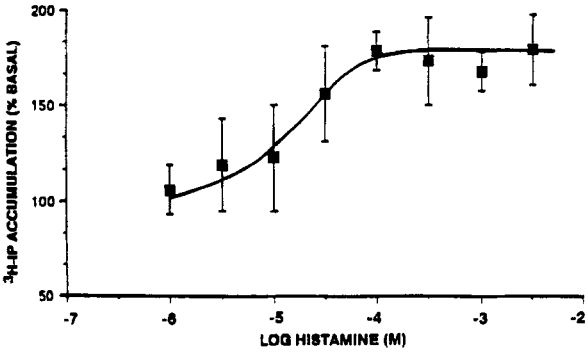


FIG. 2. Dose–response curves for agonist-stimulated PPI hydrolysis in human RPE cells. Cultured human RPE were incubated with *myo*-[^{3}H]inositol for 24 h, rinsed, and stimulated with increasing concentrations of agonist in buffer A. Labeled inositol phosphates (^{3}H-IP) were extracted as described in Materials and Methods. The EC_{50} values for carbachol and histamine (calculated by GraphPad InPlot) were 60 μM and 20 μM, respectively. Results shown are the mean ± SEM (bars) of the percentage of basal ^{3}H-IP formation from four independent experiments, where each determination was done in triplicate.

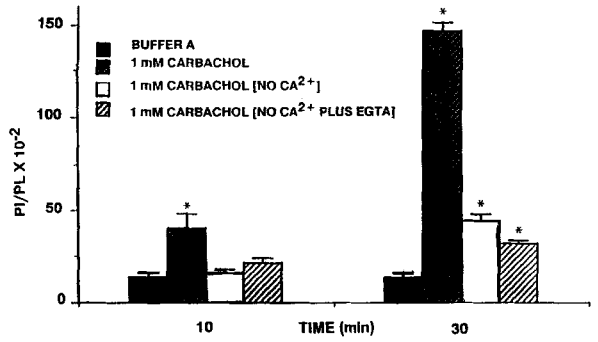


FIG. 3. Carbachol stimulation of phosphoinositide turnover is dependent on extracellular Ca^{2+}. Cultured human RPE were incubated with *myo*-[^{3}H]inositol for 24 h, rinsed, and stimulated with buffer A alone, buffer A plus 1 m*M* carbachol, buffer A minus Ca^{2+} plus 1 m*M* carbachol, or buffer A minus Ca^{2+} plus 0.5 m*M* EGTA and 1 m*M* carbachol. Results are expressed as the ratio of dpm in ^{3}H-inositol phosphate/^{3}H-lipid $\times 10^{-2}$ and are the mean of three replicates ± SEM from one of two experiments that gave similar results. *$p < 0.01$, compared with buffer A alone by Student's *t* test.

Characterization of muscarinic receptor subtype

The identity of the subtype of muscarinic receptor responsible for carbachol-stimulated PPI hydrolysis in human RPE cells was explored by using three selective muscarinic antagonists with variable affinities for muscarinic receptor subtypes. Pirenzepine, an M_1-selective (high affinity for pirenzepine) antagonist, can differentiate the M_1 subtype from the M_2 and M_3 (low affinity for pirenzepine) subtypes (Doods et al., 1987), whereas the latter can be distinguished by their relative affinities for 4-DAMP ($M_3 \gg M_2$) and AF-DX 116 ($M_2 \gg M_3$) (Doods et al., 1987). The carbachol-stimulated PPI turnover in human RPE cells was inhibited by all three antagonists at concentrations that reflected each antagonist's affinity for the M_3 muscarinic acetylcholine receptor (Fig. 4). Apparent K_i values calculated from the method of Cheng and Prusoff (1973) for each drug were as follows: 4-DAMP, 0.47 ± 0.28 n*M*; pirenzepine, 216 ± 91 n*M*; and AF-DX 116, 1.4 ± 0.7 μM. The results suggest that the M_3 muscarinic receptor subtype mediates carbachol-stimulated PPI metabolism in human RPE.

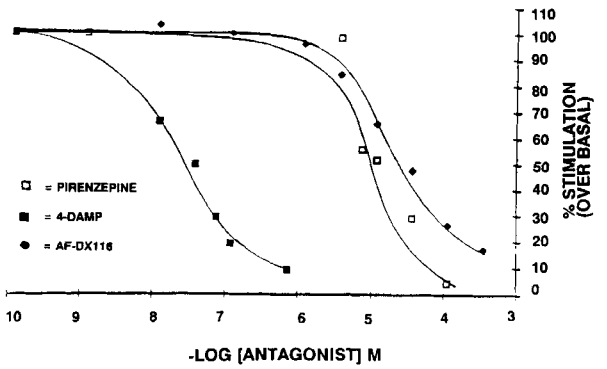


FIG. 4. Inhibition of carbachol-stimulated PPI hydrolysis in human RPE by selected muscarinic antagonists. Cultured human RPE were incubated with *myo*-[^{3}H]inositol for 24 h, rinsed, and stimulated with 1 m*M* carbachol alone or in the presence of 4-DAMP (■), pirenzepine (□), and AF-DX 116 (♦) at the concentrations shown. Reactions were terminated after 40 min and total ^{3}H-inositol phosphates were extracted as described in Materials and Methods. Each value represents a mean of three replicates from one of two experiments that gave similar results. IC_{50} values, computed from GraphPad InPlot, were 0.47 n*M*, 280 n*M*, and 1.4 μM, respectively. Calculated Hill coefficients were 0.99, 0.90, and 0.98, respectively.

Measurement of cytoplasmic Ca^{2+} concentration in quiescent and agonist-stimulated RPE

Both basal and stimulated intracellular Ca^{2+} levels were measured using fura-2 AM-loaded RPE. The overall basal intracellular Ca^{2+} was determined to be 138 ± 24 n*M* (n = 71). Carbachol and histamine stimulated peak intracellular Ca^{2+} values in fura-2 AM-loaded RPE. Figure 5 illustrates responses to 1 m*M* carbachol and histamine. After a stable baseline was determined (~1.5 min), agonist was introduced and a peak value reached within a 2–3-s time span, followed by a slow decrease to a plateau value greater than that of the baseline level. The initial rise in Ca^{2+} was de-

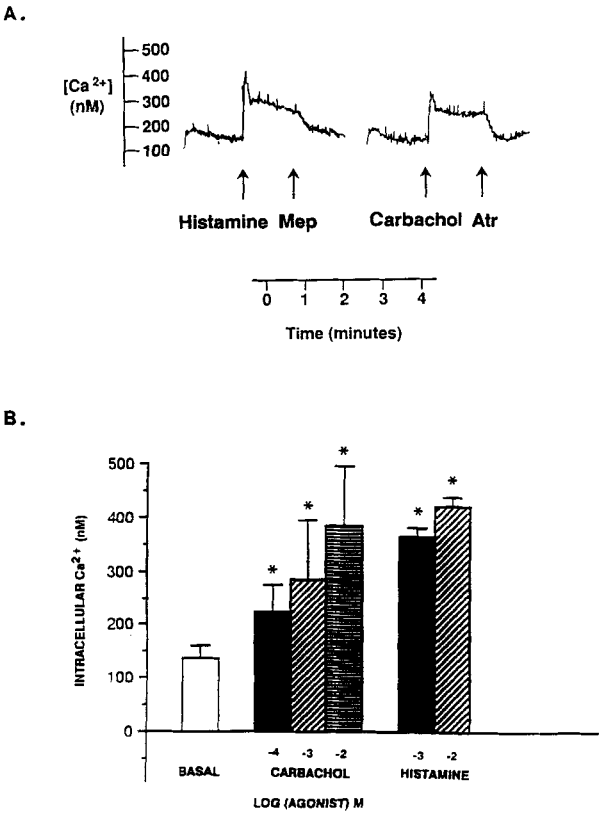


FIG. 5. Concentration-dependent increase in intracellular Ca^{2+} by carbachol and histamine. **A:** Cells were loaded with 1 μM fura-2 AM at 37°C for 15 min. For each trace, the initial arrow indicates the addition of 1 m*M* agonist, whereas the second arrow indicates the addition of the corresponding inhibitor, 10 μM mepyramine (Mep) or 10 μM atropine (Atr). **B:** The peak Ca^{2+} values achieved with agonist stimulation. Results shown are mean ± SEM for six separate experiments. *$p < 0.01$, compared with basal intracellular Ca^{2+} as calculated with the Student's *t* test.

pendent on the concentration of the agonist, but the plateau value varied little from 100 μM to 1 mM carbachol and 100 μM to 1 mM histamine (data not shown). The cumulative data for peak values are shown in Fig. 5. Carbachol (10 mM) elicited a 179 ± 37% (n = 16) increase in intracellular Ca^{2+}, whereas histamine (1 mM) increased the intracellular Ca^{2+} by 187 ± 10% (n = 6). When extracellular Ca^{2+} was decreased to 300 nM by addition of 4 mM EGTA, resting intracellular Ca^{2+} was reduced to 81 ± 17 nM (n = 10). This reduction in extracellular Ca^{2+} did not effect the rapid increase in intracellular Ca^{2+} after 10 mM carbachol stimulation (205 ± 18%, n = 4); however, the plateau phase was shortened to <90 s.

DISCUSSION

The RPE plays an active role in fluid mobilization, regulating the volume and composition of the subretinal space. Active transmembrane exchange in RPE of sodium, potassium, and chloride provides the driving force for RPE fluid transport (Hughes et al., 1988, 1989). In exocrine cells, increased membrane permeability to these same ions also results in cellular transport of protein and/or fluid. Exocrine cells possess muscarinic cholinergic receptors that, when activated, stimulate PPI hydrolysis, producing cytosolic Ca^{2+} flux and changes in membrane permeability to sodium, potassium, and chloride (Tennes and Putney, 1986). The strong similarities between RPE and exocrine cell fluid transport lead us to speculate that inositol lipid turnover and cytosolic Ca^{2+} flux occur in RPE in response to muscarinic cholinergic stimulation. In human RPE, 300 μM carbachol stimulated PPI turnover >600% of basal with a calculated EC_{50} of 60 μM. This compares favorably with activation of muscarinic cholinergic receptors in multiple exocrine cell types, including mouse (Powers et al., 1985) and rat pancreas (Rubin et al., 1984), rat parotid (Aub and Putney, 1985; Irvine et al., 1985), and rabbit gastric mucosa (Baudiere et al., 1986).

Five distinct muscarinic cholinergic receptors have been cloned (Bonner, 1989). When transfected into mammalian cells, M_1, M_3, and M_5 can successfully couple cholinergic agonists to PPI hydrolysis (Bonner et al., 1988). Pharmacologically, receptors can be differentiated by their relative affinity for specific antagonists. M_1 receptors have a high affinity for pirenzipine, M_2 for AF-DX 116, and M_3 for 4-DAMP (Doods et al., 1987). The K_i values of these selective muscarinic antagonists in human RPE implied that carbachol stimulated PPI hydrolysis through the M_3 receptor. Exocrine pancreas (Iwatsuki et al., 1989) and parotid gland (Dehaye et al., 1988) contain the M_3 receptor coupled to agonist-stimulated PPI hydrolysis and cellular secretion. In a similar pattern, carbachol stimulated PPI turnover by the M_3 receptor in human ocular ciliary epithelial cells (Wax and Coca-Orados 1989; Honkanen et al., 1990). These cells, exocrine in nature, manufacture aqueous humor, ocular fluid with a similar ionic composition to subretinal fluid, produced by the RPE (Zauberman, 1979).

In the RPE, carbachol-induced PPI hydrolysis was partially dependent on extracellular Ca^{2+}. In the absence of extracellular Ca^{2+}, carbachol-stimulated PPI turnover was decreased by ~80%. Permeabilized pancreatic acinar cells require Ca^{2+} for agonist-stimulated inositol phosphate formation (Taylor et al., 1986), and Ca^{2+} can modulate PPI turnover in exocrine pancreas and lacrimal glands (Taylor et al., 1986; Godfrey and Putney, 1984). These data and our own support the idea that phospholipase C activation may be Ca^{2+} sensitive (McDonough et al., 1988; Eberhard and Holz, 1988; Fisher et al., 1988) or that extracellular Ca^{2+} is required for efficient agonist-receptor interaction.

Histamine-coupled PPI hydrolysis is not well characterized in exocrine cells. We found, however, in human RPE, histamine stimulated PPI turnover of 179 ± 10% (n = 5) with an EC_{50} of 20 μM. A nearly identical accumulation (180%) was reported with histamine stimulation of PPI metabolism in rabbit retina (Nowak et al., 1989). The histaminergic response in human RPE likely occurred by the H_1 receptor because mepyramine, a selective H_1 antagonist, completely blocked histamine-stimulated PPI metabolism. This receptor subtype is present in bovine (Nowak and Maslinski, 1986) and rabbit (Nowak et al., 1989) retina. In a similar pattern in the CNS, H_1 receptors are linked to CNS inositol lipid turnover, especially in the cerebellum and hypothalamus, whereas cyclic AMP accumulation occurs by the H_2 receptor (Fisher and Agranoff, 1986). The mepyramine-insensitive H_2 receptor has been reported in cultured chick RPE where, as predicted, it mediated cyclic AMP accumulation (Koh and Chader, 1984).

Agonists that are known to stimulate either PPI hydrolysis or cyclic AMP accumulation in neural tissues (Fisher and Agranoff, 1986), but not exocrine cells (Tennes and Putney, 1986), did not enhance PPI turnover in human RPE. There was no effect of norepinephrine on human RPE PPI hydrolysis, whereas cultured human RPE have β-adrenergic receptors linked to adenylate cyclase turnover (Friedman et al., 1988). Similarly, dopamine, known to modulate adenylate cyclase activity in the retina (O'Connor et al., 1989), did not stimulate PPI turnover in human RPE. There was no evidence of PPI hydrolysis with glutamate or its excitatory amino acid agonist, quisqualic acid (data not shown), in human RPE, although it is well known that glutamate and quisqualic acid are coupled to PPI hydrolysis in rat cortex (Schoepp and Johnson, 1988). Serotonin has a modest effect on inositol phosphate accumulation in rabbit retinal cultures (Ghazi and Osborne, 1988) and rabbit retinal slices (Cutcliffe and Osborne, 1987), but had no effect on human RPE.

In human RPE, using fura-2 AM, basal intracellular Ca^{2+} was 138 ± 24 nM (n = 71), one-half the reported value obtained with the fluorescent indicator quin-2

(Friedman et al., 1988). Carbachol and histamine resulted in a rapid increase in the concentration of cytoplasmic Ca^{2+}. This increase was followed by a slower decline that plateaued above the basal intracellular Ca^{2+} until the agonist was displaced by the appropriate antagonist. A similar pattern of Ca^{2+} flux has been reported for both exocrine (Chew and Brown, 1986; Merritt and Rink, 1987; Sung et al., 1988) and neural cells (Fisher et al., 1988).

In summary, carbachol and histamine stimulated inositol lipid turnover and intracellular Ca^{2+} flux in human RPE by the M_3 muscarinic acetylcholine receptor and the H_1 histaminergic receptor, respectively. Cholinergic mediated PPI hydrolysis was dependent on extracellular Ca^{2+}. We speculate that inositol lipid hydrolysis in RPE may play a pivotal role in regulating the volume and ionic composition of the subretinal space.

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