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Loss of platelet-derived growth factor-stimulated phospholipase activity in NIH-3T3 cells expressing the EJ-*ras* oncogene

(prostaglandin E_2/transformation/guanine nucleotide-binding protein)

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ABSTRACT **Data indicating that the 21-kDa protein (p21) Harvey-*ras* gene product shares sequence homology with guanine nucleotide-binding proteins (G proteins) has stimulated research on the influence(s) of p21 on G-protein-regulated systems in vertebrate cells. Our previous work demonstrated that NIH-3T3 mouse cells expressing high levels of the cellular *ras* oncogene isolated from the EJ human bladder carcinoma (EJ-*ras*) exhibited reduced hormone-stimulated adenylate cyclase activity. We now report that in these cells another enzyme system thought to be regulated by G proteins is inhibited, namely phospholipases A_2 and C. NIH-3T3 cells incubated in plasma-derived serum release significant levels of prostaglandin E_2 (PGE_2) as determined by radioimmunoassay when exposed to platelet-derived growth factor (PDGF) at 2 units/ml; the levels of PGE_2 released from EJ-*ras*-transfected cells are only 3% those of controls despite a similar basal (unstimulated) release from control and EJ-*ras*-transfected cells. The lack of PDGF-stimulated PGE_2 release from EJ-*ras*-transfected cells is not due to a defect in the prostaglandin cyclooxygenase enzyme, since incubation of control cells and EJ-*ras*-transfected cells in 0.33, 3.3, or 33 μM arachidonate resulted in identical levels of PGE_2 release. The lack of PDGF-stimulated PGE_2 release from EJ-*ras*-transfected cells also does not result from the loss of functional PDGF receptors. EJ-*ras*-transformed cells bind 70% as much ^{125}I-labeled PDGF as control cells and are stimulated to incorporate [^{3}H]thymidine and to proliferate after exposure to PDGF. Moreover, this inhibition is not likely the result of a secondary cellular effect related to the transformed phenotype, since NIH-3T3 cells transformed by v-*src* released PGE_2 at wild-type levels after exposure to PDGF. Determination of total water-soluble inositolphospholipids and changes in the specific activities of phosphatidylcholine in control and EJ-*ras*-transfected cells demonstrated that PDGF-stimulated phospholipase C and A_2 activities are inhibited in the EJ-*ras*-transfected cells.**

The *ras* genes are a highly conserved gene family in eukaryotic cells. Three human *ras* genes have presently been characterized. Harvey-*ras* (Ha-*ras*) and Kirsten-*ras* (Ki-*ras*) are related to acutely transforming murine sarcoma retroviruses, and the third, N-*ras*, was isolated from a human neuroblastoma (1–4).§ *ras* genes encode proteins with molecular masses of 21 kDa (p21s). Point mutations in the cellular-*ras* genes (c-*ras*) at amino acids 12, 13, or 61 confer on p21 the ability to transform some cell types in culture (6–10). Although mutations in c-*ras* that activate its transforming potential are known, the functional change(s) in mutated p21 that mediate the transformation are not known. It is known that both mutated and normal p21 bind GTP with similar affinity and display GTPase activity. In many cases, mutated p21 has been reported to have lower GTPase activity compared with normal p21 (11–14).

Previous work from our laboratory showed that both the basal and hormone-stimulated adenylate cyclase activities were reduced in NIH-3T3 cells expressing the mutated *ras* gene from the human EJ bladder carcinoma (EJ-*ras*) (17). We also found a decrease in adenylate cyclase activity in cells expressing high levels of the normal c-*ras* gene. These data supported the concept that both normal and mutated p21 are related to mammalian G proteins (17). We now report that another vertebrate system thought to be regulated by G proteins, hormone-sensitive phospholipase activity, is also inhibited in cells expressing high levels of the EJ-*ras* gene. Thus, platelet-derived growth factor (PDGF) stimulated prostaglandin E_2 (PGE_2) and arachidonate release from EJ-*ras*-transformed NIH-3T3 cells is markedly reduced compared to that in control cells. These data, together with those previously reported (17), are consistent with the hypothesis that the biology of *ras* results in part from an overall dampening of G-protein-regulated systems in the vertebrate cell.

MATERIALS AND METHODS

The following materials were obtained from the indicated sources: Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, and trypsin/EDTA in Hanks' balanced salt solution (Irvine Scientific); penicillin/streptomycin and Hanks' balanced salt solution (HBSS) (GIBCO); nerve growth factor (NGF) and epidermal growth factor (EGF) (Collaborative Research, Waltham, MA); recombinant interleukin 1β (IL-1β) (Upjohn); highly purified PDGF was purchased from Collaborative Research, was purified in our laboratory (18) or was the kind gift of Thomas Deuel (St. Louis, MO); arachidonic acid (Nu-Chek Prep); [^{3}H]PGE_2, [^{3}H]arachidonic acid, and *myo*-[^{3}H]inositol (New England Nuclear); ^{125}I-labeled PDGF was prepared as described (18). PGE_2 antisera was provided to us by F. A. Fitzpatrick (Upjohn). Plasma-derived serum (PDS) was prepared from freshly drawn human blood as described by Habenicht *et al.* (19).

Cells and DNA Transfections. NIH-3T3 mouse fibroblasts were maintained in DMEM containing 10% calf serum (EC-10). Cells expressing the neomycin-resistance gene were selected in geneticin (GIBCO) at 0.4–1 mg/ml. Plasmids pEJ and pbc-N1, which are pBR322 derivatives that carry the EJ

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Abbreviations: PGE_2, prostaglandin E_2; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; NGF, nerve growth factor; IL-1β, interleukin 1β; G protein, guanine nucleotide-binding protein; PDS, plasma-derived serum; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; PtdInsP_2, phosphatidylinositol 4,5-bisphosphate.

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§The symbols *HRAS*, *KRAS*, and *NRAS* have been recommended for these genes in humans (5).

human bladder carcinoma oncogene and the normal human c-Ha-*ras* allele, respectively, were obtained from the American Type Culture Collection. pSRA-2, a permuted clone of Rous sarcoma virus DNA, was obtained from M. Bishop (University of California). pUCNeo is a pBR322 derivative that carries the neomycin-resistance gene from transposon Tn5 and the long terminal repeat from the Harvey murine sarcoma virus; neomycin resistance was used as the selectable gene in cotransfection experiments (17). Transfection of NIH-3T3 cells with cloned DNA was carried out by the calcium phosphate coprecipitation technique, and expression of the c-*ras* and EJ-*ras* genes in transfected cells was confirmed by immunoblotting as described (17).

For cell growth experiments, the cells were grown in 1.5 ml of EC-10 in 35-mm culture wells in the presence of PDGF. At the appropriate times, trypsin was added to the cultures and the cells were collected and pelleted by low-speed centrifugation. The cell pellets were gently resuspended in fresh EC-10 and the cell numbers were determined by using a Coulter Counter, model ZBI.

Metabolism of Arachidonic Acid. The metabolism of arachidonic acid in cells was studied by measuring PGE_2 levels by radioimmunoassay (RIA) as described (20). Cells were grown in EC-10 in 35-mm culture dishes. Twenty-four hours prior to an experiment, the EC-10 was replaced with DMEM containing 1.25% PDS. To begin the experiment the DMEM/PDS medium was replaced with fresh DMEM containing PDGF at 2–5 units/ml in 0.1 M acetic acid. Control plates received DMEM containing only an equivalent amount of acetic acid. Medium was collected at various times and frozen at −70°C until analysis by RIA. PDGF content is expressed in units as defined by Antoniades *et al.* (21).

Exogenous arachidonate metabolism was studied by adding 0.33–33.0 μM arachidonic acid to cultures treated as described above, except no PDGF was added. Medium was collected and frozen at −70°C, and the PGE_2 concentrations were determined by RIA.

Determination of Water-Soluble Inositolphospholipid Levels in Cells. The levels of water-soluble inositol phosphates generated in cells after PDGF stimulation were determined as described by Berridge *et al.* (22). Cells were grown in EC-10 in 35-mm culture wells to about 75% confluency. The medium was removed, 1.5 ml of DMEM/PDS containing 3 μCi (1 Ci = 37 GBq) of *myo*-[^{3}H]inositol was added to each well, and the cells were incubated at 37°C for 24 hr. The cells were washed three times with HBSS and then incubated for 1 hr at 37°C in 1.5 ml of HBSS. The HBSS was removed, the cells were washed once, and 1 ml of HBSS was added. The cultures were incubated at 37°C for 15 min and then 0.1 M acetic acid (controls) or 0.1 M acetic acid containing PDGF (final concentration 2–5 units/ml) was added. The reactions were terminated by adding 5 ml of 15% trichloroacetic acid to each well followed by incubation at 4°C for 30 min. The trichloroacetic acid solutions were removed and the cells were further washed two times with an equal volume of distilled water. Inositol tris[^{32}P]phosphate (provided by Rita Huff, Upjohn) was added as an internal standard, and the trichloroacetic acid solutions were extracted four times with an equal volume of water-saturated diethyl ether. After extraction, the aqueous samples were applied to a 1.5-ml Dowex anion-exchange columns (formate form). The water-soluble metabolites were eluted and the levels of [^{3}H]inositolphospholipids in the samples were determined by scintillation counting.

Thin-Layer and Two-Dimensional Paper Chromatography of Phospholipids. The release of [^{3}H]arachidonic acid in response to PDGF was followed by both thin-layer chromatography (TLC) and two-dimensional paper chromatography. Cells were grown in 100-mm culture dishes to about 75% confluency. The medium was removed and replaced with 4 ml of DMEM/PDS containing 1 μCi of [^{3}H]arachidonic acid, and the cells were incubated at 37°C for 18 hr. The cultures were washed twice with 5 ml of DMEM, and finally 4 ml of DMEM/PDS was added to each culture. At this point (time 0) PDGF in 0.1 M acetic acid (final concentration 2 units/ml) or an equivalent volume of 0.1 M acetic acid (without any PDGF) was added to matched triplicate sets of the cultures. The reactions were terminated by the addition of 5 ml of methanol chilled to 4°C, and the lipids were extracted by the method of Bligh and Dyer (23). The resulting extracts were taken to dryness under nitrogen and resuspended in benzene. One half of the sample was then used for TLC analysis and the remaining half was used for two-dimensional paper chromatography as previously described (24, 25). Phospholipids were stained with 0.001% rhodamine 6G, visualized under UV light at 366 nm, and cut out and quantitated in a liquid scintillation spectrometer. The papers were removed from the scintillant and digested in 70% perchloric acid/0.01% ammonium molybdate and then phosphorus was determined (26). Data were calculated as specific activity (cpm/μg of phosphorus per 10^7 cells) and presented as a percent of the zero time value.

RESULTS

NIH-3T3 Cells Transfected with EJ-*ras* DNA Release Only Low Levels of PGE_2 After PDGF Stimulation. Control NIH-3T3 cells (transfected with only calf thymus DNA and pUCNeo DNA) grown for 24 hr in 1.25% PDS release significant amounts of PGE_2 as determined by RIA, when exposed to PDGF at 2 units/ml (Fig. 1). The presence of PGE_2 in the culture medium is observed within minutes of PDGF addition, with maximal levels occurring 2 hr after exposure. In contrast, NIH-3T3 cells transfected with EJ-*ras* and pUCNeo DNA release only low levels of PGE_2 in response to PDGF; after 2 hr these cells have released only 3% as much PGE_2 as controls. This is despite the fact that the basal levels of PGE_2 release in EJ-*ras*-transformed cells were

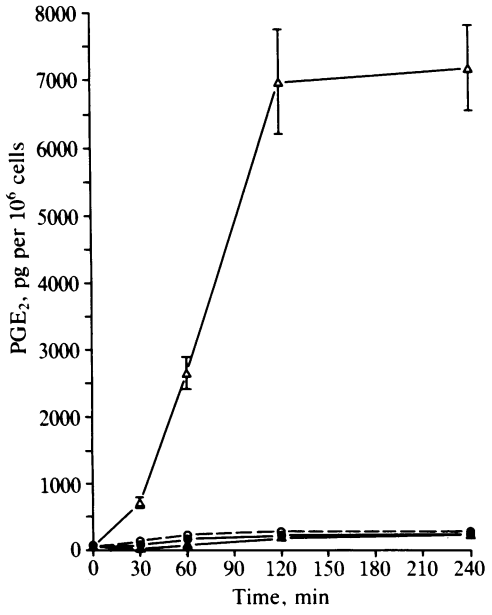

FIG. 1. PDGF-stimulated PGE_2 release in control and EJ-*ras*-transformed cells. NIH-3T3 cells (approximately 1.5×10^6 cells per 35-mm well) were incubated for 24 hr in 1.25% PDS and then stimulated with PDGF at 2.0 units/ml. PGE_2 was quantitated by RIA at the indicated times. Data are reported as mean ± SEM of triplicate determinations. ▲, Control basal; △, control + PDGF; ●, EJ-*ras*-transformed basal; ○, EJ-*ras*-transformed + PDGF.

not significantly different from those in the control cells (Fig. 1). Similar data were obtained with all preparations of PDGF.

To study why cells transformed by EJ-*ras* DNA release only low levels of PGE_2 after PDGF stimulation, we initially examined the cyclooxygenase enzyme activities in these cells. Exposure of both control and EJ-*ras*-transfected cells to 0.33, 3.3, or 33 μM arachidonate resulted in identical levels of PGE_2 release into the culture media (Fig. 2). These data indicated that the cyclooxygenase enzyme capacities of these cells were equivalent. Thus, the defect in the EJ-*ras* DNA-transformed cells in PDGF-stimulated PGE_2 biosynthesis occurs at an earlier point in the biochemical pathway than the cyclooxygenase enzymic conversion of arachidonic acid to PGE_2. The defect(s) in the EJ-*ras*-transformed cells may involve, therefore, cellular phospholipase(s), the PDGF receptor, or both.

Although it is apparent that the growth of EJ-*ras*-transfected cells is not completely arrested in DMEM/PDS, their growth rate does increase to an extent similar to that of the control cells after the addition of PDGF (Fig. 3). Thus, the numbers of both control and EJ-*ras*-transfected cells increased about 2-fold 48 hr after PDGF addition to the cultures. Transforming growth factor β (β-TGF), NGF, EGF, or recombinant IL-1β did not induce a similar mitogenic or PGE_2-stimulatory response in control or EJ-*ras*-transformed cells maintained in PDS. Control and EJ-*ras*-transfected cells also exhibited a 494 ± 89% and a 185 ± 24%, respectively, increase in [^{3}H]thymidine incorporation into their DNA after exposure to PDGF (data not shown). In addition, EJ-*ras*-transfected cells bound 70% as much ^{125}I-labeled PDGF as control cells (0.85×10^5 sites per cell vs. 1.22×10^5 sites per cell); these cells also exhibited the same PDGF dissociation constant (17 ± 0.2 nM). These data indicate that the EJ-*ras*-transformed cells have functional PDGF receptors.

Reduced Levels of PGE_2 Release from PDGF-Stimulated NIH-3T3 Cells Expressing the EJ-*ras* Oncogene Compared to Cells Expressing the Normal Human c-*ras* Gene or the v-*src* Gene. To investigate whether the low level of PGE_2 released from cells after PDGF stimulation was specific to cells expressing the EJ-*ras* gene, we determined the levels of PDGF-stimulated PGE_2 release from cells after transfection with the normal c-*ras* gene DNA or with Rous sarcoma virus DNA (Fig. 4). Unlike NIH-3T3 cells transformed with the EJ-*ras* gene, cells that were transfected with the c-*ras* gene displayed only a slightly reduced level of PDGF-stimulated PGE_2 release; these levels were consistently lower than those released from control cells but significantly higher than those from the EJ-*ras*-transformed cells. The difference in the levels of PDGF-stimulated PGE_2 release from cells transfected with EJ-*ras* DNA compared to cells transfected with the c-*ras* DNA are not accounted for by differences in the

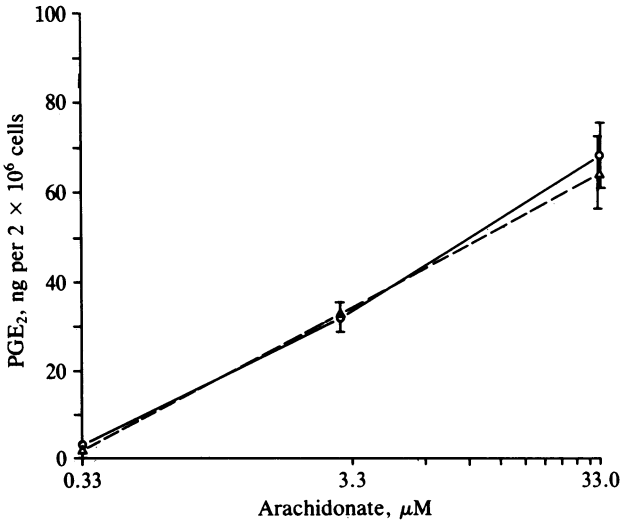

FIG. 2. Arachidonic acid-stimulated PGE_2 synthesis in control and EJ-*ras*-transformed cells. Cells were incubated for 24 hr in 1.25% PDS and then exposed to 0.33, 3.3, or 33.0 μM arachidonic acid for 30 min at 37°C. The medium was then harvested and PGE_2 was quantitated by RIA. Data are presented as the mean ± SEM of triplicate determinations. ○, Control; △, EJ-*ras*-transformed.

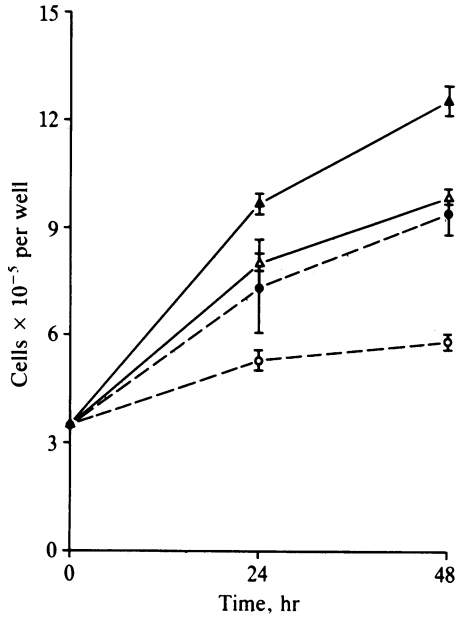

FIG. 3. Proliferation of control and EJ-*ras*-transformed cells in response to PDGF. Cells (3.2×10^5 cells per 35-mm well) were grown for 24 hr in 1.25% PDS and then exposed to PDGF at 5 units/ml. Cells were counted at 24 and 48 hr after addition. Data are reported as the mean ± SEM of triplicate wells. ○, Control basal; ●, control + PDGF; △, EJ-*ras*-transformed basal; ▲, EJ-*ras*-transformed + PDGF.

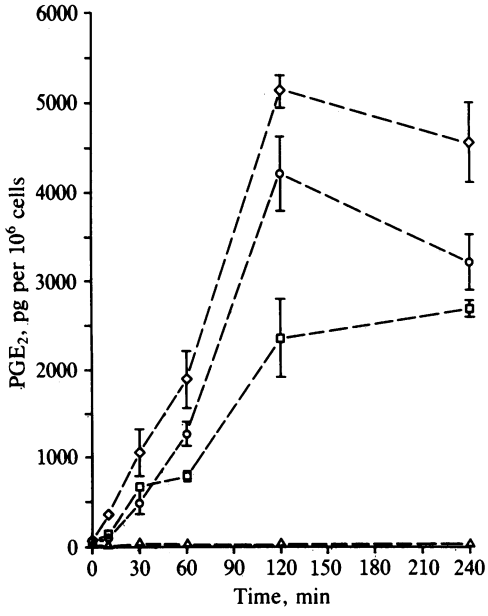

FIG. 4. PDGF-stimulated PGE_2 release from control, EJ-*ras*-, v-*src*-, and c-*ras*-transfected cells. Cells ($0.6–1 \times 10^6$ cells per 35-mm well) were grown for 24 hr in 1.25% PDS and then stimulated by PDGF at 2.0 units/ml. PGE_2 levels were quantitated by RIA at the indicated times. Data are presented as mean ± SEM of triplicate determinations. ○, Control + PDGF; ◇, v-*src*-transfected + PDGF; □, c-*ras*-transfected + PDGF; △, EJ-*ras*-transfected + PDGF.

levels of expression of the *ras* genes in the transfected cells. Immunoblots of the total protein found in cell lysates of the EJ-*ras*- and c-*ras*-transfected cells indicated that these cells were synthesizing equivalent levels of p21 (data not shown).

Slightly higher levels of PDGF-stimulated PGE_2 release were found compared with controls in experiments with cells transfected by Rous sarcoma virus DNA (which carries the v-*src* oncogene) (Fig. 4). The cells used in these experiments were morphologically transformed, expressed v-*src* RNA as determined by dot blot analysis using a v-*src*-specific probe, and formed macroscopic colonies in soft agar (data not shown). These data indicate that the inability of NIH-3T3 cells expressing high levels of the EJ-*ras* gene to respond to PDGF-stimulated PGE_2 biosynthesis is not a general property of transformed cells expressing an oncogene.

PDGF-Stimulated Phospholipase A_2 and C Enzymic Activities Are Markedly Reduced in EJ-*ras*-Transfected NIH-3T3 Cells Compared to Control Cells. The above results suggested that mutated *ras* p21 may interfere with the stimulation of cellular phospholipase activity by PDGF. To investigate this further, we labeled phospholipid pools by incubating control cells and cells expressing either the EJ-*ras* or the c-*ras* gene with [^{3}H]arachidonate. After labeling, the cells were exposed to PDGF at 2.5 units/ml for 1 hr at 37°C, and PGE_2 and arachidonic acid were quantitated as described in *Materials and Methods* (Table 1).

Consistent with our measurements of PGE_2 released from control and EJ-*ras*-transfected cells after PDGF stimulation presented above, these data indicated that both the basal and PDGF-stimulated levels of PGE_2 and arachidonate synthesized in EJ-*ras*-transfected cells were lower than the corresponding levels in control cells (Table 1). Cells transfected with c-*ras* DNA also showed reduced basal and PDGF-stimulated PGE_2 and arachidonate release when compared to control cells; these levels were increased after PDGF stimulation and were consistently higher than the levels synthesized in the EJ-*ras*-transformed cells (Table 1).

To assess whether the phospholipase C enzymic activities were reduced in the *ras*-transfected cells, we labeled cells with *myo*-[^{3}H]inositol and then determined the levels of water-soluble inositolphospholipids after PDGF stimulation. These results indicated that control cells synthesized more water-soluble inositolphospholipids after PDGF stimulation (301 ± 15 dpm compared to 360 ± 1 dpm per 10^6 cells; $P < 0.005$). In contrast, cells transfected with EJ-*ras* DNA did not show a significant increase after exposure to PDGF (379 ± 43 dpm compared with 410 ± 10 dpm per 10^6 cells; $P > 0.10$).

We also investigated whether cells transfected with EJ-*ras* DNA exhibited reduced phospholipase A_2 activities compared to control cells after PDGF stimulation. Thus, control and EJ-*ras*-transfected cells were labeled with [^{3}H]arachidonic acid and changes in the specific activities of phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), phosphatidylethanolamine (PtdEtn), and phosphatidylcholine (PtdCho) were determined after PDGF stimulation (Fig. 5). Control cells stimulated with PDGF at 2.5 units/ml showed a statistically significant 24% decrease in the specific activity of PtdCho after 30 sec ($P < 0.005$); this reduction in PtdCho specific activity persisted for 3 min, and then the specific activity returned to normal. EJ-*ras*-transfected cells showed no decrease in PtdCho specific activities after PDGF stimulation (Fig. 5). There was no statistically significant change in PtdIns specific activity in either control or EJ-*ras*-transfected cells (Fig. 5). The specific activities of PtdEtn and PtdSer also remained unchanged in these cells after PDGF stimulation (data not shown).

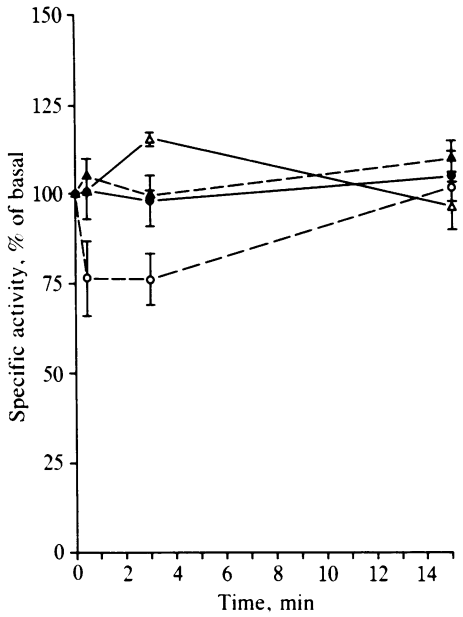

FIG. 5. PDGF-stimulated PtdCho hydrolysis in control and EJ-*ras*-transformed cells. Cells (respectively, 4 and 6.5 × 10^6 cells per 100-mm well) were grown for 24 hr in 1.25% PDS and then exposed to PDGF at 2.5 units/ml. The specific activities of PtdCho and PtdIns were measured at 0.5, 3, and 15 min after addition. Data presented are the mean ± SEM specific activity, expressed as percent of basal. The data are representative of two separate experiments with $n = 6$. ○, PtdCho in control cells + PDGF; ●, PtdIns in control cells + PDGF; △, PtdCho in EJ-*ras*-transformed cells + PDGF; ▲, PtdIns in EJ-*ras*-transformed cells + PDGF.

DISCUSSION

Previous work from our laboratory showed that hormone-stimulated adenylate cyclase activity was reduced in NIH-3T3 cells expressing high levels of the EJ-*ras* oncogene (17). Since p21 shares sequence homology with the α subunit of G proteins, we hypothesized that other vetebrate G-protein-regulated systems, besides adenylate cyclase, would be modulated by *ras* p21s. Work by Okajima *et al.* (27) and Bokoch and Gilman (28) showed that the ADP-ribosylation of the α subunit of the N_i G protein blocked the release of arachidonate from neutrophils. These data suggested that G proteins regulate cellular phospholipase activity and, thus, phospholipase activity may also be modulated by *ras* p21s.

Table 1. PDGF-stimulated [^{3}H]PGE_2 and [^{3}H]arachidonate release

	Metabolite released, dpm/10^6 cells					
	Control		c-*ras*		EJ-*ras*	
Stimulation	PGE_2	Arachidonate	PGE_2	Arachidonate	PGE_2	Arachidonate
None	663 ± 81	4,993 ± 549	360 ± 60	3619 ± 325	251 ± 13	1807 ± 162
PDGF	2356 ± 259	14,864 ± 1189	1645 ± 148	8381 ± 587	517 ± 25	2352 ± 259

Monolayer cultures in PDS were incubated for 6 hr with 1 μCi of [^{3}H]arachidonic acid per 10^6 cells. The cells were then washed twice, and incubated with DMEM containing 0.5% fetal calf serum. Some cells were exposed to PDGF at 2.0 units/ml. After 1 hr at 37°C PGE_2 and arachidonate were quantitated. Values are mean ± SEM.

We have used PDGF-stimulated phospholipid hydrolysis and arachidonate/PGE_2 release from cells to investigate the influence of mutated and normal p21 on phospholipase activity in NIH-3T3 cells. Our data suggest that phospholipase enzymic activity is deficient in cells expressing the EJ-*ras* gene at high levels. Four independent biochemical measurements support this hypothesis: (*i*) NIH-3T3 cells transfected with EJ-*ras* DNA released only low levels of PGE_2 compared to control cells after PDGF stimulation; (*ii*) NIH-3T3 cells whose phospholipids were labeled with [^{3}H]-arachidonate released higher levels of PGE_2 and arachidonate after PDGF stimulation compared to EJ-*ras*-transfected cells; (*iii*) cells expressing the EJ-*ras* gene also formed less water-soluble inositolphospholipids than control cells after PDGF stimulation; and (*iv*) EJ-*ras*-transfected cells also showed no decrease in the specific activity of PtdCho after PDGF stimulation, while control cells showed a 24% decrease under identical conditions.

EJ-*ras*-transformed cells contain functional PDGF receptors as evidenced by direct ^{125}I-labeled PDGF binding studies and their proliferation after exposure to PDGF. The transformed cells do show a modest reduction in the total number of receptors per cell, but previous work has shown no clear relationship between the total number of receptors and mitogenesis (18). Thus it is highly unlikely that the reduction of PDGF-stimulated phospholipase A_2/C activities observed in these cells results solely from a loss of PDGF receptors, receptor desensitization, or the occupation of PDGF receptors by an unknown autocrine growth factor. Interestingly, our data may dissociate the growth-stimulating activity of PDGF from PDGF-stimulated phospholipase activation, and they suggest that PDGF may be coupled to multiple pathways of cellular activation.

Previous work by Berridge *et al.* (22) has shown that PDGF stimulates phospholipase C activity in Swiss 3T3 cells, resulting in an increase in concentrations of water-soluble inositolphospholipids and intracellular Ca^{2+}. Although we detected a small stimulation in phospholipase C activity in control cells after PDGF exposure, the data from Fig. 5 suggest that the majority of the released arachidonate is derived from PtdCho and the action of phospholipase A_2. This statement is based upon the rapid reversible 24% decline in the specific activity of PtdCho labeled with [^{3}H]arachidonate and a parallel increase in lysophosphatidylcholine after PDGF stimulation. No concommitant reduction in the specific activity of other phospholipid species was observed. Similar conclusions have been published by Hasegawa-Sasaki (29). Moreover, cells expressing the EJ-*ras* gene product showed no reduction in the specific activity of PtdCho after PDGF stimulation. Taken together, these data strongly suggest that both phospholipase C and A_2 activities are depressed in EJ-*ras*-transformed NIH-3T3 cells. Recently, Fleischman *et al.* (15) suggested that mutated *ras* may directly stimulate a phospholipase C that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PtdInsP_2). However, this postulate was based upon a small change in the ratio of diacylglycerol to PtdInsP_2 and a modest increase in total water-soluble inositolphospholipids. These effects were evident only in cells of high density; exponentially growing cells showed no difference from control values. No attempt was made to evaluate growth factor-stimulated PtdIns turnover (15). Due to the density dependence, and the lack of data concerning growth factor stimulation, it is difficult to relate their experiments to our own. We did measure a small increase in the basal level of total water-soluble inositolphospholipids in *ras*-transformed cells, but our experiments suggest that mutated *ras* does not stimulate phospholipase; instead it reduces growth factor-stimulated phospholipase activity. Similar conclusions were recently presented in abstract form by Parries and Racker (16).

In summary, the transforming activity of mutated *ras* has now been associated with two vertebrate cellular systems thought to be regulated by G proteins, namely phospholipases A_2/C and adenylate cyclase. In both cases the enzyme activity is reduced in cells expressing mutated *ras* at high levels. Since cells expressing c-*ras* at high levels also exhibited reduced phospholipase and adenylate cyclase activities, we believe that c-*ras* may normally help modulate systems that are regulated by G proteins and that *ras* transformation may result from a concerted aberration of guanine-nucleotide-regulated systems.

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