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Opposing effects of a *ras* oncogene on growth factor-stimulated phosphoinositide hydrolysis: Desensitization to platelet-derived growth factor and enhanced sensitivity to bradykinin

(inositolphospholipids/GTP-binding proteins/receptors/phosphorylation)

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ABSTRACT **Expression of a transforming Harvey or Kirsten *ras* gene caused opposing effects in the ability of platelet-derived growth factor (PDGF) and bradykinin to activate phospholipase C-mediated phosphoinositide hydrolysis. In [^{3}H]inositol-labeled rat-1 fibroblasts, PDGF (5 ng/ml) resulted in a 2-fold increase in the level of [^{3}H]inositol trisphosphate (InsP_3) after 2 min and, in the presence of LiCl, a 3- to 8-fold increase in the level of [^{3}H]inositol monophosphate (InsP_1) after 30 min. However, in EJ-*ras*-transfected rat-1 cells, which exhibit near normal levels of PDGF receptors, PDGF resulted in little or no accumulation of either [^{3}H]InsP_3 or [^{3}H]InsP_1. Similarly, marked stimulations by PDGF were observed in NIH 3T3 cells, as well as in v-*src*-transformed 3T3 cells, but not in 3T3 cells transformed by Kirsten sarcoma virus or by transfection with v-Ha-*ras* DNA. This diminished phosphoinositide response in *ras*-transformed cells was associated with a markedly attenuated mitogenic response to PDGF. On the other hand, both phosphoinositide metabolism and DNA synthesis in *ras*-transformed fibroblasts were stimulated several-fold by serum. In NIH 3T3 cells carrying a glucocorticoid-inducible v-Ha-*ras* gene, a close correlation was found between the expression of p21^{ras} and the loss of PDGF-stimulated [^{3}H]InsP_1 accumulation. In contrast to this *ras*-induced desensitization to PDGF, *ras*-transformed NIH 3T3 cells exhibited an enhanced sensitivity to bradykinin; this effect was associated with an elevated level of high-affinity [^{3}H]bradykinin binding. We propose that a *ras* gene product (p21) can, directly or indirectly, influence growth factor-stimulated phosphoinositide hydrolysis, as well as DNA synthesis, via alterations in the properties of specific growth factor receptors.**

The family of mammalian H-, K-, and N-*ras* genes encode highly conserved 21-kDa proteins (p21^{ras}) (1, 2) that appear to be intimately involved in the control of cellular growth and differentiation (3–5). Certain mutated *ras* genes, including the v-*ras* genes of the Harvey and Kirsten murine sarcoma viruses (1) as well as various "activated" *ras* genes found in many human tumor cells (2, 6–8), code for altered p21 proteins capable of transforming morphologically normal cells into tumorigenic cells.

The cellular targets of normal or transforming *ras* proteins are not known. Normal p21^{ras} proteins associate with the cytoplasmic surface of the plasma membrane (9, 10), bind guanine nucleotides (11, 12), and catalyze the hydrolysis of GTP (13). These observations, together with the homologies between *ras* proteins and the α-subunits of certain guanine nucleotide regulatory proteins (G-proteins) (14), have led to the suggestion that p21^{ras} might participate in a G-protein-like manner in some guanine nucleotide-dependent signal-transducing mechanism. In *Saccharomyces cerevisiae*, *ras* proteins are required for GTP-stimulated adenylate cyclase activity (15), although the mechanism of this interaction is not yet clear. The *S. cerevisiae* cyclase can also be activated by mammalian p21^{ras} (16), but a similar role for *ras* proteins in mammalian cells (16, 17) or even in other yeasts (18) has not been demonstrated. Additional clues to possible targets of the *ras* gene product in mammalian cells have been obtained through analysis of *ras*-induced metabolic alterations. Decreased levels of cAMP (19), elevated secretion of type α transforming growth factor (20), enhanced rates of glycolysis and system A amino acid transport (21), as well as increased activities of phospholipases A_2 (22) and C (23, 24) have been observed. Alterations by *ras* of phospholipase A_2 and C activities are of particular interest because in some cells these enzymes are influenced by guanine nucleotides (25–28). Due to the fact that many or all of these metabolic events are regulated by serum growth factors, however, it is essential to differentiate between an effect of p21^{ras} on the metabolic pathway itself and on the responses of cells to growth factors.

In this paper, we report that expression of a *ras* oncogene gives rise to marked alterations in the ability of platelet-derived growth factor (PDGF) and bradykinin to activate phospholipase C-mediated phosphoinositide hydrolysis. These and other altered growth factor responses may be relevant to the diverse metabolic aberrations observed in *ras*-transformed cells.*

MATERIALS AND METHODS

Growth Factors and Cell Lines. Pure human PDGF (31) and [^{125}I]PDGF (32) were gifts of R. Ross and E. Raines. Purified porcine PDGF was provided by M. Greenhalgh (Bioprocessing, Consett, U.K.). Insulin-like growth factor I was a gift of M. Czech. Bombesin, thrombin, prostaglandin $F_{2\alpha}$, and bradykinin were obtained from Sigma and [2,3-*prolyl*-3,4-^{3}H(N)]bradykinin (49.7 Ci/mmol; 1 Ci = 37 GBq) from New England Nuclear.

Cell lines were obtained as follows: rat-1 and R1-EJ2 (EJ-*ras*; ref. 33), R. Weinberg; NIH 3T3, Kirsten murine sarcoma virus (Ki-MuSV)-transformed 3T3 ("DT" line; ref. 34), and NIH 3T3 [v-Ha-*ras* transfectants 433.3 (ref. 35) and 568], R. Bassin; NIH 3T3 [long terminal repeats (LTR)-c-Ha-*ras*, clone pBW192], D. Lowy; and NIH 3T3 {clone NIH[pMv-*src*(focus)A]; ref. 36}, D. Shalloway.

Abbreviations: PDGF, platelet-derived growth factor; DMEM, Dulbecco's modified Eagle's medium; InsP_1, inositol monophosphate; InsP_3, inositol trisphosphate; Ki-MuSV, Kirsten murine sarcoma virus; p21^{ras}, 21-kDa *ras* proteins; LTR, long terminal repeats.

*Preliminary accounts of part of this work were presented at the 1986 Annual Meeting of the American Society of Biological Chemists, Washington, DC, June 1986 (29), and at the Sixth International Conference on Cyclic Nucleotides, Calcium and Protein Phosphorylation, Bethesda, MD, September 1986 (30).

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Tissue Culture. Rat-1 and R1-EJ2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 mM Hepes (pH 7.4), penicillin (50 units/ml), streptomycin (50 μg/ml), and 10% calf serum (Hyclone)—designated as complete medium. NIH 3T3 cells and variants were grown in an identical medium, except that 10% fetal bovine serum (GIBCO) was used. For experiments, cells were seeded onto 35-mm culture dishes (Falcon) at a density of 1–2 × 10^5 cells per dish in 2 ml of complete medium and grown to confluency. All cultures were maintained in a humidified atmosphere of 5% CO_2/95% air at 37°C.

Analysis of Phosphoinositide Hydrolysis. Cells were labeled to isotopic equilibrium with *myo*-[2-^{3}H]inositol (Amersham) and incubated with growth factors as described in the legends to individual experiments. After incubation, the culture medium was replaced with 0.4 ml of ice-cold 10% trichloroacetic acid. Cells were harvested by scraping, followed by two rinses of the culture dishes with 0.4-ml aliquots of 10% trichloroacetic acid. The acid-precipitated cell suspensions were centrifuged at 10,000 × *g* for 5 min, and the resulting residue was rinsed with 0.4 ml of 10% trichloroacetic acid. The combined extracts were washed three times with 2-ml aliquots of diethyl ether to remove trichloroacetic acid and then adjusted to pH 7 with 1 M NaOH. Separation of [^{3}H]-inositol phosphates was via polyethyleneimine-cellulose column chromatography, with a LiCl gradient to specifically elute individual [^{3}H]inositol phosphates (unpublished method).

Assay of p21 Protein. Levels of $p21^{ras}$ were determined by immunoprecipitation of [^{35}S]methionine-labeled proteins with $p21^{ras}$-specific monoclonal antibody Y13-259 (a gift of M. Furth) (10). ^{35}S-labeled $p21^{ras}$ immunoprecipitates were electrophoresed on 12% polyacrylamide gels in the presence of $NaDodSO_4$ (37). The gels were subjected to fluorography on Kodak X-Omat AR film, and relative levels of $p21^{ras}$ were determined by scanning video densitometry.

Receptor Binding Studies. Equilibrium binding of [^{125}I]-PDGF (32) or [^{3}H]bradykinin (38) to cultured cells was as previously described.

DNA Synthesis. Quiescent cell cultures were treated with or without growth factors and, after 12 hr, pulsed with 1 μCi of [*methyl*-^{3}H]thymidine (87 Ci/mmol, Amersham). After an additional 12 hr, trichloroacetic acid-insoluble radioactivity was determined as described (31).

RESULTS AND DISCUSSION

Inhibitory Effect of a *ras* Oncogene on PDGF-Stimulated Phosphoinositide Hydrolysis. As has been shown previously for quiescent Swiss 3T3 cells (39, 40), confluent cultures of rat-1 and NIH 3T3 cells respond to PDGF with a marked activation of phosphoinositide-specific phospholipase C. However, *ras*-transformed counterparts of these cells, which exhibit normal levels of cell-surface PDGF receptors (see below), have become refractory to this growth factor. As shown in Table 1, incubation of [^{3}H]inositol-labeled rat-1 cells for 2 min with human platelet PDGF (5 ng/ml) resulted in an enhancement of phosphatidylinositol 4,5-bisphosphate hydrolysis as evidenced by a 2.1-fold increase ($P < 0.005$, Student's *t* test) in the level of [^{3}H]InsP_3; in contrast, PDGF treatment of *ras*-transformed rat-1 cells (R1-EJ2 line, obtained by transfection with the cloned human EJ-*ras* gene; ref. 33) resulted in no significant accumulation of [^{3}H]InsP_3. A similar situation was observed when [^{3}H]InsP_1 levels were measured. In cells treated with lithium chloride to block InsP_1 phosphatase activity (41), incubation with PDGF (10 ng/ml) for 30 min resulted in a 3.2-fold increase in the level of [^{3}H]InsP_1 in rat-1 cells, with no corresponding increase in EJ-*ras*-transformed cells (Table 1). PDGF also induced a marked accumulation of [^{3}H]InsP_1 in NIH 3T3 cells, but not in 3T3 cell lines transformed by Ki-MuSV or by transfection with viral Ha-*ras* DNA. In rat-1 cells, the stimulation by purified human PDGF was dose-dependent, with half-maximal activation at ≈6 ng/ml (0.2 nM) and with maximal activation at ≈25 ng/ml (0.8 nM) (Fig. 1). In EJ-*ras*-transformed cells, there was no significant increase in [^{3}H]-InsP_1 formation even with levels of PDGF that were saturating for rat-1 cells (up to 92 ng/ml; 3 nM).

Table 1. Inhibitory effect of a *ras* oncogene on [^{3}H]inositol phosphate accumulation in PDGF-stimulated rat-1 and NIH 3T3 fibroblasts

	[^{3}H]InsP_3,* cpm × 10^{-3} per mg of protein, 2-min incubation		[^{3}H]InsP_1, cpm × 10^{-3} per mg of protein, 30-min incubation	
Cell line	Control	PDGF	Control	PDGF
A Rat -1				
Parental	0.72 ± 0.05	1.55 ± 0.03	21.9 ± 1.2	70.7 ± 2.2
EJ-*ras*	1.07 ± 0.12	1.01 ± 0.26	21.4 ± 0.9	19.3 ± 0.2
B NIH 3T3				
Parental	ND	ND	11.4 ± 0.3	90.2 ± 3.3
Ki-MuSV	ND	ND	5.9 ± 0.2	6.0 ± 0.5
Parental	ND	ND	13.4 ± 0.5	78.2 ± 3.2
v-Ha-*ras*	ND	ND	10.3 ± 0.4	10.0 ± 0.4

Cells were grown to near confluency in complete medium and then incubated for 40–48 hr in DMEM containing 2% serum and [^{3}H]-inositol (2.5 μCi/ml or 1.25 μCi/ml for InsP_3 and InsP_1 determinations, respectively). The cell monolayers were rinsed with serum-free DMEM and then, for InsP_3 measurements, incubated for 2 min in 0.7 ml of DMEM either with or without partially purified human platelet PDGF (fraction CMS-III, ref. 31; equivalent to 5 ng/ml of pure PDGF). For InsP_1 measurements, labeled cells were incubated for 10 min in 1 ml of DMEM containing 25 mM LiCl, and then for an additional 30 min either with or without PDGF [A, purified human platelet PDGF at 10 ng/ml; B, partially purified human platelet PDGF (equivalent to 5 ng/ml of pure PDGF) for Ki-MuSV cells or purified porcine PDGF at 40 ng/ml for v-Ha-*ras*]. Data represent the means ± SD of triplicate determinations. ND, not determined.
*Stereoisomer composition not characterized.

Receptor binding experiments with [^{125}I]PDGF were done to determine whether the lack of PDGF response in *ras*-transformed cells might be due to an absence of cell-surface receptors. In parental and EJ-*ras*-transformed rat-1 cells, equilibrium binding of [^{125}I]PDGF showed that the level of specific binding (up to 3 ng/ml; 96 pM) in EJ-*ras* cells was ≈80–90% of that in the parental cells (Fig. 1 *Inset*). In parental and v-Ha-*ras*-transformed NIH 3T3 cells (clone 568), the levels of specific [^{125}I]PDGF binding (at 2 ng/ml) were 28.5 ± 1.0 and 26.8 ± 0.4 fmol/mg of protein ($n = 2$), respectively. The results suggest that the lack of PDGF response is not due to an absence of cell-surface receptors but is due to some defect distal to PDGF binding.

A diminished response of *ras*-transformed rat-1 or NIH 3T3 cells to PDGF was also evident when phosphoinositide metabolism was measured as an incorporation of [^{3}H]inositol into total lipids. In other experiments utilizing this assay, we observed that NIH 3T3 cells transfected with the normal rat c-Ha-*ras* gene (42), promoted by the Harvey sarcoma virus LTR, exhibit a 40% loss of PDGF-stimulated phosphoinositide metabolism as compared with that in control cells, implying that overexpression of a normal c-*ras* gene is sufficient to render cells at least partially refractory to PDGF (Table 2).

Responses to PDGF were also examined in parental NIH 3T3 cells and in 3T3 cells that were transformed via transfection with the Rous sarcoma virus v-*src* gene {clone NIH[pMv-*src*(focus)A]; ref. 36}. As shown in Table 2, v-*src*-transformed cells responded to PDGF with a 3.8-fold stimulation of phosphoinositide metabolism, similar to that noted in the parental cell line. Thus, the lack of PDGF-stimulated phosphoinositide metabolism in *ras*-transformed cells is not a general consequence of cellular transformation,

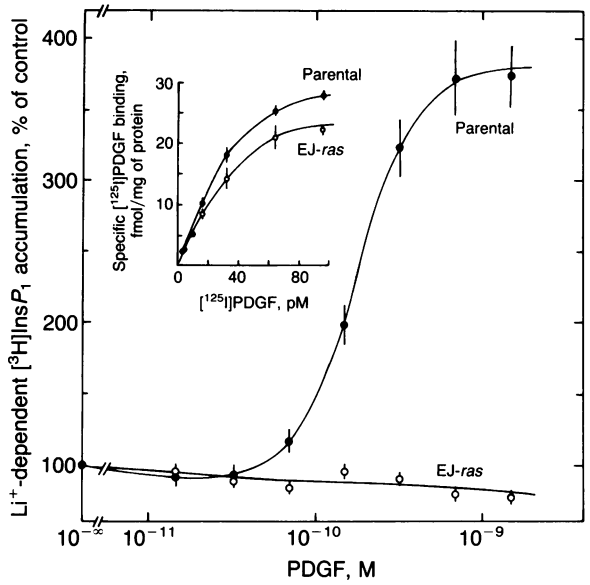

FIG. 1. Dose–response analysis of PDGF effects on [^{3}H]InsP_1 levels in parental (●) and EJ-*ras*-transformed (○) rat-1 cells. Cells were labeled with [^{3}H]inositol (1.25 μCi/ml) and then treated with 25 mM LiCl as outlined in the legend to Table 1. Incubations were continued for an additional 30 min in the presence of pure human platelet PDGF. [^{3}H]InsP_1 levels were measured as described. Results were calculated from the averages ± SD of triplicate incubations. Control values (without PDGF) were 21,900 ± 1180 and 21,500 ± 900 cpm per mg of protein for parental and EJ-*ras* cells, respectively. (*Inset*) [^{125}I]PDGF binding. Cells were grown to approximately 90% of confluency in 24-well dishes (Falcon), incubated for 10–12 hr in DMEM containing 0.5% calf serum, and then for 1 hr in serum-free DMEM. Specific binding of [^{125}I]PDGF (1000 cpm/fmol) was determined as described (32). The binding curves represent the average ± SD of three independent experiments, each carried out in triplicate.

Table 2. Comparison of transfected *src* and *ras* oncogenes on PDGF-stimulated phosphoinositide synthesis and DNA synthesis in rat-1 and NIH 3T3 fibroblasts

	Chloroform-soluble [^{3}H]inositol, cpm × 10^{-3} per mg of protein		Stimulation of [^{3}H]dThd incorporation, *x*-fold	
Cell line	Control	PDGF	PDGF	serum
A Rat-1				
Parental	1.05 ± 0.19	5.40 ± 1.75	15 ± 2	42 ± 4
EJ-*ras*	1.31 ± 0.32	1.35 ± 0.35	1.4 ± 0.1	2.8 ± 0.1
B NIH 3T3				
Parental	1.38 ± 0.07	18.4 ± 0.6	24 ± 1	6.8 ± 0.4
v-Ha-*ras*	1.64 ± 0.06	2.26 ± 0.01	2.5 ± 0.9	5.5 ± 1.0
Parental	1.62 ± 0.01	5.98 ± 0.04	ND	ND
c-Ha-*ras*	1.52 ± 0.06	3.98 ± 0.03	ND	ND
Parental	6.00 ± 0.27	19.5 ± 0.6	ND	ND
v-*src*	4.57 ± 0.12	17.2 ± 0.2	ND	ND

Confluent monolayers of cells were incubated for 1 hr in serum-free DMEM containing [^{3}H]inositol (2.5 μCi/ml) and then for an additional 30 min in the presence or absence of partially purified human platelet PDGF (fraction CMS-III, ref. 31; equivalent to 5 ng/ml pure PDGF). Cells were rinsed twice with 1 ml of Dulbecco's phosphate-buffered saline, scraped from the plates in 0.8 ml of 10 mM EDTA/2 mM EGTA (pH 7), and then extracted by vortexing first with 3 ml of chloroform/methanol/12 M HCl (1:2:0.01, vol) and then after the addition of 1 ml of 2.4 M HCl and 1 ml of chloroform. After a brief centrifugation to separate the phases, the chloroform layer and interfacial material were washed three times with 4 ml of 0.1 M HCl, dried, and quantitated for ^{3}H radioactivity. Values are the means ± range of duplicate determinations. DNA synthesis in response to purified porcine PDGF (40 ng/ml) or 10% fetal bovine serum was determined as described. Basal levels of [^{3}H]dThd incorporation (as cpm × 10^{-3} per 35 mm) were 23.3 ± 1.9 and 296 ± 8 for parental and EJ-*ras*-transformed rat-1 cells, respectively, and 33.7 ± 1.2 and 40.8 ± 7.5 for parental and v-Ha-*ras*-transformed NIH 3T3 cells, respectively (n = 3, ±SD).

but may be specifically related to the actions of a transforming *ras* gene product.

The Diminished Phosphoinositide Response in *ras*-Transformed Cells Is Associated with an Attenuated Mitogenic Response to PDGF. It was of interest to determine whether the diminished phosphoinositide response in *ras*-transformed fibroblasts might also be accompanied by an attenuated mitogenic response to PDGF. In parental and v-Ha-*ras*-transfected NIH 3T3 cells, which were serum-starved to obtain a low basal rate of DNA synthesis, purified PDGF resulted in a 24-fold stimulation of acid-insoluble [^{3}H]thymidine incorporation in the parental cells but only a 2.5-fold increase in the *ras*-transfected line; both cell lines, however, were stimulated to similar extents by serum (Table 2). The influence of a *ras* gene on the relative mitogenic effects of PDGF and serum in rat-1 cells was less clear since EJ-*ras*-transfected rat-1 cells maintained a high basal level of [^{3}H]thymidine incorporation even after serum starvation (Table 2, legend); thus, in EJ-*ras* cells the apparent effects of both serum and PDGF were greatly diminished.

Kinetics of Phosphoinositide Turnover and Stimulation of *ras*-Transformed Cells by Serum. If the phosphoinositide cycle in *ras*-transformed cells were in a persistently activated state, this could account for an apparent lack of PDGF stimulation. In *ras*-transformd rat-1 and NIH 3T3 cells, however, this is not the case because the basal levels of phosphoinositide synthesis were nearly the same in the parental and transformed cell lines (Table 2). Moreover, when the kinetics of lithium-dependent [^{3}H]InsP_1 accumulation in serum-free medium were determined (Fig. 2), there was no significant increase in [^{3}H]InsP_1 radioactivity over a 30-min interval in either EJ-*ras*-transformed (R1-EJ2) or parental rat-1 cells, indicating that both cell lines have a similar basal rate of turnover. In rat-1 cells, a combination of LiCl and PDGF resulted in a rapid accumulation of [^{3}H]-InsP_1, while in R1-EJ2 cells the level of [^{3}H]InsP_1 remained at a value similar to that in the unstimulated state. On the other hand, phosphoinositide hydrolysis in both parental and EJ-*ras*-transformed rat-1 cells was stimulated 3- to 5-fold by either 5% bovine or human serum or by 5% human plasma-derived serum (results not shown), indicating that both cell lines possess an activatable phosphoinositide cycle.

Correlation of p21^{ras} Expression with the Loss of PDGF-Stimulated Phosphoinositide Hydrolysis. To gain insight into the relationship between the expression of p21^{ras} protein and the loss of PDGF-stimulated phosphoinositide metabolism, we have examined these two parameters in NIH 3T3 cells (clone 433.3) containing a v-Ha-*ras* gene promoted by the glucocorticoid-inducible mouse mammary tumor virus LTR. As shown in Fig. 3*A*, treatment of [^{35}S]methionine-labeled cells with 30 nM dexamethasone resulted in a progressive accumulation of [^{35}S]p21^{ras} protein between 0 and 18 hr. In parallel cultures labeled with ^{3}H-labeled inositol, treatment with dexamethasone for 8 or 18 hr resulted in an ≈85% decrease in the level of PDGF-stimulated [^{3}H]InsP_1 accumulation; under these conditions there was little or no decrease in the level of [^{125}I]PDGF binding (Fig. 3*A Inset*). In control NIH 3T3 cells (Fig. 3*B*), dexamethasone treatment also resulted in a significant decrease in the level of PDGF stimulation, as well as an approximately 10-fold increase (after 18 hr) in the level of [^{35}S]p21^{ras}. No significant loss of [^{125}I]PDGF binding was observed (Fig. 3*B Inset*). This effect of dexamethasone on p21^{ras} in control 3T3 cells may be due to an enhanced expression of an endogenous mouse cellular c-*ras* gene(s), and is consistent with the earlier observation regarding the expression of an LTR-linked c-Ha-*ras* gene

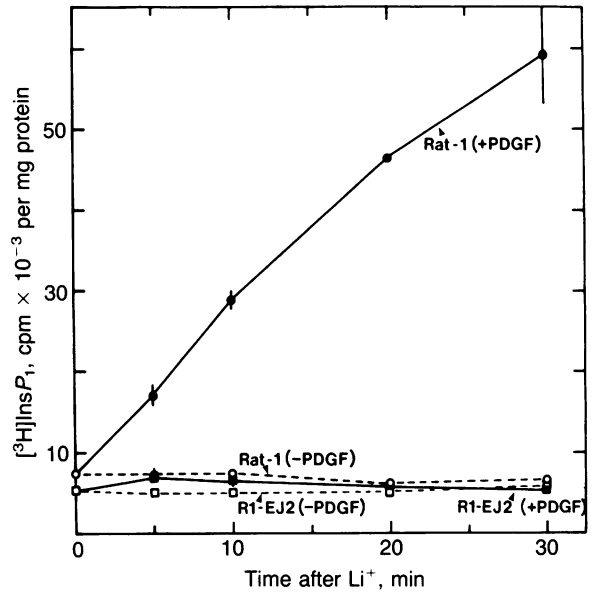

FIG. 2. Kinetics of InsP_1 accumulation in the presence of lithium. Rat-1 (○, ●) and R1-EJ2 (□, ■) cells were labeled for 44 hr with [^{3}H]inositol (2.5 μCi/ml) in DMEM containing 2% calf serum. Either LiCl (25 mM) or a mixture of LiCl and partially purified human platelet PDGF (fraction CMS-III, ref. 31; equivalent to 5 ng/ml of pure PDGF) were then added to the medium for the indicated times. Incubations were terminated and [^{3}H]InsP_1 levels determined as outlined. Values are the means ± range of duplicate determinations.

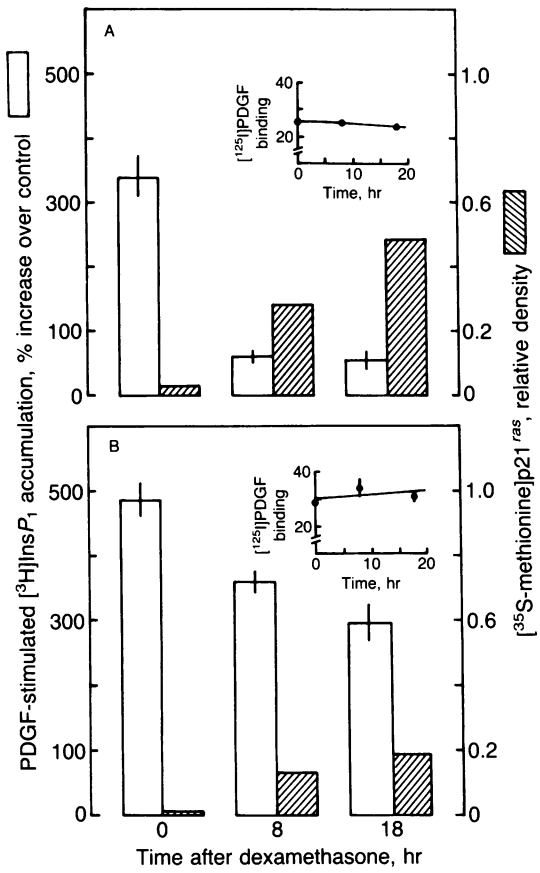

FIG. 3. Correlation of p21$^{v\text{-}Ha\text{-}ras}$ expression with loss of PDGF-stimulated InsP_1 accumulation in NIH 3T3 cells carrying a mouse mammary tumor virus LTR-linked v-Ha-*ras* gene, clone 433.3 (*A*) and parental NIH 3T3 cells (*B*). Cells were grown to ≈90% of confluency and then labeled for 42 hr with [^{3}H]inositol as in Table 1. Dexamethasone (30 nM) was added at times during the labeling period to achieve the indicated time of exposure at the end of the 42 hr. Cells were then treated with LiCl, with or without purified porcine platelet PDGF (40 ng/ml), and assayed for [^{3}H]InsP_1 formation. Values were calculated from the means ± SD of triplicate incubations. For determination of p21^{ras} expression, parallel cultures of cells were labeled for 25 hr with [^{35}S]methionine (100 μCi/ml, Amersham) in Eagle's basal medium containing 2% dialyzed calf serum; 30 nM dexamethasone was added so that the indicated time of exposure was reached at the end of the 25-hr labeling period. Relative levels of p21^{ras} were determined by immunoprecipitation as indicated; relative densities are in arbitrary units. (*Insets*) Specific [^{125}I]PDGF receptor binding. Cells were treated with dexamethasone under conditions identical to those described above and then assayed directly for [^{125}I]PDGF binding (at 2 ng/ml) (32). Data are expressed as fmol of [^{125}I]PDGF bound per mg of protein; values are the means ± range of duplicate incubations.

(Table 2) that overproduction of a normal cellular *ras* protein can be associated with a partial loss of PDGF response. While these data do not establish a cause and effect relationship between p21^{ras} expression and the loss of PDGF-stimulated phosphoinositide metabolism, they are nevertheless consistent with the hypothesis that p21^{ras} may, directly or indirectly, cause an uncoupling of PDGF receptors from the activation of phosphoinositide-specific phospholipase C.

In other experiments, we have observed that the stimulation of PDGF receptor autophosphorylation is markedly reduced in membranes isolated from *ras*-transformed cells, as compared with those from control cells. If the PDGF activation of phospholipase C is dependent on the protein tyrosine kinase activity of the PDGF receptor, then a defect in receptor autophosphorylation could account for the lack of PDGF stimulation.

Opposing Effects of *ras*-Transformation on Phosphoinositide Metabolism Coupled to Receptors for Bradykinin and PDGF. To ascertain whether *ras* might alter the activation of phosphoinositide-specific phospholipase C via receptors other than for PDGF, we surveyed a variety of factors for differential activation of phosphoinositide metabolism in normal and *ras*-transformed cells. Although PDGF was the only factor whose response was found to be diminished in the transformed cells, *ras*-transformed NIH 3T3 cells exhibited a marked increase in sensitivity to bradykinin, a nonapeptide hormone that has previously been shown to induce inositol-phospholipid turnover in neuroblastoma–glioma cells (43, 44) and aortic endothelial cells (45). As shown in Fig. 4, bradykinin (1 μM) had no apparent effect on the lithium-dependent accumulation of [^{3}H]InsP_1 in parental NIH 3T3 cells, but induced a 2-fold increase in [^{3}H]InsP_1 levels in 3T3 cells transformed by Ki-MuSV. Half-maximal stimulation occurred with ≈10 nM agonist. Similarly, an enhanced sensitivity to bradykinin was observed in NIH 3T3 cells transformed by viral Ha-*ras* DNA (clone 568); EJ-*ras*-transformed rat-1 cells also showed some increased sensitivity to bradykinin, although to a lesser extent than in NIH 3T3 cell lines (data not shown). In contrast to the *ras*-induced desensitization to PDGF, which does not involve an alteration in [^{125}I]PDGF binding, the elevated level of bradykinin-stimulated phosphoinositide metabolism in Ki-MuSV-transformed cells was paralleled by an increase in high-affinity [^{3}H]bradykinin binding (K_d ≈ 3 nM) (Fig. 4*B Inset*). Ki-MuSV-transformed 3T3 cells also showed a slightly increased sensitivity to bombesin (1 μM), thrombin (250 ng/ml), prostaglandin $F_{2\alpha}$ (10 μM), and insulin-like growth factor I (50 ng/ml).

Similar to our findings in NIH 3T3 cells, Chiarugi *et al.* (46) have recently reported that in BALB/3T3 cells an EJ-*ras* gene results in an increased hydrolysis of inositolphospholipids in response to muscarinic cholinergic stimulation. In addition, NIH 3T3 cells that overproduce normal p21$^{N\text{-}ras}$ exhibit an enhanced sensitivity to bombesin and gastrin-

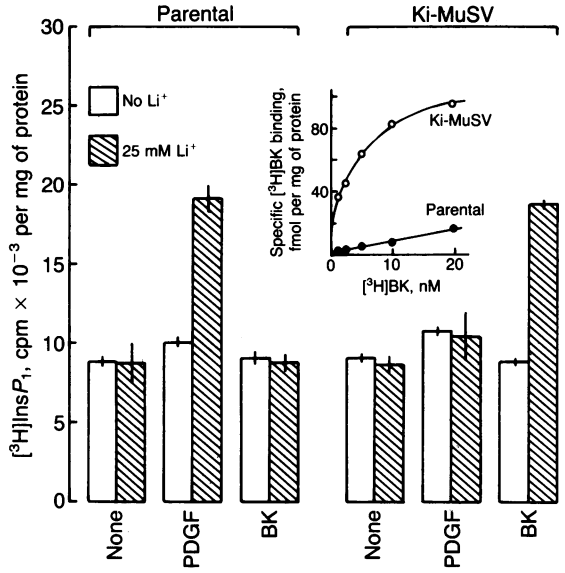

FIG. 4. Lithium-induced accumulation of [^{3}H]InsP_1 in response to bradykinin or PDGF. Cells were labeled for 36 hr with [^{3}H]inositol (1.25 μCi/ml) in DMEM containing 2% fetal bovine serum, refed with serum-free DMEM (with or without 25 mM LiCl), and then incubated for 30 min with either purified porcine PDGF (20 ng/ml), 1 μM bradykinin, or no addition. [^{3}H]InsP_1 levels were measured as described. Values are given as the means ± SD of triplicate incubations. (*Inset*) [^{3}H]bradykinin binding. Parallel cultures of cells were incubated in DMEM containing 2% serum as above and then for an additional hour in serum-free medium. After cooling the cells to 4°C, equilibrium binding of [^{3}H]bradykinin (final activity of 49.7 Ci/mmol) was determined. Nonspecific binding, measured in the presence of 3 μM unlabeled bradykinin, averaged 0.018% of the total input ^{3}H cpm in both cell lines and was subtracted from all values. Results are the averages of duplicate determinations, which varied by less than 10%. BK, bradykinin.

releasing peptide (without any apparent changes in receptor number or affinity), as well as to bradykinin (47).

Conclusions. We have shown here that a *ras* oncogene can induce marked alterations in the normal patterns of growth factor-stimulated phosphoinositide metabolism. The molecular mechanisms underlying the altered responses are not clear, but the data suggest either a direct or indirect influence of p21^{ras} on the properties of the relevant growth factor receptors. Modifications in growth factor responses may represent a contributing factor in various serum-dependent metabolic abnormalities that have been observed and may also be relevant to the generation of aberrant regulatory signals thought to be involved in transformation.

Finally, we propose that each oncogene can induce a characteristically altered profile of receptors that, in turn, influences the pattern of cell metabolism. Both qualitative and quantitative differences in receptor profiles, as expressed in their responses to growth factors and hormones, should contribute to a picture of cell individuality that may provide us with a rational approach to the understanding and treatment of the tumor.

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