Mechanism of Desensitization of the Ca\textsuperscript{2+}-mobilizing System to Bombesin by Ha-ras

INDEPENDENCE FROM DOWN-MODULATION OF AGONIST-STIMULATED INOSITOL PHOSPHATE PRODUCTION

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Expression of a transforming Ha-ras gene in NIH 3T3 cells transfected with an inducible Ha-ras construct leads to a rapid desensitization of the intracellular Ca\textsuperscript{2+}-mobilizing system to bombesin and serum growth factors. Half-maximal depression of the Ca\textsuperscript{2+} response is observed 2 h after induction of p21\textsuperscript{Ha}. A maximum is obtained after 6 h. Bombesin-induced elevation of inositol 1,4,5-trisphosphate formation is also depressed in cells expressing Ha-ras. This, however, is a relatively late phenomenon and not yet detectable when maximal depression of the Ca\textsuperscript{2+} signal is observed. We conclude that the rapid desensitization of the Ca\textsuperscript{2+}-releasing system to bombesin by Ha-ras is not caused by down-modulation or uncoupling of phospholipase C-coupled bombesin receptors. The inositol 1,4,5-trisphosphate-mediated release of intracellular Ca\textsuperscript{2+} is reduced in permeabilized cells expressing the Ha-ras oncogene. A depletion of intracellular Ca\textsuperscript{2+} stores by Ha-ras is unlikely since (i) the Ha-ras-induced growth factor-independent stimulation of inositol phosphate formation occurs several hours after reduction of the Ca\textsuperscript{2+} response and (ii) the Ca\textsuperscript{2+} load of intracellular nonmitochondrial Ca\textsuperscript{2+} stores was found to be unaffected by Ha-ras. We conclude that the desensitization of the Ca\textsuperscript{2+}-mobilizing system is caused either by partial inhibition of inositol 1,4,5-trisphosphate-regulated Ca\textsuperscript{2+} channels or by interference of Ha-ras with Ca\textsuperscript{2+} translocation between intracellular Ca\textsuperscript{2+} compartments.

In previous publications (1, 2), we have demonstrated that expression of the transforming Ha-ras oncogene leads to a progressive desensitization of the intracellular Ca\textsuperscript{2+}-mobilizing system to serum growth factors. The detailed mechanism underlying the depression of the Ca\textsuperscript{2+} response is not entirely clear.

Several authors (3–7) have shown that cells expressing transforming ras oncogenes display reduced PDGF\textsuperscript{-} or bombesin-stimulated inositol phosphate formation and depression of the mobilization of intracellular Ca\textsuperscript{2+} by these growth factors. These effects have been discussed as resulting from a functional uncoupling of the corresponding growth factor receptors from inositol phospholipid hydrolysis by phospholipase C (3–7). Evidence for protein kinase C-mediated inhibition of bombesin- and PDGF-stimulated phosphatidylinositol 4,5-bisphosphate breakdown by p21\textsuperscript{Ha} (8) provides a biochemical basis for such a mechanism.

The study presented here, however, demonstrates that Ha-ras-mediated reduction of bombesin-induced mobilization of intracellular 

EXPERIMENTAL PROCEDURES

Materials—Phosphatidylinositol, phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate, all other phospholipids, dexamethasone, A23187, Na\textsubscript{3}VO\textsubscript{4}, saponin, leupeptin, pepstatin, and bombesin were purchased from Sigma. Silica Gel-coated glass plates (high performance thin-layer chromatography plates, 10 × 20 cm, No. 5641) were from E. Merck AG (Darmstadt, Federal Republic of Germany). myo-[2-\textsuperscript{3}H]inositol (12.8 Ci/mmol) and [\textsuperscript{32}P]orthophosphate (acid-free) were from Du Pont-New England Nuclear. Ca\textsuperscript{2+} (10–40 mCi/mg), D-my-o-[\textsuperscript{3}H]inositol 1,4,5-trisphosphate assay system, \textsuperscript{125}I-labeled protein A, and autoradiography films (Hyperfilm-MP) were from Amersham Corp. Fura-2 was purchased from Molecular Probes (Eugene, OR). Inositol-free DMEM was from Amirened (Basel, Switzerland).

Cell Culture—NIH 3T3 fibroblasts were transfected with the transforming human Ha-ras oncogene or the Ha-ras proto-oncogene subjected to transcriptional regulation by glucocorticoids by \textit{in vitro} recombination with MMTV-LTR as described (21). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS in the presence of 6% CO\textsubscript{2}. One day after plating, cells were made quiescent by incubation in inositol-free DMEM containing 0.5% FCS (in the case of inositol phosphate measurement; see below) or DMEM plus 0.5% FCS (for Ca\textsuperscript{2+} determinations) for 48 h.

Determination of Cytosolic Ca\textsuperscript{2+} Concentrations—Cells were prepared for cytosolic Ca\textsuperscript{2+} concentration measurements by growing on glass coverslips (9 × 18 mm). [Ca\textsuperscript{2+}] was determined by fluorescence spectrophotometry employing fura-2 (22). Cells attached to rectangular coverslips were loaded with fura-2 by incubation in HEPES-buffered saline (HBSS) (140 mM NaCl, 5 mM KCl, 1 mM CaCl\textsubscript{2}, 0.5 mM MgCl\textsubscript{2}, 5.5 mM glucose, 10 mM HEPES, pH 7.4) containing 5 μM fura-2 acetoxymethyl ester for 80 min at 37 °C (1). For fluorescence measurements, one cell-supporting coverslip was placed into a 1 × 1 × 4 cm quartz cuvette. Fluorescence (excitation of 350 or 385 nm, slit width of 1 nm; emission of 510 nm, slit width of 1 nm) was measured by a Spex Fluorolog 2 spectrophotometer (CM-1) equipped with two excitation monochrometers and a chopper system. Nanomolar values of cytosolic free [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]\textsubscript{i}) were calculated from the ratio of observed fluorescence intensities (\textit{I}\textsubscript{508/340}) of intracellular fura-2 as follows: [Ca\textsuperscript{2+}]=K\textsubscript{D} × f × ((\textit{I}(508/340) – \textit{I}(508/340)\textsuperscript{c})/(\textit{I}(508/340) – \textit{I}(508/340)\textsuperscript{c})),

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where \( K_s = 225 \text{ nM} \) for \( Ca^{2+}/fura-2 \) under cytoplasmic ionic conditions, and the factors \( f_1 = 12.7, f_\text{fluo-3} = 28.5 \) and \( f_\text{fluo-4} = 1 \) were determined as a separate EGTA/Ca\(^{2+}\) calibration procedure as described (22).

**Efflux of \( ^{45}Ca^{2+} \) from Nonmitochondrial Stores**—The experiments were performed by a modified procedure as described (23). Quiescent cells were loaded with \( ^{45}Ca^{2+} \) (1 Ci/ml) for 7 h in the presence of 10 \( \mu \text{M} \) antimycin in DMEM containing 0.5% FCS. The radioactive medium was removed, and the cells were washed three times with HBS containing 10 mM EGTA (HBS/EGTA). The efflux of \( ^{45}Ca^{2+} \) was measured by incubating the cells in 1 ml of HBS/EGTA (25 °C), which was replaced at 2-min intervals with fresh solution. The 1-ml efflux samples were added to 10 ml of scintillation fluid and counted for \( ^{45}Ca^{2+} \) radioactivity. At the end of the experiment, the cell number was determined using a Coulter Counter.

**Measurements of \( ^{45}Ca^{2+} \) Uptake and IP\(_3\)-induced \( ^{45}Ca^{2+} \) Release in Permeabilized Cells—**NIH 3T3 cells, transfected with Ha-ras, were grown in 35-mm culture dishes (6-well plates) at a density of 1.4-1.8 \( \times 10^5 \) cells/dish. Where indicated, Ha-ras expression was induced by 1 \( \mu \text{M} \) dexamethasone for 0 or 24 h. \( ^{45}Ca^{2+} \) uptake and IP\(_3\)-induced \( ^{45}Ca^{2+} \) release experiments were performed as described (25) with minor modifications. Before experiments, the medium was removed, and the cells were washed with 1 ml of buffer A (20 mM NaCl, 100 mM KCl, 5 mM MgSO\(_4\), 1 mM Na_2PO_4, 25 mM HEPES, 1% bovine serum albumin, pH 7.2). After a preincubation period of 10 min in buffer A in the presence of 1 mM EGTA, the experiment was started by incubation in 1 ml of buffer B (buffer A supplemented with 0.02% saponin, 3 mM ATP, and 1 \( \mu \text{Ci} \) of \( ^{45}Ca^{2+} \)/ml). \( ^{45}Ca^{2+} \) uptake in the absence of ATP was 24% of the ATP dependent uptake (data not shown). Where indicated, IP\(_3\), and 0.1 mM vanadate were added. The experiment was stopped by removing buffer B and washing three times with buffer A without bovine serum albumin. Then the cells were collected, and one part was added to 9 ml of scintillation fluid and counted for \( ^{45}Ca^{2+} \) radioactivity. The other part was used for determination of protein content as described (26).

**Isolation of Inositol Phosphates and Analysis of Phosphatidylinositol—**Fibroblasts were seeded onto 35-mm culture dishes (6-well plates) at a density of 1.4-1.8 \( \times 10^5 \) cells/dish. After 24 h, cells were washed twice with phosphate-buffered saline (140 mM NaCl, 2.7 mM KCl, 4.6 mM NaHPO\(_4\), 1.7 mM Na_2PO_4, 1 mM CaCl\(_2\), 0.5 mM MgCl\(_2\), 5.5 mM glucose, pH 7.4) and incubated for 48 h in 1 ml of inositol-free DMEM containing 0.5% FCS in the presence of 10 \( \mu \text{Ci} \) of myo-[2-\(^3\)H]inositol/ml. Cell density at this time was \( 2-3 \times 10^5 \) cells/dish. Oncogene expression was induced by 1 \( \mu \text{M} \) dexamethasone for 6 or 24 h. 45Ca\(^{2+}\) uptake in the absence of ATP was 24% of the ATP dependent uptake (data not shown). Where indicated, IP\(_3\), and 0.1 mM vanadate were added. The experiment was stopped by removing buffer B and washing three times with buffer A without bovine serum albumin. Then the cells were collected, and one part was added to 9 ml of scintillation fluid and counted for \( ^{45}Ca^{2+} \) radioactivity. The other part was used for determination of protein content as described (26).

**Analysis of Inositol Phosphates—**Cells (1.4-1.8 \( \times 10^5 \)) were seeded onto 35-mm culture dishes (6-well plates) and treated as described above. Instead of [2-\(^3\)H]inositol labeling, the cells were labeled for 2 h with \( ^{32}P\text{O}_4\) (10 \( \mu \text{Ci} \)/ml of medium). Phosphoinositides were separated by ion-exchange chromatography on AG 1 \( \times 8 \) formate (Bio-Rad) following the procedure of Berridge and Irvine (9). Where indicated, the identity of inositol phosphate isoforms was determined by HPLC analysis according to Heslop et al. (10) with the following modifications. The dichloroethane wash was neutralized (20 mM Tris-HCl, pH 7.4), filtered (0.2 \( \mu \text{m} \)), lyophilized, and subsequently stored at \(-20^\circ\text{C} \). Before application to a Whatman Partisil SAX HPLC column, each probe was resuspended in Hz0 containing 1 mM EGTA and a mixture of AMP, ADP, and ATP to inhibit autophosphorylation. A sample volume equivalent to 50 \( \mu \text{g} \) of each crude membrane preparation was finished by centrifugation of the resulting supernatant for 1 h at 100,000 \( \times g \) (Beckman L-5-55). Homogenization of the membrane pellets in lysis buffer was done by sonification for 10 s with a Branson sonifier at 25 watts. Protein content of the samples was determined as described (26). A sample volume equivalent to a protein content of 50 \( \mu \text{g} \) of each crude membrane preparation was used per lane and mixed with a corresponding volume of 5-fold concentrated Laemmli sample buffer, boiled for 3 min, and electrophoretically separated on 16-cm sodium dodecyl sulfate-15% polyacrylamide gels according to Laemmli (27). Subsequent electroblotting of proteins was done on Immobilon polyvinylidene difluoride transfer membranes (Millipore, Bedford, MA) according to Pfeeren (28) with minor modifications in a Bio-Rad Trans-Blot system for 90 min at a constant current of 3 mA/cm\(^2\) of gel. After transfer, p21\(^{\text{ras}}\) protein was immunodetected by exposure to rabbit polyclonal p21\(^{\text{ras}}\) antibody with subsequent treatment with \( ^{125}\text{I} \)-labeled protein A and autoradiography at \(-70^\circ\text{C} \) for 24 h on Hyperfilm-MP.

**RESULTS**

Addition of a saturating concentration of bombesin to growth-arrested NIH 3T3 fibroblasts causes a transitory increase in IP\(_3\) with a maximum around 10 s (Fig. 1, upper). HPLC analysis revealed that inositol 1,4,5-trisphosphate is the predominant IP\(_3\) isomer. The IP\(_3\) peak is followed by a rise in cytosolic [\( Ca^{2+} \)] (Fig. 2, upper). Fig. 2 (upper) also shows that bombesin and fetal calf serum cause similar \( Ca^{2+} \) responses. The accumulation of p21\(^{\text{ras}}\) in Ha-ras-transfected NIH 3T3 fibroblasts is shown in Fig. 3. For these experiments,
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FIG. 2. Time course of transient peak Ca\textsuperscript{2+} after expression of transforming Ha-ras(A) oncogene (upper) or Ha-ras(N) proto-oncogene (lower) by dexamethasone. NIH 3T3 cells were grown, and the cytosolic Ca\textsuperscript{2+} concentration was determined as described under "Experimental Procedures." The cells were stimulated with bombesin (2.5 \mu M) or 2.5% FCS (arrows). Typical tracings of uninduced control cells and cells 6 h after induction of the Ha-ras(A) oncogene (upper) or the Ha-ras(N) proto-oncogene (lower) by 1 \mu M dexamethasone (dex) are shown.

FIG. 3. Accumulation of p21\textsuperscript{ras} in Ha-ras-transfected NIH 3T3 cells. NIH 3T3 fibroblasts were grown and prepared for Western blot analysis of p21\textsuperscript{ras} as described under "Experimental Procedures." Expression of transforming Ha-ras and the Ha-ras proto-oncogene was induced by addition of 1 \mu M dexamethasone for 1, 3, 5, 6, or 24 h.

the transforming Ha-ras oncogene and a normal human Ha-ras proto-oncogene were subjected to transcriptional regulation by glucocorticoids by in vitro recombination with the promoter region of MMTV-LTR, and the resulting construct was transfected into NIH 3T3 cells. Some biochemical and biological consequences of this system have been described previously (1, 2, 11, 21, 24). Fig. 3 demonstrates that p21\textsuperscript{ras} is already clearly expressed 5 h after induction by dexamethasone in cells carrying transforming Ha-ras as well as in those transfected with the Ha-ras proto-oncogene. As described previously (21, 24), cells expressing transforming Ha-ras require a transformed morphological phenotype and progress into S-phase at low serum within ~24 h after addition of the hormone. Both effects are less expressed after induction of the proto-oncogene (24).

The effects of expression of transforming Ha-ras on the bombesin-induced IP\textsubscript{3} and Ca\textsuperscript{2+} signals are shown in Fig. 4.

As can be seen, both signals are depressed after induction of Ha-ras. These effects are specific for transforming ras and are not seen in cells overexpressing the Ha-ras proto-oncogene (Figs. 1 (lower) and 2 (lower)). Similar effects have been obtained with serum-stimulated Ha-ras-transformed NIH 3T3 cells (1). It is clear from Fig. 3 that the failure of the Ha-ras proto-oncogene to affect the bombesin-induced elevation of IP\textsubscript{3} and Ca\textsuperscript{2+} is not due to smaller quantities of p21\textsuperscript{ras} formed. On the contrary, the product encoded by the proto-oncogene accumulates to higher levels than the product of the oncogene. This is in accordance with previous observations in this system (21).

Fig. 4 exhibits the time course for the desensitization of the Ca\textsuperscript{2+}-mobilizing system by Ha-ras. As can be seen, reduction of the Ca\textsuperscript{2+} response is half-maximal 2 h after expression of the oncogene and fully expressed at 6 h, whereas formation of labeled IP\textsubscript{3} is nearly unchanged up to 6 h following induction of the oncogene in cells preincubated with \(^{3}H\)inositol for 48 h (Fig. 4). Identical results are obtained if the levels of IP\textsubscript{3} are determined by a mass measurement procedure (Table I).

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vation of inositol phosphate formation (1). The data of Fig. 5 are, however, not in accordance with such a mechanism. A significant elevation of inositol phosphate formation and $^{32}$P uptake into phosphatidylinositol 4,5-bisphosphate is not detectable earlier than 6 h after induction of the oncogene by dexamethasone. The desensitization of the Ca$^{2+}$-mobilizing system, however, is already expressed 2 h after addition of the inducer (Fig. 4). These results do not support the assumption of an IP$_3$-mediated depletion of intracellular Ca$^{2+}$ stores by Ha-ras. This conclusion was further substantiated by direct determinations of the load of intracellular nonmitochondrial Ca$^{2+}$ stores. For this purpose, cells were incubated with $^{45}$Ca$^{2+}$ in the presence of antimycin. The stored $^{45}$Ca$^{2+}$ was released by addition of the Ca$^{2+}$ ionophore A23187 and quantified by measuring the $^{45}$Ca$^{2+}$ released into the medium. Fig. 6 demonstrates that prestimulation of quiescent cells with serum growth factors leads to a significant decrease in the $^{45}$Ca$^{2+}$, which can be mobilized by subsequent treatment with A23187. Thus, a depletion of IP$_3$-sensitive Ca$^{2+}$ stores by Ha-ras should be detectable by this procedure. Table II demonstrates, however, that A23187 mobilizes identical amounts of Ca$^{2+}$ from nonmitochondrial stores of quiescent cells and cells expressing the Ha-ras oncogene for 26 h. These data indicate that expression of Ha-ras does not deplete the Ca$^{2+}$ load of IP$_3$-sensitive Ca$^{2+}$ stores.

Depression of intracellular Ca$^{2+}$ release under conditions where neither the formation of IP$_3$ nor the load of intracellular Ca$^{2+}$ stores is affected points to an interference of Ha-ras with IP$_3$ receptors or IP$_3$-regulated Ca$^{2+}$ channels. This assumption is supported by the data shown in Fig. 7 demonstrating that less Ca$^{2+}$ can be released by addition of IP$_3$ to permeable cells expressing transforming Ha-ras. This effect is expressed within 6 h following Ha-ras induction by dexamethasone.

**TABLE II**

<table>
<thead>
<tr>
<th>NIH 3T3 fibroblasts</th>
<th>Amount of $^{45}$Ca$^{2+}$ (cpm/10$^6$ cells)</th>
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<tr>
<td>p21$^{mm}$ not expressed</td>
<td>1916 ± 129</td>
</tr>
<tr>
<td>p21$^{mm}$ expressed for 26 h</td>
<td>2036 ± 175</td>
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**FIG. 6.** $^{45}$Ca$^{2+}$ release from nonmitochondrial stores by A23187. Quiescent NIH 3T3 cells were grown and prepared as described under "Experimental Procedures." Where indicated, 2.5% FCS (C) or 1 $\mu$M A23187 (G and D) was added. The amount of $^{45}$Ca$^{2+}$ efflux during each 2-min interval is expressed as counts/minute/10$^6$ cells.

**FIG. 7.** IP$_3$-induced $^{45}$Ca$^{2+}$ release in $^{45}$Ca$^{2+}$-labeled permeabilized NIH 3T3 cells. NIH 3T3 cells, transfected with transforming Ha-ras, were prepared as described under "Experimental Procedures." Upper, Ca$^{2+}$ release by 1 $\mu$M IP$_3$ in permeabilized cells 6 and 24 h after induction of p21$^{mm}$ versus noninduced controls. Steady-state distributions of ATP-dependent Ca$^{2+}$ uptake were attained within 12 min at 25°C (O, control; C, 24-h dexamethasone (dex) treatment). Addition of IP$_3$ (final concentration) in the presence of 0.1 mM vanadate immediately induced Ca$^{2+}$ release from intracellular, IP$_3$-sensitive stores (l). This effect was reduced if the Ha-ras oncogene was expressed by induction with 1 $\mu$M dexamethasone for 6 h (a) or 24 h (d). Data represent means ± S.E. (n ≥ 6) or means of two independent experiments. Lower, IP$_3$-mediated Ca$^{2+}$ release as function of IP$_3$ concentration in cells expressing Ha-ras and corresponding controls. Ca$^{2+}$ release was calculated as the difference between Ca$^{2+}$ uptake and residual Ca$^{2+}$ after IP$_3$ stimulation (2 min of incubation) at the concentrations indicated. Data (O, control; •, 24-h dexamethasone treatment) represent means of at least seven independent experiments ± S.E.

**DISCUSSION**

Ha-ras has been shown to alter the responsiveness of transformed cells to exogenous growth factors. The response to...
bradykinin was found to be greatly enhanced in cells overexpressing transforming Ki-ras (6, 12) and Ha-ras. This effect was explained by an increase in bradykinin receptor density (12). Cells transformed by Ha-ras have been shown to be desensitized to PDGF or bombesin (3–7). These suppressed responses were not paralleled by a reduction of the corresponding receptors (3), but seemed to indicate an uncoupling of the receptors from their effector phospholipase C. The depression of phospholipase C activity in these cases has been discussed as the result of a protein kinase C-mediated negative feedback loop (8). Such a receptor down-modulation would easily explain the desensitization of the Ca$^{2+}$-mobilizing system to serum growth factors or bombesin that has been observed after expression of Ha-ras (1–5). It is shown here that the desensitization of the Ca$^{2+}$-releasing system to bombesin which is observed 2–6 h after expression of Ha-ras cannot be explained by down-regulation of phospholipase C-coupled receptors. This conclusion is based on the observation that the reduction of Ca$^{2+}$ release is seen under conditions where the IP$_3$ response is unchanged. Thus, the depression by Ha-ras of the bombesin-induced mobilization of intracellular Ca$^{2+}$ is caused by a partial depletion of intracellular Ca$^{2+}$ stores or due to an interference with the Ca$^{2+}$-releasing mechanism downstream of phosphoinositidase C.

In previous publications (1, 2), we have demonstrated that NIH 3T3 cells transfected with a MMTV-LTR Ha-ras construct exhibit a growth factor-independent elevation of inositol phosphate formation and a concomitant increase in phosphatidylinositol turnover after expression of the oncogene by dexamethasone. Similar findings have been reported by other authors (16, 17). Since inositol phosphate levels showed a continuous increase over >36 h, an IP$_3$-mediated depletion of intracellular Ca$^{2+}$ stores seemed conceivable (1). The data presented here, however, are not in accordance with this supposition because (i) the desensitization of the Ca$^{2+}$-mobilizing system precedes the elevation of inositol phosphates by several hours and (ii) the Ca$^{2+}$ load of nonmitochondrial intracellular stores was found to be unchanged after expression of Ha-ras. As far as the time courses of inositol phosphate formation and Ca$^{2+}$ desensitization are concerned, it may be argued that the elevated inositol phosphate levels consisted almost exclusively of inositol mono- and bisphosphates. The IP$_3$ peak may have appeared much earlier and may have been missed for technical reasons. This, however, is unlikely. Careful analysis at earlier and closely timed intervals after expression of the oncogene never revealed IP$_3$ levels exceeding 5–10% of control values. Furthermore, metabolic labeling of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) with $^{32}$PO$_4$ exceeds control levels in orthophosphate uptake not before 6 h following expression of the oncogene (Fig. 5), indicating that the turnover of the IP$_3$ precursor (PIP$_2$) is increased at about the same time when a significant rise in total inositol phosphates is detectable.

In view of the data presented here, it seems possible that Ha-ras interrelies with the Ca$^{2+}$-mobilizing system by directly affecting IP$_3$-regulated Ca$^{2+}$ channels. This assumption is supported by the finding pointing to the addition of IP$_3$ to permeabilized cells mobilizes less Ca$^{2+}$ from intracellular stores in cells expressing a transforming Ha-ras gene than in corresponding controls. It is not yet clear, however, whether this phenomenon is responsible for the desensitization of the Ca$^{2+}$-mobilizing system to bombesin by Ha-ras. Data on the actual IP$_3$ concentrations at the IP$_3$ receptor are required before further conclusions can be drawn. Furthermore, it remains to be elucidated whether the reduction of IP$_3$-mediated Ca$^{2+}$ release is caused by a decrease in IP$_3$ receptors, is due to a change in the affinity to IP$_3$, or is the result of reduced receptor-effector coupling. Finally, IP$_3$-sensitive Ca$^{2+}$ stores may represent a small percentage of total nonmitochondrial Ca$^{2+}$ compartments. The total Ca$^{2+}$ response which is seen after stimulation by bombesin or serum growth factors may in part depend on translocation of Ca$^{2+}$ from IP$_3$-insensitive to IP$_3$-sensitive pools. Ha-ras may interfere with this Ca$^{2+}$ translocation. Evidence for the existence of two intracellular Ca$^{2+}$ compartments distinguishable by IP$_3$ sensitivity has been reported (19). Interestingly, Ca$^{2+}$ translocation between the compartments requires GTP (18). Circumstantial evidence suggests that small ras-like GTP-binding proteins of Mr 21,000–28,000 are involved in mediating the Ca$^{2+}$ exchange between intracellular Ca$^{2+}$ pools (19, 20). Thus, the effect of transforming Ha-ras on the mobilization of intracellular Ca$^{2+}$ may be as closely related to the biological function of p21ras.

Our findings demonstrating a reduction of the IP$_3$-mediated Ca$^{2+}$ release by Ha-ras are not in agreement with data reported by Olinger et al. (7), who found that a microinjection of IP$_3$ can induce a normal Ca$^{2+}$ response in EJ-ras-transformed NIH 3T3 cells exhibiting a desensitization to PDGF. However, the system employed by Olinger et al. (7) differs from the system used here in several aspects. They employed a stable EJ-ras-transformed cell line under the influence of PDGF, whereas our data have been obtained with bombesin during the first hours after expression of an inducible Ha-ras construct.

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