DIFFERENTIAL REGULATION OF PITUITARY-SPECIFIC GENE EXPRESSION BY INSULIN-LIKE GROWTH FACTOR 1 IN RAT PITUITARY GH4C1 AND GH3 CELLS

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ABSTRACT
We have compared the influence of insulin-like growth factor 1 (IGF-1) on pituitary gene expression in the rat cell lines GH4C1 and GH3. Incubation with IGF-1 increased PRL messenger RNA (mRNA) levels in GH4C1 cells by 4- to 5-fold but decreased the levels of PRL transcripts in GH3 cells. In addition, the levels of GH-mRNA that were not affected by IGF-1 in GH4C1 cells were significantly inhibited by the growth factor in GH3 cells. IGF-1 also decreased PRL and GH-mRNA response to T3, retinoic acid, and Fk in GH3 cells. Stability of PRL or GH transcripts was not altered by IGF-1 in GH3 cells, suggesting that the inhibitory effect is exerted at a transcriptional level. The pituitary-specific transcription factor GHF-1/Pit-1 activates both the GH and PRL promoters. As analyzed by Western blot, IGF-1 did not alter GHF-1/Pit-1 protein levels in GH4C1 cells but reduced the levels of the transcription factor in GH3 cells. This decrease is secondary to a reduction of GHF-1/Pit-1 transcripts in IGF-1-treated GH3 cells. Thus, a different effect of IGF-1 on the expression of GHF-1/Pit-1 in GH3 and GH4C1 cells is likely involved in the different regulation of GH and PRL gene in both cell types. IGF-1 increases the activity of the PRL promoter in transient transfection assays in GH4C1 cells by a Ras-dependent mechanism. Expression of oncogenic RasVal12 mimics the effect of IGF-1, and the dominant negative RasAom17 blocks IGF-1-mediated stimulation of the PRL promoter in GH4C1 cells. Although IGF-1 did not stimulate the PRL promoter in GH3 cells, RasVal12 strongly activated the promoter in these cells. Hence, the machinery to activate Ras-dependent signaling is intact in GH3 cells. Moreover, IGF-1 stimulates the mitogen-activated protein kinase in GH5 cells, showing that the components linking the IGF-1 receptor to Ras are also active. These results suggest that, in addition to the Ras/mitogen-activated protein kinase pathway, IGF-1 could activate a different pathway and that the combination of both is required to elicit PRL gene expression by the growth factor. This second pathway may be defective in GH3 cells that respond to Ras but not to IGF-1. (Endocrinology 138: 5442–5451, 1997)
have demonstrated that activation of the PRL promoter by IGF-1 in GH4C1 cells requires activation of the Ras/MAP-K pathway, because dominant negative mutants of these proteins block stimulation of PRL gene expression by IGF-1. Our results also suggest that IGF-1 could stimulate additional, independent pathway(s), which cooperate with the Ras/MAP-K to elicit PRL gene expression.

In this study, we have compared the regulation of PRL, GH, and GHF-1/Pit-1 gene expression in pituitary GH4C1 and GH3 cells. Both cell lines are closely related and have retained the ability of normal somatolactotrophs to produce GH and PRL. The GH3 cells were cloned from pituitary tumor cells transferred twice between animal and tissue culture, and the GH4C1 cells are a cloned variant of the GH3 cells (14). Our results show a strikingly different regulation of these genes by IGF-1. In GH4C1 cells, the growth factor induces PRL gene expression without significantly affecting the GH gene. In contrast, IGF-1 strongly inhibits PRL and GH gene expression in GH3 cells. This occurs despite the fact that oncogenic Ras activates the PRL promoter in GH3 cells and that IGF-1 activates the MAP-Ks in a manner indistinguishable from that found in GH4C1 cells. A differential regulation of GHF-1/Pit-1 might contribute to the different regulation of GH and PRL gene expression in both cell lines. Expression of the GHF-1/Pit-1 gene is suppressed by IGF-1 in GH3 cells, where the growth factor inhibits pituitary-specific gene expression, but is not reduced in GH4C1 cells, where it activates the PRL gene.

Materials and Methods

RNA extraction and hybridization

GH4C1 cells were grown in DMEM containing 10% FCS, and GH3 cells were cultured in RPMI medium containing 15% donor horse serum and 2.5% FCS (GIBCO BRL, Grand Island, NY). The cells were incubated for 24 h in a medium containing 10% AG1-X8 resin-charcoal stripped FCS (GIBCO BRL, Grand Island, NY). The cells from each electroporation were split into different culture plates and incubated overnight in DMEM containing 10% AG1-X8 resin-charcoal stripped newborn calf serum. This hormone-depleted medium was replaced by serum-free medium, and the treatments were administered. In some assays, in which the cells were cotransfected with the PRL reporter construct and expression vectors, the amount of DNA was kept constant by addition of the same amount of an empty noncoding vector (RSV-0). Each transfection also received 2.5 μg of a luciferase vector to monitor transfection efficiency. Each treatment with the ligands was performed, at least, in duplicate cultures that normally exhibited less than 10% variation in CAT activity. CAT activity was determined by incubation of the cell extracts with [3H]chloramphenicol. The unreacted and acetylated [3H]chloramphenicol were separated by TLC, identified by autoradiography, and quantified. The data are expressed as the percentage of acetylated forms after each treatment. Each experiment was repeated at least two or three times with similar relative differences in regulated expression.

Western blot analysis

The levels of GHF-1/Pit-1 were determined by Western blot analysis. GH4C1 or GH3 cell extracts (10 μg) were run in a 12% polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane and incubated with a 1:1000 dilution of a polyclonal antibody that recognizes the transcription factor, and GHF-1/Pit-1 was identified by chemiluminescence (24).

Determination of MAP-K activity

GH4C1 and GH3 cells were plated in 60-mm diameter dishes in growth medium. After 24 h, cells were switched to a resin-charcoal depleted medium, and after an additional 12 h period, were serum starved for 18 h and exposed to 13 nM IGF-1 or 100 nM phorbol 12-myristate 13-acetate (TPA) for different time periods. Cells were then lysed in 300 μl of cold lysis buffer (10 mM EGTA, 40 mM β-glycerophosphate, 1% NP-40, 2.5 mM MgCl2, 2 mM orthovanadate, 1 mM Dithiothreitol, 20 mM HEPES, pH 7.5) containing protease inhibitors (leupeptin, aprotinin, benzamidine, and phenylmethylsulfonyl fluoride). Cell extracts (100 μg) were incubated with 5 μl ERK-2 (C-14) rat polyclonal antibody (Santa Cruz Biotechnology, Germany) at 4°C for 1 h. Protein A-Sepharose was added and the samples incubated for an additional hour and centrifuged. The pellets were washed three times with PBS, 1% NP-40, and 2 mM orthovanadate; and once with 1% Triton-X-100 (pH 7.5), 0.5 mM LiCl, and kinase buffer (2 mM Dithiothreitol, 20 mM glycerophosphate, 20 mM NaPP, 20 mM MgCl2, 0.1 mM orthovanadate, and 20 mM HEPES, pH 7.6). The immunoprecipitates were then incubated in 30 μl of kinase buffer containing 20 μM ATP, 2 μCi of γ-32P-ATP, and 1 μg myelin basic protein (MBP) as a substrate. After incubation for 20 min at 30°C, the samples were subjected to SDS-PAGE in 15% acrylamide gels, dried, and autoradiographed. MBP band density on the autoradiographs was quantitated by densitometry.

Results

Influence of IGF-1 on PRL- and GH-mRNA levels in GH4C1 and GH3 cells

Figure 1 compares the effect of a 48-h incubation with 13 nM IGF-1 on PRL and GH-mRNA levels in GH4C1 and GH3 cells. IGF-1 significantly increased (4- to 5-fold) PRL transcripts in GH4C1 cells. In contrast with these results, IGF-1 caused a strong decrease of PRL mRNA levels in GH3 cells. A reduction of GH-mRNA levels also was observed in IGF-1-treated GH3 cells, whereas this factor did not significantly alter GH transcripts in GH4C1 cells. Quantification of Northern blots from five different experiments showed that IGF-1 reduced by 40–75% PRL- and GH-mRNA levels in GH3 cells.
In GH41 cells, GH-mRNA levels only varied between 0 and 15% after treatment with IGF-1.

The effect of IGF-1 in combination with T₃ on PRL and GH gene expression also was analyzed in both cell lines. Treatment with 5 nM T₃ stimulated PRL mRNA levels in both GH4C1 and GH3 cells. In GH3 cells, this response was strongly suppressed in the presence of IGF-1, whereas IGF-1 did not inhibit this response in GH4C1 cells. T₃ consistently induced GH gene expression in both cell lines, and IGF-1 was able to partially inhibit the stimulatory effect of T₃ on GH gene expression in GH3 cells. In the experiment illustrated in the figure, induction by T₃ decreased from 6-fold in the absence of IGF-1, to 4-fold in its presence. This inhibition was less marked in GH4C1 cells and was not consistently observed in independent experiments.

We also examined the response to IGF-1 in RA-treated cells. Figure 2 shows GH and PRL-mRNA levels in GH3 and GH4C1, incubated for 48 h with 1 μM RA in the presence and absence of IGF-1. In results similar to those obtained with T₃, IGF-1 reduced the GH response to RA in GH3 cells. In addition, we observed that RA increases PRL transcripts in both GH4C1 and GH3 cells. The induction of PRL-mRNA by RA is less marked than that of GH-mRNA, but it was consistently observed in different experiments. IGF-1 also decreased significantly the PRL response to RA in GH3 cells, whereas it acted additively with RA to increase PRL transcripts in GH4C1 cells.

Figure 3 shows the influence of incubation with IGF-1 on the PRL response to 10 μM Fk. Again, IGF-1 significantly reduced the basal levels of PRL transcripts in GH3 cells. Incubation with Fk for 48 h increased by about 2-fold PRL-mRNA and significantly attenuated the inhibitory response of IGF-1. In contrast, IGF-1 induced PRL-mRNA levels more strongly than Fk in GH4C1 cells, and these levels were maximal with the combination of Fk and IGF-1.

Regulation of PRL promoter activity in GH4C1 and GH3 cells

The 5′-flanking region of the PRL gene mediates transcriptional responses to several hormones and second messengers. Experiments, in which GH4C1 and GH3 cells are transfected with a Prl-CAT construct containing 3000 bp of the rat PRL promoter, are shown in Fig. 4A. Incubation with IGF-1 produced a significant increase of CAT activity in GH4C1 cells transfected with the 2300Prl-CAT construct. The proximal region of the PRL gene is sufficient to mediate this response, because IGF-1 increases the activity of a PRL promoter construct containing only 176 bp (−176Prl-CAT) to an extent similar to that found with −3000Prl-CAT (data not
shown). Incubation with Fk activated the −3000Prl-CAT construct in GH4C1 cells (28, 29), and CAT activity was maximal in cells incubated with the combination of IGF-1 and Fk. However, Fk caused an apparent attenuation of the IGF-1 response. Fk treatment seems to decrease the IGF-1 response from 3-fold in the absence of the cAMP stimulator, to only 2-fold in its presence.

According to the data presented in Figs. 1–3, where IGF-1 decreased PRL-mRNA levels in GH3 cells, it was expected that IGF-1 should decrease PRL promoter activity in these cells. However, we have not been able to find regulation of the PRL promoter by IGF-1 in transient transfection assays. As shown in panel A, Fk stimulated the PRL promoter, but IGF-1 was ineffective in regulating either basal activity or the response to the activator in GH3 cells.

The stimulatory effect of IGF-1 on the PRL promoter in GH4C1 cells was specific for this promoter. Figure 4B shows that the growth factor does not significantly induce GH promoter activity in these cells. However, IGF-1 was ineffective in regulating either basal activity or the response to the activator in GH3 cells.

It was conceivable that, if insulin receptors are expressed at substantial levels in GH4C1 cells, the stimulation of the PRL promoter by IGF-1 may reflect binding of IGF-1 to the insulin receptor. However, as shown in Fig. 5, this possibility can be dismissed. IGF-1 caused a dose-dependent stimulation of the −3000Prl-CAT construct in GH4C1 cells, with a half-maximal response found at approximately 2 nM. However, incubation with insulin, at concentrations similar to those of IGF-1, was unable to affect the PRL promoter in these cells. Neither IGF-1 nor insulin had a significant effect on the activity of the PRL promoter in GH3 cells (data not shown).

One potential mechanism for the differences in the regulation of PRL promoter activity between both cell lines could be that GH3 cells may have inadequate levels of IGF-1 receptors, relative to GH4C1 cells. To test this possibility, both cell types were cotransfected with the −3000Prl-CAT construct and an expression vector for the IGF-1 receptor. Upon overexpression of the receptor, IGF-1 strongly increased pro-
in control GH3 cells and in cells incubated with IGF-1, T3, or their combination (right panel). GH-mRNA also was very stable in GH3 cells, whereas the growth factor had little, if any, effect in GH3 cells (panel A). Interestingly, IGF-1 further increased promoter activity in the presence of oncogenic Ras. Although IGF-1 was unable to regulate PRL promoter activity in GH3 cells, expression of oncogenic Ras<sup>Val12</sup> strongly induced the activity of the PRL promoter in GH4C1 cells (panel B), showing that the signaling pathway downstream of Ras is intact in these cells. As expected (29), Fk did not stimulate the promoter in GH4C1 or GH3 cells expressing Ras, and in agreement with the data shown in Fig. 4A, the response to IGF-1 (expressed as fold-induction) decreased in Fk-treated cells. The dominant negative Ras<sup>Asn17</sup> mutant blocked specifically the induction caused by IGF-1 in GH4C1 cells, confirming that activation of the PRL promoter by this factor requires functional endogenous Ras; but this mutant did not affect the PRL promoter in GH3 cells, where IGF-1 was inactive.

**Stimulation of MAP-K activity by IGF-1 in GH3 and GH4C1 cells**

The lack of stimulation of the PRL gene by IGF-1 in GH3 cells could reflect uncoupling of the receptor from the Ras/MAPK pathway. If this were the case, a downstream component of this pathway, such as the MAP-K, should not be activated in response to IGF-1 in these cells. Alternatively, this response could be preserved in GH3 cells; and an additional pathway, different from the Ras/MAPK pathway, also required for PRL gene regulation by IGF-1, would be lost. We have therefore examined the MAP-K response to IGF-1 in GH3 cells. Figure 9 compares MAP-K activity obtained in lysates from GH3 and GH4C1 cells incubated with IGF-1 for increasing time periods. IGF-1 produced a rapid and transient MAP-K activation in GH3 cells that was not
significantly different from that found in GH4C1 cells. In both cell types, a maximal effect was found at 2–6 min, and the values returned to normal after 10 min. An incubation with 100 nM TPA for 10 min was used as a control of MAP-K activation in these cells. At this time, TPA caused a stimulation that was at least two-fold stronger than the maximal IGF-1 induction.

**Influence of IGF-1 on GHF-1/Pit-1 expression in GH4C1 and GH3 cells**

The different behavior of the PRL and GH genes in GH4C1 and GH3 cells could be related to a different regulation of GHF-1/Pit-1 gene expression by IGF-1. For instance, the decrease in PRL and GH transcripts in IGF-1-treated GH3 cells could be secondary to a reduction in the concentration of GHF-1/Pit-1 that activates both genes. To test this hypothesis, Western blot analysis was performed with extracts from GH4C1 and GH3 cells. Proteins obtained from control cells and from cells treated with 13 nM IGF-1 for 48 h were assayed using a specific anti-GHF-1/Pit-1 antibody. Figure 10 (panel A) shows that the concentration of the 33-kDa protein doublet of GHF-1/Pit-1 protein decreased in GH3 cells treated with IGF-1. Quantification of this blot and additional experiments showed that treatment with IGF-1...
reduced, by 30–50%, GHF-1/Pit-1 levels in GH3 cells. However, IGF-1 did not decrease the abundance of this transcription factor in GH4C1 cells.

The influence of IGF-1 on GHF-1/Pit-1 mRNA levels also was determined in both cell types. Figure 10 (panel B) shows that two major classes of GHF-1/Pit-1 transcripts were found: a more prominent 3.1-kb band and a less abundant band of 1.2-kb. A fainter transcript of approximately 4 kb also was often detected. In agreement with the GHF-1/Pit-1 protein concentrations, the level of GHF-1/Pit-1 transcripts was reduced by IGF-1 in GH3 but not in GH4C1 cells. As in the case of the PRL and GH promoters, the activity of the GHF-1/Pit-1 promoter in transient transfection assays was not reduced by IGF-1 in GH3 cells (data not shown).

Figure 11 shows the levels of GHF-1/Pit-1 transcripts in GH4C1 and GH3 cells treated with T 3, RA, and Fk in the presence and absence of IGF-1. As illustrated in panel A, in GH3 cells, the inhibition of GHF-1/Pit-1 transcripts by IGF-1 was less marked than that caused by T 3, and the combination of both did not produce further decreases. In addition, IGF-1-mediated reduction of GHF-1/Pit-1 mRNA levels was significantly attenuated in the presence of RA or Fk. Figure 11B shows that IGF-1 alters neither basal levels of GHF-1/Pit-1 mRNA nor RA-dependent stimulation or T 3-dependent inhibition in GH4C1 cells.

The lack of response of the PRL promoter to IGF-1 in GH3 cells may reflect the presence of limiting amounts of GHF-1/Pit-1 in these cells. To directly test this hypothesis, the effect of IGF-1 on the –3000Prl-CAT construct also was examined in GH3 and GH4C1 cells transfected with an expression vector encoding GHF-1/Pit-1. As shown in Fig. 12, expression of GHF-1/Pit-1 had little, if any, effect on PRL promoter activity in GH4C1 cells. However, basal promoter activity increased in GH3 cells transfected with GHF-1/Pit-1, and under these conditions, IGF-1 did not decrease the activity of the PRL promoter but, instead, increased promoter activity by approximately 2-fold.

Discussion

This work presents evidence of alternate inhibition or stimulation of the PRL gene by IGF-1 in two different rat pituitary cell lines. Whereas in GH4C1 cells, expression of this gene is strongly induced by IGF-1, in GH3 cells, the growth factor reduces PRL transcripts and represses the response to different PRL inducers, such as T 3, RA, or Fk (28–31). The GH gene, another pituitary-specific gene, also is differentially regulated by IGF-1. The expression of GH is not significantly affected by IGF-1 in GH4C1 cells but is reduced in GH3 cells. We have previously shown that RA induces GH gene transcription in GH1 and GH4C1 cells, and we have mapped the RA response element to the same sequences that mediate regulation by T3 (26, 27). Our present results show that RA and T 3 also induce GH gene expression in GH3 cells, and we have mapped the RA response element to the same sequences that mediate regulation by T 3 (26, 27).
GH3 cells and that this decrease is secondary to a reduction in GHF-1/Pit-1 mRNA levels. This observation is in agreement with a recent report (32) showing that IGF-1 inhibited basal GHF-1/Pit-1 mRNA concentration and blunted the response to GH-RH in primary cultures of rat anterior pituitary cells. However, GHF-1/Pit-1 levels were not reduced by IGF-1 in GH4C1 cells. Furthermore, we have previously described that whereas T3 decreases (33), RA increases (34) GHF-1/Pit-1 mRNA levels, and these responses are not modified by IGF-1 in GH4C1 cells.

The reduction in the concentration of the transcription factor that is required for the expression of both the GH and PRL genes is certainly in agreement with the decreased levels of their transcripts observed in IGF-1-treated GH3 cells. Unexpectedly, the activity of the PRL and GH promoters, which contain the binding sites for GHF-1/Pit-1, was not inhibited by IGF-1 in GH3 cells in transient transfection assays. Although we do not dismiss the possibility that the sequences responsible for the inhibitory effect of IGF-1 on pituitary gene expression in GH3 cells could be outside the promoter fragments examined, it seems likely that the results obtained in the transient transfection assays do not exactly reflect the behavior of the endogenous gene. Discrepancies between the regulation of the endogenous PRL gene and the transiently transfected promoter have been previously reported to occur for the regulation of this gene by insulin. It has been found that insulin (as observed by us with IGF-1 in these cells) stimulates expression of the endogenous gene but, under identical conditions, does not stimulate PRL-CAT plasmids (35, 36). The authors attribute the lack of response of the transfected gene to the existence of low levels of insulin receptors in these cells, because cotransfection with an insulin receptor expression plasmid was required to observe insulin stimulation of the PRL promoter. Furthermore, it has been previously shown (37) that GH3 cells are relatively

![Fig. 11. Regulation of GHF-1/Pit-1 mRNA levels by IGF-1, T3, RA, and forskolin in GH4C1 and GH3 cells. RNA obtained from GH3 cells (panel A) and GH4C1 cells (panel B) was used for Northern blot analysis with the GHF-1/Pit-1 cDNA probe. The RNA was obtained from control untreated cells and from cells incubated with 5 nm T3, 1 µm RA, or 10 µm forskolin (Fk) in the presence or absence of 13 nM IGF-1, as indicated.](image)

![Fig. 12. Effect of expression of GHF-1 on the PRL promoter response to IGF-1. GH4C1 and GH3 cells were transfected with 10 µg of 3000Prl-CAT alone (–) or in combination with 10 µg of a vector expressing GHF-1. CAT activity was determined 48 h after transfection in control cells and in cells treated with 13 nM IGF-1. The data represent the mean ± SD of the values obtained from three independent cultures.](image)
resistant, as compared with GC cells, to the action of IGF-1 because they contain a lower number of IGF-1 receptors. Therefore, it was likely that the concentration of the IGF-1 receptors may be insufficient in GH3 cells to cause a reduction in the activity of the GH and PRL promoters in transient transfection assays. However, this possibility can be dismissed, because the overexpression of IGF-1 receptors does not confer a significant promoter response to IGF-1 in GH3 cells. In contrast, IGF-1 stimulation of the PRL promoter is observed in GH3 cells transfected with an expression vector encoding GHF-1/Pit-1. This result, which further suggests a role of the endogenous GHF-1/Pit-1 concentration in the differential response to IGF-1, is in agreement with the finding that overexpression of the transcription factor also enhances activation of the PRL promoter by Ras in GH4C1 cells (12).

In contrast to the findings in GH3 cells, the PRL promoter is significantly activated by IGF-1 in GH4C1 cells, and this response is further enhanced upon overexpression of the IGF-1 receptor. Stimulation by the growth factor in GH4C1 cells requires activation of the Ras/MAP-K pathway, because it is blocked by a dominant negative Ras mutant. It was then possible that some component of this pathway was defective in GH3 cells. Our results show that the pathway downstream of Ras is intact, because oncogenic Ras clearly activates the PRL promoter in GH3 cells. It also was possible that a component linking the IGF-1 receptor to Ras was uncoupled in these cells. If this were the case, IGF-1 should not activate this signaling pathway. However, the growth factor transiently stimulated MAP-K activity in GH3 cells. Both the intensity and the time-course of the activation were similar in GH3 and GH4C1 cells. From these results, it can be deduced that the defect occurs in other pathway(s), in addition to the Ras/MAP-K cascade. Although IGF-1 is a potent growth factor for other cell types, it exerts little mitogenic effects in pituitary cells (6). The rapid induction of MAPK activity, after incubation with growth factors, is followed by a slower and persistent late phase of activity that correlates with mitogenic activity, and no second phase of activation is observed in GC, GH4C1, or GH3 cells (Ref. 6, and this study). Therefore, the influence of IGF-1 on pituitary gene expression should not merely reflect changes in cell growth and the cell cycle.

The existence of an additional signaling pathway that diverges after receptor activation and contributes to the stimulation of the PRL promoter by IGF-1 is suggested by the finding that, in GH4C1 cells, IGF-1 can further increase the activity of the PRL promoter in the presence of oncogenic Ras. It is interesting that EGF, also a ligand of a tyrosine kinase receptor, activates the PRL promoter by a Ras-independent mechanism in pituitary GH4 cells (38). The exact mechanism by which this factor regulates the PRL gene is still unknown, but it is well established that ligand-activation of different receptor tyrosine kinases allows several proteins to become associated with specific phosphotyrosines and to initiate different signaling cascades. Our results are compatible with a model in which IGF-1 uses both the Ras/MAP-K and other pathway(s) to activate the IGF-1 gene. Stimulation of such pathways would be required for the IGF-1 action. GH3 cells would be deficient in the second pathway and, therefore, in stimulation of the PRL promoter, whereas GH4C1 cells would respond to IGF-1 by stimulating both pathways and, consequently, the PRL gene. That the second pathway is alone insufficient for this stimulation is shown by the finding that the dominant negative Ras mutant abolishes the IGF-1 response in GH4C1 cells. The finding that oncogenic Ras, but not IGF-1, is able to activate the PRL promoter in GH3 cells suggests that a strong and sustained stimulation of Ras, that is not obtained when the endogenous Ras is transiently activated by IGF-1, is required to achieve PRL promoter activation.

Although different pituitary cell lines are normally indistinctly used in studies of hormonal regulation of gene expression, there are previous reports of differential regulation in different pituitary cell lines. This is especially true for the PRL gene. For instance, T3 decreases PRL gene expression in GH1 cells, whereas it increases PRL transcripts in GH3 and GH4C1 cells (Refs. 21 and 30, and this study). Furthermore, insulin has been shown to stimulate the PRL gene in GH4C1 cells (31, 35, 39) without affecting PRL expression in primary pituitary cultures (8). Pituitary gene response to IGF-1 in primary cultures of rat anterior pituitary cells is rather similar to that found by us in GH3 cells and strikingly different to that observed in GH4C1 cells. IGF-1 has an inhibitory effect on GH and GHF-1/Pit-1 gene expression in GH3 and normal pituitary cells, but it has a specific stimulatory effect on PRL transcription without affecting GH or GHF-1/Pit-1 expression in GH4C1 cells. The different behavior of the two cell lines could be related to the fact that, although both are tumor cells, the GH3 cells are not variant cell lines and thus are presumably one step closer than GH4C1 cells to their normal counterparts. Regulation of pituitary gene expression provides a powerful tool for future studies on the delineation of the molecular mechanisms by which the same hormone or growth factor can repress or stimulate the same gene in closely related cell types.

Finally, the action of IGF-1 in the pituitary axis must be complex and should take into account other factors such as IGF-2 or the IGF-binding proteins (IGFBPs). Although our experiments have been performed in the absence of serum, how each of the different binding proteins functions to augment or inhibit the effect of IGF-1 in pituitary gene expression, or how IGF-1 works in conjunction with IGF-2 or other factors, has not yet been delineated and will require future studies.

References


10. Deleted in proof


23. Feig L, Cooper GM 1988 Inhibition of NIH 3T3 cell proliferation by a mutant Ras protein with preferential affinity for GDP. Mol Cell Biol 8:3235–3243.


