Activin increases phosphorylation and decreases stability of the transcription factor Pit-1 in MtTW15 somatotrope cells

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Activin is a polypeptide growth factor which exerts endocrine, paracrine, and autocrine effects in a variety of tissues. In the pituitary somatotrope, activin represses proliferation and growth hormone (GH) biosynthesis and secretion. We previously demonstrated that decreases in GH biosynthesis in MtTW15 somatotrope cells are due at least in part to decreased binding of the tissue-specific transcription factor, Pit-1, to the GH promoter, resulting in decreased transcription of the GH gene. The objective of the current study was to determine the extent to which activin-mediated decreases in GH transcription were the result of decreased Pit-1 activity and/or decreased Pit-1 protein content in MtTW15 cells. Activin caused rapid increases in Pit-1 phosphorylation, which were temporally correlated with decreases in GH DNA binding. Pit-1 phosphorylation preceded marked decreases in steady-state levels of Pit-1 protein. The rate of Pit-1 synthesis was only moderately decreased by activin, with a time-course similar to that observed for decreases in GH biosynthesis. However, Pit-1 stability was markedly decreased after more than 4 h of activin treatment. These data demonstrate that activin decreases GH expression in MtTW15 cells through multilevel regulation of Pit-1, which may represent a more general mechanism whereby activin and other transforming growth factor β family members modulate gene expression through regulation of transcription factor activity as well as content.

Activins and inhibins are members of the TGF-β superfamily of growth and differentiation factors which exert endocrine, paracrine, and autocrine effects in a variety of tissues. Other members of this growing superfamily include Müllerian inhibiting substance (1), glial-derived neurotrophic factor (2), several bone morphogenetic proteins (3), dorsalin (4), nodal (5), the Drosophila decapentaplegic (6) and Vgr60A (7) gene products, and Xenopus Vg1 (8). Activins are dimeric proteins, composed of two β subunits (homo- or heterodimers of βA:βA, βA:βB, or βB:βB) whereas the related inhibins are heterodimers composed of one α subunit and one of the β subunits. Although first purified from gonadal sources, activins are also found in brain, uterus, placenta, adrenals, bone marrow, and the gonadotropes of the anterior pituitary gland (9). Activins exert effects on the secretion and production of a variety of endocrine products, as well as altered growth, differentiation, and function of a variety of cells. These include ovarian theca and granulosa cells, testicular Leydig cells, erythroid progenitor cells, gonadotropin-releasing hormone-producing hypothalamic cells, and pituitary gonadotropes, somatotropes, and corticotropes (10), as well as oxytocin-producing hypothalamic paraventricular neurons (11). In addition, within the last few years, a number of investigators have demonstrated a major role for activin in the developing embryo as a dorsalizing mesoderm-inducing signal (12–14).

The molecular mechanisms by which activin exerts these multiple actions in a variety of cell types are incompletely understood. Activin forms a heteromeric receptor complex with a high affinity ligand binding component (type II, ~75-kDa protein) and a second component (type I, ~55-kDa protein) whose activin binding is dependent upon the presence of type II receptor and whose role is thought to be that of propagating the activin signal to downstream targets. Multiple cDNAs of both types of activin receptors have been cloned (type II (15–17) and type I (18–21)) and were found to be members of the same gene superfamily, along with receptors for related ligands such as TGF-β1 and Müllerian inhibiting substance. These receptor molecules are serine/threonine kinases, each with a single transmembrane domain. The type II receptor proteins (II and IIB) have been shown to be autophosphorylated on serine and threonine residues when expressed in mammalian cells, as well as in vitro kinase assays (22–24) similar to the TGF-β1 system (25). The type I receptors (ActRI and ActRIB) show limited autophosphorylation, but are highly phosphorylated on serine and threonine residues (26) in the presence of type II receptor kinase activity. Evidence in both the activin (26) and the TGF-β systems (27, 28) indicates that the kinase domains and/or activities of both type I and type II receptors are required for downstream signaling. Thus, the effects of activin (as well as TGF-β) are thought to be mediated by a signaling pathway initiated by phosphorylation of both types of receptors.

Activin has been demonstrated to be a negative regulator of the pituitary somatotrope, in which activin represses cellular proliferation and growth hormone (GH) biosynthesis and secretion (29, 30). The somatotrope has been the subject of extensive studies to characterize the regulation of GH gene transcription (reviewed in Ref. 31) and has provided a useful system for analyzing the regulatory mechanisms controlling cell commitment, proliferation, and differentiation in vertebrates (32, 33). Using a transplantable rat somatotrope tumor model, MtTW15, which is responsive to both growth hormone releasing factor (34) and activin, we have previously demonstrated that activin decreases GH biosynthesis at least in part through
decreased transcription of the GH gene in primary cultures of MtTW15 cells (35). Electrophoretic mobility shift analyses indicated that decreased transcription was the result of decreased interaction of the tissue transcription factor Pit-1, also called GHF-1, to its proximal and distal cis-acting elements on the GH promoter. Pit-1 is a member of the POU-domain family of transcriptional and cell-specific regulators which constitute a subclass of the homeobox genes (32, 33, 36). Expression of transcriptional and cell-specific regulators which constitute the GH promoter. Pit-1 is a member of the POU-domain family called GHF-1, to its proximal and distal sequences.

The purpose of the current study was to determine the causes of activin repression of Pit-1-GH promoter interactions. We report that activin exerts multilevel regulation of Pit-1 involving early decreases in Pit-1 DNA binding activity associated with increased Pit-1 phosphorylation, later decreases in the overall Pit-1 content as a result of decreased stability, and, to a lesser extent, synthesis of Pit-1.

**EXPERIMENTAL PROCEDURES**

**Materials—** Recombinant human activin A was provided by J. Mather, Genentech Inc. (132-Pit antisera and purified recombinant Pit-1 were generously provided by M. G. Rosenfeld, UCSD (37). Anti-monkey anti-rat GH antibody was a gift from the NIDDK hormone distribution program.

**Cell Culture—** MtTW15 somatotropic tumors were passaged and cultured as described previously (35). Cells were routinely cultured at a density of 3–5×10^5 cells per 10-cm dish in 10% FCS-β-PIT J2 ule (40) for 3 days prior to treatments in medium containing 1% FCS.

**Nuclear Extract Preparation and Mobility Shift Assays—** MtTW15 nuclear extracts were prepared, and mobility shift analysis was performed as described previously (35). Briefly, binding reactions were performed at 4°C for 30–45 min using 0.05, 0.15, 0.5, or 1 μg of nuclear extract and 25,000–50,000 cpm of [32P]-labeled rat GH promoter (183/66) of the rat GH promoter (36, 44). As previously reported, gel shifts using 1 μg of control protein A (Amersham) for 1–2 h, washed twice in TTBS, and once in TBS (no Tween) prior to autoradiography.

**Activin Labeling—** For 35S labeling, MtTW15 cells were plated in 6-well dishes at a density of 0.5 × 10^5 cells/well and allowed to incubate in 10% FCS-β-PIT J2 ule (40) for 48–72 h. Cells were then washed in phosphate-free Dulbecco’s modified Eagle’s medium and incubated in this medium for 30 min prior to the addition of 0.3 μCi/ml [35S]cysteine and -methylionine label mixture (EXPRES35S35S, Du Pont NEI) in 1 ml of medium. To analyze the time course of GH and Pit-1 protein synthesis, cells were incubated with labeled amino acids for the last hour of activin stimulation prior to lysis for immunoprecipitation as described below. For pulse-chase analysis, cells were incubated with labeled amino acids for 1 h, washed three times with complete medium (0.5% FCS-β-PIT J2 ule), and incubated in complete medium ± 20 ng/ml activin for the duration of the chase period prior to lysis for immunoprecipitation.

**Results—** Following metabolic 35S-labeling, cells were washed three times in HEPES-buffered saline and solubilized in 1 ml of lysis buffer (0.1% SDS, 20 mM Tris, pH 7.8, 2 mM EDTA, 300 mM NaCl, 5 μg/ml leupeptin, and 5 μg/ml pepstatin A) for 30 min at 0°C in the presence of heat-killed Phophococcus aureus (Pansornib, Calbiochem) that was preadsorbed with normal rabbit serum. Lysates were spun at 12,000 × g for 5 min, and supernatants containing equivalent amounts of trichloroacetic acid-prefetable counts for each sample were used for immunoprecipitation. 32P-Labeled cells were lysed in buffer described above in the presence of phosphatase inhibitors (10 mM sodium fluoride, 10 mM sodium pyrophosphate, 40 mM sodium vanadate) and were treated with 50 μg/ml RNase A during solubilization to decrease nucleic acid-derived background (43). Samples were incubated rotating for 2 h at 4°C in 1:1000 rabbit anti-rat Pit-1 antibody (37) in the presence or absence of 4 μl of recombinant rat Pit-1 protein (33-kDa species). Following incubation with Pit-1 antibody, 45 μl of 25% Protein A-Sepharose (Pharmacia Biotech Inc.) was added, and the samples were incubated rotating for 1 h at 4°C. For 32P-labeled samples, beads were washed twice with lysis buffer and once with Buffer II (150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 0.4 mM sodium vanadate) (37). For 32P-labeled samples, beads were washed three times in Buffer II containing 0.1% Triton X-100, 0.02% SDS, and once in detergent-free Buffer II as described (37). Remaining supernatants after Protein A-Sepharose precipitation were sometimes incubated with either 1-50 monkey anti-rat antibody to GH (30) or to mAtk11 (1:250 of antibody 199D) in 1 ml of medium. To analyze the time course of GH and Pit-1 synthesis, gel electrophoretic mobility shift assays were performed. MtTW15 nuclear extracts were incubated with a [32P]-labeled oligonucleotide corresponding to the proximal Pit-1 binding site (97 to –66) of the rat GH promoter (36, 44).

**Pit-1 Binding to GH Promoter DNA—** We have previously determined that activin-mediated decreases in Pit-1 binding to the GH promoter in MtTW15 somatotrope cells. To characterize activin-mediated decreases in Pit-1-DNA binding, gel electrophoretic mobility shift assays were performed. MtTW15 nuclear extracts were incubated with a [32P]-labeled oligonucleotide corresponding to the proximal Pit-1 binding site (97 to –66) of the rat GH promoter (36, 44). As previously reported, gel shifts using 1 μg of control protein A (Amersham) for 1–2 h, washed twice in TTBS, and once in TBS (no Tween) prior to autoradiography.

**RESULTS**

**Pit-1 Binding to GH Promoter DNA—** We have previously determined that activin-mediated decreases in Pit-1 binding to the GH promoter in MtTW15 somatotrope cells. To characterize activin-mediated decreases in Pit-1-DNA binding, gel electrophoretic mobility shift assays were performed. MtTW15 nuclear extracts were incubated with a [32P]-labeled oligonucleotide corresponding to the proximal Pit-1 binding site (97 to –66) of the rat GH promoter (36, 44). As previously reported, gel shifts using 1 μg of control protein A (Amersham) for 1–2 h, washed twice in TTBS, and once in TBS (no Tween) prior to autoradiography.
extract resulted in the formation of two major protein-DNA complexes, I and II, and a minor complex III (Fig. 1A and Ref. 35). The formation of complexes I and II were specifically competed with unlabeled Pit-1 oligonucleotide. However, incubation of extracts with a mutant GH promoter Pit-1 oligonucleotide (GH-1), previously demonstrated to be unable to bind Pit-1 (36), did not result in complex formation with nuclear extracts from control or activin-treated MtTW15 cells. The complexes formed with wild-type Pit-1 oligonucleotide both contained Pit-1 protein; preincubation of nuclear extracts with an antiserum specific for Pit-1 (a 132-Pit) prior to addition of labeled Pit-1 oligonucleotide shifted the mobility of both complexes I and II to higher molecular weight species. Following activin treatment, complex formation decreased progressively from 4–12 h and was barely detected after 24 h of activin treatment (Fig. 1A).

Because Pit-1 is highly abundant in nuclear extracts of somatotrope cells, it was possible that a subset of Pit-1 molecules were affected by the activin treatment, which would not be reflected in measurements of microgram quantities of nuclear extracts. Therefore, additional mobility shift analyses were performed to determine the minimum amount of Pit-1-containing nuclear extract required to form a complex with an intact GH promoter fragment in the presence or absence of activin. An intact 32p-labeled GH promoter fragment (–183/+6) containing the proximal and distal binding sites for Pit-1 binding (36, 44) was incubated with increasing amounts of nuclear extract from control or activin-treated MtTW15 cells. As previously reported, gel shifts with control MtTW15 nuclear extracts demonstrated the formation of a major protein-DNA complex, visualized as a doublet (Fig. 1B and Ref. 35). By contrast to Fig. 1A, in which microgram quantities of extract were capable of forming strong complexes after short exposure to activin, use of smaller amounts of nuclear extract in Fig. 1B facilitated the earlier detection of an activin-dependent decrease in Pit-1 binding to DNA. Progressive decreases in Pit-1-DNA binding were first observed within 15 min, and DNA binding was abolished within 24 h of activin treatment (Fig. 1B). Similar titrations using the (–97 to –66) proximal Pit-1 oligonucleotide probe were also observed (data not shown).

Steady-state Pit-1 Content—To determine if the lack in Pit-1 binding to GH promoter DNA reflected a loss in steady-state levels of Pit-1 protein, immunoblot analyses were performed using whole cell or nuclear extracts from unstimulated or activin-treated MtTW15 cells. As previously reported, gel shifts with control MtTW15 nuclear extracts demonstrated the formation of a major protein-DNA complex, visualized as a doublet (Fig. 1B and Ref. 35). By contrast to Fig. 1A, in which microgram quantities of extract were capable of forming strong complexes after short exposure to activin, use of smaller amounts of nuclear extract in Fig. 1B facilitated the earlier detection of an activin-dependent decrease in Pit-1 binding to DNA. Progressive decreases in Pit-1-DNA binding were first observed within 15 min, and DNA binding was abolished within 24 h of activin treatment (Fig. 1B). Similar titrations using the (–97 to –66) proximal Pit-1 oligonucleotide probe were also observed (data not shown).
Activin Regulation of Pit-1 in MtTW15 Cells

FIG. 2. Activin decreased Pit-1 protein content in MtTW15 cells as detected by immunoblot analysis. Nuclear extracts were prepared from MtTW15 cells following activin stimulation for the times indicated, and immunoblot analysis was performed as described under "Experimental Procedures" using 150 μg of protein and 1 μg/ml Pit-1 antiserum from Santa Cruz Biotechnology. A representative blot from 3 experiments is shown. Migration of molecular mass markers (×1000) are indicated on the right, and Pit-1 protein (33-kDa species) is indicated on the left.

(Pit-1β) (38, 39) was not routinely observed in these assays, consistent with its much lower level of expression in the anterior pituitary. In response to activin, Pit-1 content appeared relatively constant for up to 12 h of stimulation. However, longer stimulation with activin resulted in progressive decreases in Pit-1 content, which were observed following 24 h and 48 h of activin stimulation. These data indicated that the rapid decreases in Pit-1 binding to the GH promoter (15 min-1 h) and 48 h of activin stimulation. These data indicated that the rapid decreases in Pit-1 binding to GH promoter DNA (Fig. 1B) were not the result of rapid loss of Pit-1 protein content.

Synthesis and Degradation—To determine the cause of diminishing Pit-1 content during the above time course, MtTW15 cells were metabolically labeled with [35S]labeled amino acids to analyze the rates of Pit-1 synthesis and degradation. For synthesis studies, [35S]labeled amino acids were added during the last hour of activin administration. Coordinate decreases in GH and Pit-1 synthesis were detectable within 4 h of activin stimulation; however, the decreases in Pit-1 synthesis observed within 24 h of activin treatment did not precede decreases in GH synthesis, which were of a greater magnitude (Fig. 3). In scanning densitometric analysis of multiple experiments, Pit-1 synthesis was diminished by ~50% after 48 h of treatment whereas GH synthesis was diminished by >80%. Pit-1 synthesis was not abolished by activin treatment over the time course analyzed. The synthesis of the 31- and 33-kDa isoforms of Pit-1 expressed in these cells appeared to be decreased similarly in response to activin.

The analyses of Pit-1 content and rate of synthesis indicated that decreased Pit-1 synthesis was not solely sufficient to account for the low levels of Pit-1 detected in the immunoblot analysis after 24–48 h of activin stimulation. Therefore, the rate of Pit-1 degradation was analyzed by pulse-chase labeling of MtTW15 cells with [35S]labeled amino acids. Analyses of four experiments indicated that control MtTW15 cells demonstrated a Pit-1 half-life of ~4 h (Fig. 4, upper panel). The half-life for Pit-1 was unchanged in MtTW15 cells treated with activin for up to 4 h. However, within 12 h of activin exposure, Pit-1 stability decreased, leading to a much shorter half-life (less than 2 h). The half-lives of both Pit-1 species were similarly affected by activin treatment. These data suggest that Pit-1 stability is unaffected by short exposure to activin (4 h or less), but that within 12 h of treatment, Pit-1 becomes more sensitive to degradation. This activin-destabilization effect was specific to Pit-1, since the stability of ActRII (used as a control protein) in these same samples was not decreased by activin treatment (Fig. 4, lower panel). Taken together, these data indicate that the rapid activin-mediated decreases in Pit-1 binding to GH promoter DNA (Fig. 1B) could not be accounted for by rapid decreases in Pit-1 synthesis or stability.

Phosphorylation—Pit-1 content was unchanged in MtTW15 cells following short-term activin treatment (15 min–1 h), suggesting that activin-induced modification of Pit-1 might be responsible for decreasing its affinity for DNA binding. One possible modification is phosphorylation, since Pit-1 is a phosphoprotein whose phosphorylation can be increased by protein kinases A and C (45). Increased Ser/Thr phosphorylation of Pit-1 has been associated with decreased ability of Pit-1 to bind to the proximal cis-acting element on the GH promoter (45). Because the rapid activin-mediated decreases in Pit-1 binding to GH DNA were not correlated with a rapid loss of Pit-1 protein, activin-mediated Pit-1 phosphorylation represented a potential mechanism whereby increased phosphorylation might decrease its affinity for DNA. To address this possibility, MtTW15 cells were metabolically labeled with [32P]orthophosphate in the presence or absence of activin, followed by Pit-1 immunoprecipitation and analysis by SDS-PAGE. A representative gel from 5 experiments is shown in Fig. 5. Low levels of Pit-1 phosphorylation were observed in untreated MtTW15 cells. Activin treatment increased phosphorylation of both 31- and 33-kDa Pit-1 species within 7–15 min, a time at which decreases in Pit-1 binding to GH promoter DNA were first detected. This increased phosphorylation was maintained for up to 4 h, during which time additional decreases in Pit-1-DNA binding to GH DNA were observed. The autoradiogram exposure times were 16 h for A and 1 h for B.
FIG. 4. Activin increased the degradation of Pit-1 protein in MtTW15 cells as analyzed by 35S pulse-chase analysis. MtTW15 cells were stimulated with activin for the indicated times and incubated with 35S-labeled amino acids for 1 h followed by the indicated chase periods with complete unlabeled medium as described under “Experimental Procedures.” Lysates were prepared and equivalent protein contents were sequentially immunoprecipitated with α132Pit antiserum (upper panel) followed by antibody 199D to ActRII (lower panel) as described under “Experimental Procedures” and analyzed by SDS-PAGE. A representative gel from 3 experiments is shown. Autoradiogram exposure times were 5 days for both panels. Estimates of Pit-1 half-life (t1/2) were determined by scanning densitometry to be ~4 h in unstimulated and in 4-h activin-treated cells. After 12 h of activin treatment, Pit-1 t1/2 was reduced to ~2 h, whereas the t1/2 for ActRII was unchanged.

FIG. 5. Activin increased Pit-1 phosphorylation in MtTW15 cells. A, MtTW15 cells were metabolically labeled with 32P orthophosphate and stimulated with 20 ng/ml activin or a 100 nM concentration of the protein kinase C activator 12-O-tetradecanoylphorbol-13-acetate. Lysates of equivalent protein content were immunoprecipitated with 1:1000 α132Pit antiserum in the presence (+) or absence (−) of blocking 33-kDa recombinant Pit-1 protein followed by Protein A-Sepharose and analyzed by SDS-PAGE. A representative gel from 4 experiments is shown. Phosphorylated and dephosphorylated Pit-1 from control extracts exhibited comparable protein-DNA complex formation. Addition of the potato acid phosphatase inhibitor 4-nitrophenyl phosphate also had little effect. Nuclear extracts from 15-min activin-treated cells exhibited decreased complex formation compared to that observed using control extracts. Importantly, dephosphorylation of nuclear extracts by potato acid phosphatase resulted in the restoration of Pit-1 binding to DNA to a level comparable to control extracts. Potato acid phosphatase activity was partially blocked by co-incubation with 4-nitrophenyl phosphate, indicating that restored DNA binding in the potato acid phosphatase-treated extracts was dependent upon protein dephosphorylation within the complex. Similar restoration of complex formation was also observed using 30-min and 1-h activin-stimulated nuclear extracts (data not shown).

Taken together, these data indicate that short-term stimulation of MtTW15 cells with activin increases Pit-1 phosphorylation, which causes coordinate decreases in Pit-1-containing complex formation with GH promoter DNA. Dephosphorylation of activin-treated extracts restores Pit-1 binding to control levels, consistent with phosphorylation reducing the affinity of Pit-1 for DNA. In addition, long-term activin stimulation causes decreases in Pit-1 stability and synthesis, resulting in decreased Pit-1 content, which contributes to less Pit-1 available for binding and transactivation of the GH promoter.

DISCUSSION

Our previous observations demonstrated long-term (6-day) effects of activin on decreasing GH mRNA and GH transcription in MtTW15 somatotrope cells (35). These effects were associated with decreased binding of the tissue-specific transcription factor Pit-1 to the GH promoter. The current studies indicate that decreased Pit-1 binding, and thus decreased GH biosynthesis in MtTW15 cells, is the result of multilevel effects of activin on Pit-1 activity as well as on total Pit-1 content, which may involve alterations in the autoregulatory loop maintaining Pit-1 transactivation. The first measurable effects of
Activin were temporally correlated increases in Pit-1 phosphorylation (Fig. 5) and decreases in GH promoter binding after 15 min of activin treatment (Figs. 1B and 6). These effects were followed by decreases in Pit-1 stability and synthesis, and finally increased Pit-1 content and continued diminished rates of synthesis (Figs. 2, 3, and 4).

Activin treatment of MtTW15 cells caused rapid decreases in Pit-1-GH complex formation in the absence of altered Pit-1 protein content. Since transcription factor phosphorylation is a well-utilized mechanism for regulation of DNA binding and transactivation (reviewed in Refs. 46–48), these data suggested that altered phosphorylation of Pit-1 might be involved in decreased GH promoter binding. Indeed, earlier phosphorylation studies of Pit-1 in vitro and in metabolic labeling of GC cells demonstrated increased protein kinase A– or protein kinase C-dependent phosphorylation of Pit-1 within 7 min of cAMP or 12-O-tetradecanoylphorbol-13-acetate treatment, respectively (45). Associated with this increased Pit-1 phosphorylation was decreased binding affinity for the proximal GH promoter (45). Associated with this increased Pit-1 phosphorylation was decreased binding affinity for the proximal GH promoter (45). Associated with this increased Pit-1 phosphorylation was decreased binding affinity for the proximal GH promoter (45).

These differences have been attributed to variations within the consensus sequences for Pit-1 binding in the TSH–β, GH, prolactin, and Pit-1 genes (49). It has also been suggested that additional factors interacting with Pit-1 are the true protein kinase A and protein kinase C phosphorylation targets which are involved in regulating GH and prolactin promoter activity (50–53).

Although the intracellular intermedietes of activin signaling have not been identified, protein kinase A and protein kinase C do not appear to play major roles in mediating a variety of activin effects. The current studies demonstrated that activin increased Pit-1 phosphorylation, which was temporally correlated with significant decreases in Pit-1 binding to the GH promoter (15 min–4 h). Phosphatase treatment of short-term activin-treated nuclear extracts restored binding to control levels. This result highlights the importance of phosphorylation in mediating the early decreases in Pit-1 binding to DNA and suggests that activin-dependent Pit-1 phosphorylation decreases its affinity for binding to the GH promoter. Activin may also target the phosphorylation of a Pit-1 co-factor, such as Zn-15 (54), which subsequently decreases the affinity of the Pit-1-cofactor complex to bind to the GH promoter. Although previous activin-mediated decreases in Pit-1-DNA complex formation have been correlated with repression of GH gene transcription (35), the requirement of Pit-1 phosphorylation for activin-dependent repression of GH promoter transactivation has yet to be confirmed.

Increased Pit-1 phosphorylation upon activin treatment was temporally associated with decreases in Pit-1 binding to GH DNA. Since not all of the nuclear Pit-1 was inhibited from binding to DNA after short-term activin treatment, this may indicate that phosphorylation of Pit-1 is incomplete after 15 min, or that sites are also rapidly dephosphorylated. In addition, increased Pit-1 phosphorylation on some sites may alter Pit-1 sensitivity to proteases. Several examples of phosphorylation-dependent degradation have been reported. The stability of c-fos is decreased when the protein dimerizes with phosphorylated c-jun (55). Induced phosphorylation of cytoplasmic IκB when complexed with NFκB targets IκB for degradation, resulting in NFκB activation and translocation to the nucleus (56, 57). Phosphorylation of the tumor suppressor protein, p53, by p34cdc2 is also associated with rapid protein degradation (58). However, in MtTW15 cells, the time delay between the increased Pit-1 phosphorylation, observed within 15 min of activin treatment, and the increased degradation of Pit-1, observed within 9–12 h of activin treatment, suggests that inducible degradation of Pit-1 may be controlled by different mechanisms.

Activin decreases not only the stability of Pit-1, but also its rate of synthesis. While this effect on Pit-1 synthesis may reflect an activin action directly on Pit-1 transcription or translation, other mechanisms are also possible. Because Pit-1 positively autoregulates its synthesis, activin-mediated degradation of Pit-1 would disrupt this loop because less Pit-1 is available, further decreasing the level of Pit-1 in the cell. In addition, activin may exert a direct inhibitory effect on regulatory sequences in the Pit-1 gene to down-regulate Pit-1 transcription.

In conclusion, activin exerts multilevel regulation of the tissue-specific transcription factor, Pit-1, involving increased phosphorylation and decreased stability of the protein, resulting in decreased Pit-1 available for transactivation of the GH gene in the somatotrope. Activin-mediated phosphorylation of tissue-specific transcription factors may provide a more general mechanism whereby other tissue-specific genes, such as α-inhibin in the gonad (59) and globin genes during erythrocyte differentiation (60, 61), are regulated by activin through phosphorylation-induced modulation of transcription factor activity and/or content.

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