Transforming Growth Factor-β and Activin Inhibit Basal Secretion of Prolactin in a Pituitary Monolayer Culture System (43295)

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Abstract. **Incubations of rat anterior pituitary cells with transforming growth factor (TGF)-β1 for 48 hr suppressed the secretion of basal prolactin (PRL) in a dose-dependent manner (ED_{50}, 100 pg/ml). Activin, a gonadal hormone processing cysteine distribution similar to TGFβ, also suppressed basal PRL secretion, but it was less effective (ED_{50}, 4 ng/ml). Treatment with TGFβ1 significantly suppressed basal PRL secretion from the pituitary after 24 hr and up to 72 hr of incubation. TGFβ1 also inhibited thyrotropin-releasing hormone-mediated PRL secretion and activin inhibited thyrotropin-releasing hormone-mediated PRL secretion slightly, but significantly. In addition, we also measured the secretion of growth hormone by cultured pituitary cells treated with TGFβ1 or activin for 24 to 72 hr. TGFβ1 and activin showed an opposite effect on growth hormone secretion; TGFβ stimulated and activin inhibited basal secretion of growth hormone. These results suggest that TGFβ1 is a potent inhibitor of basal secretion of PRL by the pituitary, and both TGFβ1 and activin play a multifunctional role in basal secretion of pituitary hormones.** [P.S.E.B.M. 1991, Vol 198]

Inhibin and activin, isolated and characterized from ovarian follicular fluid on the basis of their ability to regulate secretion of follicle-stimulating hormone (FSH) by the pituitary, have a similar cysteine distribution to transforming growth factor (TGF)-β (1). Although TGFβ was originally identified by its ability to reversibly induce certain nonneoplastic cells to express a transforming phenotype (2), this growth factor also regulates FSH secretion by the pituitary (3), mediates FSH-induced steroidogenesis in the gonadal cells (4, 5), and has been shown to have an important immunomodulatory effect on several types of cells in the immune system (2). These findings point to a potential interrelationship between pituitary hormones and immune response.

On the other hand, prolactin (PRL) has been reported to have an important role in maintaining immune function (6–8). Although Kitaoka *et al.* (9) have reported that activin inhibits thyrotropin-releasing hormone-mediated PRL secretion in a pituitary monolayer culture system (9), there is no information about the effect of TGFβ on PRL secretion. We reported here that TGFβ1 and activin suppress basal secretion of PRL by pituitary cells *in vitro*.

Materials and Methods

Purified recombinant human TGFβ1 (10) was obtained from Genentech, Inc. (South San Francisco, CA). Activin was purified, as described previously (11). Thyrotropin-releasing hormone (TRH) was purchased from Sigma Chemical Co. (St. Louis, MO).

Pituitaries from 22-day-old female Sprague-Dawley rats (Holtzman Co., Madison, WI) were removed aseptically after decapitation. The anterior pituitary lobes were collected and dispersed enzymatically, as described previously (3). The cells (2.5×10^5) were seeded into 24-well tissue culture plates (Flow Laboratories, Inc., McLean, VA) with Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY), which was supplemented with 15% fetal bovine serum. On the second day of culture, the medium was removed and samples of TGFβ1 or activin at varying doses, dissolved in 1 ml of Dulbecco's modified Eagle's medium with 1% fetal bovine serum, were added after

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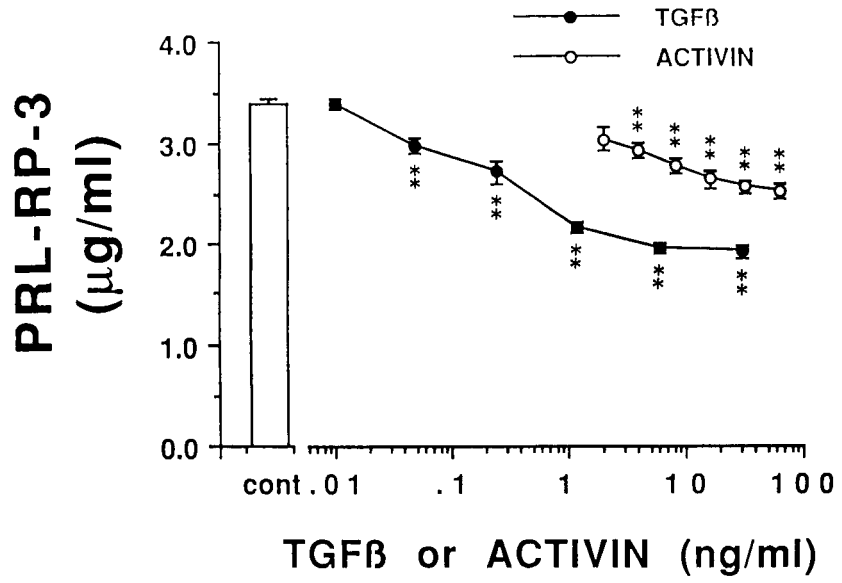

Figure 1. The effect of 48-hr incubation with TGFβ1 or activin on basal PRL secretion in cultured rat anterior pituitary cells. Data are the mean ± SE from three or more independent experiments performed in triplicate. Asterisks denote significant differences from controls (**$P < 0.01$).

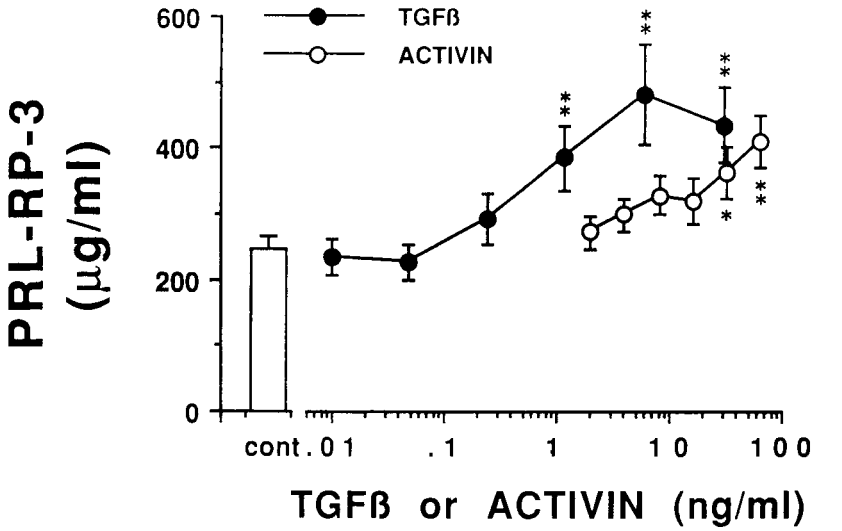

Figure 2. The effect of 48-hr incubation with TGFβ1 or activin on the intracellular contents of PRL in cultured rat anterior pituitary cells. Data are the mean ± SE from three independent experiments performed in triplicate. Asterisks denote significant differences from control (*$P < 0.05$. **$P < 0.01$).

being individually passed through a 0.22-μm filter (Millipore, Bedford, MA). After a 48-hr incubation, medium was collected for measurements by radioimmunoassay (RIA). In experiments for measuring acute PRL secretion, after medium collection, cells were washed twice with Dulbecco's modified Eagle's medium and incubated for 4 hr with or without 200 nM TRH. For the extraction of cell content, after a 48-hr incubation with hormones, medium was collected and 0.5 ml of 0.1 N acetic acid was added to each well and incubated for 10 min at room temperature. The lysed cells were lyophilized and reconstituted in 1 ml of RIA buffer for hormone measurements.

RIA for pituitary hormones were performed with antiserum provided by Dr. A. F. Parlow through the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Disease). For the separation of free and antibody-bound hormone, goat antirabbit γ-globulin-precipitating antibodies (Antibodies Inc., Davis, CA) for PRL RIA (12) and the preparation of sacrificed *Staphylococcus aureus* (IgGsorb; Enzyme Center Inc., Boston, MA) for growth hormone (GH) RIA (13) were used, as described previously. Fifty or 100 μl of diluted samples (1/1000 and 1/100 dilution for PRL and GH, respectively) were used for each assay. Interassay coefficients of variation

Table I. The Effect of 48-hr Incubation with TGFβ1 or Activin on Basal PRL Secretion and Cellular and Total PRL Levels[a]

	PRL-RP-3 (μg/ml/48 hr)		
	Secretion	Cellular	Total
Control	3.35 ± 0.06	0.25 ± 0.02	3.56 ± 0.06
TGFβ1 (ng/ml)			
0.048	2.98 ± 0.06[b]	0.23 ± 0.03	3.20 ± 0.11
1.2	2.17 ± 0.08[b]	0.39 ± 0.05[c]	2.55 ± 0.12[e]
30	1.92 ± 0.08[b]	0.43 ± 0.06[c]	2.36 ± 0.13[e]
Activin (ng/ml)			
2	3.06 ± 0.11	0.27 ± 0.02	3.33 ± 0.11
8	2.66 ± 0.09[b]	0.33 ± 0.03	2.99 ± 0.09[e]
32	2.55 ± 0.07[b]	0.36 ± 0.04[d]	2.92 ± 0.09[e]

[a] The effects of 48-hr incubation with various doses of TGFβ1 or activin on basal PRL secretion and cellular and total (cellular plus secreted) PRL levels are shown. The data are the mean ± SE from three separate experiments performed in triplicate.
[b] $P < 0.01$, compared with the control value of basal secretion.
[c] $P < 0.01$, compared with the control value of cellular content.
[d] $P < 0.05$, compared with the control value of cellular content.
[e] $P < 0.01$, compared with the control value of total level.

were 10.8% and 8.6% in PRL and GH RIA, respectively.

DNA synthesis was determined from the extent of [^{3}H]thymidine incorporation into trichloroacetic acid-soluble cellular fractions (14). The cells were incubated with [^{3}H]thymidine (1 μCi/well) (New England Nuclear, Boston, MA) for 48 hr with or without TGFβ1, subsequently washed twice with ice-cold phosphate-buffered saline and twice with ice-cold 10% trichloroacetic acid, and solubilized with 1 *N* NaOH for scintillation counting.

Data were analyzed by one-way analysis of variance, followed by the Duncan's test when differences were significant.

Results

The incubation of rat anterior pituitary cells for 48 hr with varying doses of TGFβ1 decreased the basal secretion of PRL in a dose-dependent manner. The minimal concentration required to significantly suppress the basal PRL secretion is 48 pg/ml (1.9 p*M*), with a ED_{50} at 100 pg/ml (4 p*M*) (Fig. 1). However, treatment with doses higher than 1.2 ng/ml of TGFβ1 showed no further suppression of PRL secretion. Incubation with activin also suppressed basal PRL secretion with the ED_{50} at 4 ng/ml (0.14 n*M*), but its effect was less potent than that of TGFβ (Fig. 1).

Intracellular contents of PRL were significantly increased by treatment with doses of TGFβ1 and activin higher than 1.2 ng/ml (48 p*M*) and 32 ng/ml (1.1 n*M*), respectively (Fig. 2). However, total PRL levels (cellular plus secreted) were significantly decreased by treatment with doses of TGFβ1 and activin higher than 1.2 ng/ml and 8 ng/ml (0.29 n*M*), respectively (Table I).

Treatment with 0.25 ng/ml (10 p*M*) of TGFβ1 inhibited basal secretion of PRL from 24 hr of incubation up to 72 hr of incubation (Fig. 3). Figure 4 showed that treatment with 2.5 ng/ml (89 p*M*) and 25 ng/ml (0.89 n*M*) of activin for 48 hr significantly inhibited basal secretion of PRL, but treatment with 25 ng/ml of activin for 72 hr inhibited PRL secretion slightly, but not significantly.

Secretions of PRL for 4 hr of incubation in the presence or absence of TRH were significantly inhibited by the pretreatment with TGFβ1 for 48 hr (Table II).

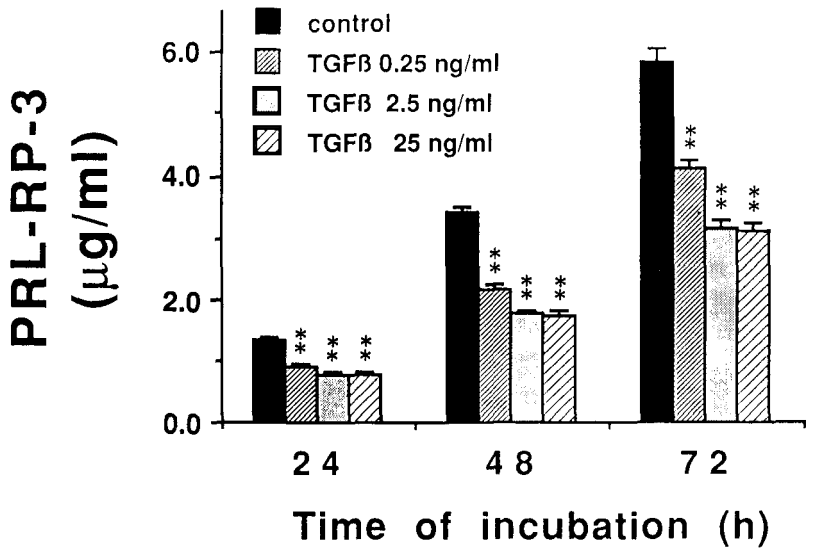

Figure 3. The effects of 24-, 48-, or 72-hr incubation with TGFβ1 (0.25, 2.5, or 25 ng/ml) on basal PRL secretion in cultured rat anterior pituitary cells. Data are the mean ± SE from three or more independent experiments performed in triplicate. Asterisks denote significant differences from the corresponding control (**$P < 0.01$).

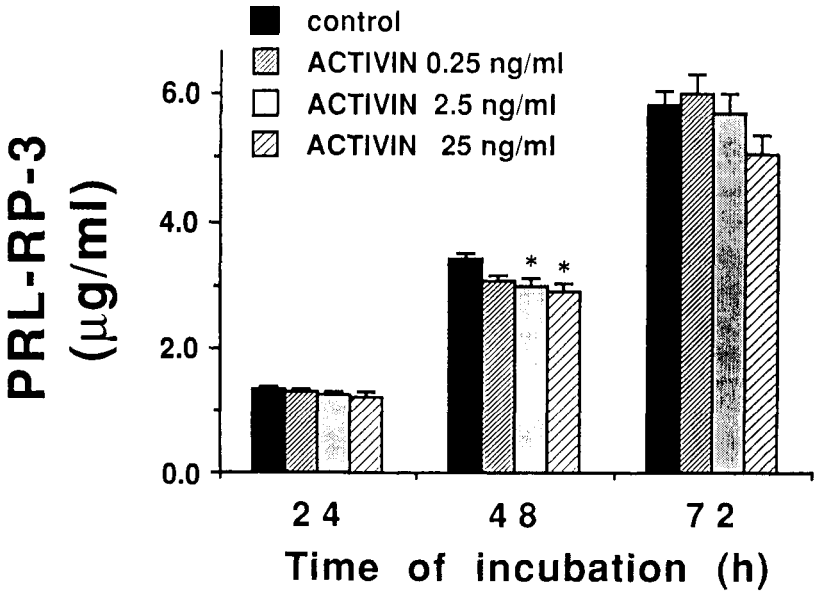

Figure 4. The effects of 24-, 48-, or 72-hr incubation with activin (0.25, 2.5, or 25 ng/ml) on basal PRL secretion in cultured rat anterior pituitary cells. Data are the mean ± SE of wells from three or more independent experiments performed in triplicate. Asterisks denote significant differences from the corresponding control ($P < 0.05$).

Table II. Effects of Pretreatment with TGFβ1 and Activin on PRL Secretion for 4-hr Incubation in the Presence or Absence of TRH[a]

	PRL-RP-3 (μg/ml/4 hr)	
	Without TRH	With TRH
Control	193 ± 5.9	285 ± 9.7
TGFβ1 (ng/ml)		
0.25	176 ± 13	234 ± 18
2.5	124 ± 12[b]	162 ± 15[c]
25	113 ± 10[b]	160 ± 9.9[c]
Activin (ng/ml)		
0.25	197 ± 4.5	254 ± 9.5
2.5	193 ± 4.5	259 ± 17
25	192 ± 5.6	249 ± 7.2[d]

[a] The cells were incubated with various doses (0.25, 2.5, or 25 ng/ml) of TGFβ1 or activin for 48 hr. After washing the cells, they were incubated in the presence and absence of 200 *nM* TRH for 4 hr. The data are the mean ± SE from three separate experiments performed in triplicate.
[b] $P < 0.01$, compared with the value of controls without TRH.
[c] $P < 0.01$, compared with the value of controls with TRH.
[d] $P < 0.05$, compared with the value of controls with TRH.

Pretreatment with activin for 48 hr inhibited TRH-mediated PRL secretion slightly, but significantly, only at dose of 25 ng/ml. However, pretreatment with activin ranging from 0.25 to 25 ng/ml for 48 hr did not affect PRL secretion for 4 hr of incubation (Table II).

In addition, we measured growth hormone secretion after 24–72 hr of incubation. Treatment with 2.5 and 25 ng/ml of TGFβ1 for 24 to 72 hr significantly stimulated basal GH secretion (Fig. 5). But treatment with 2.5 and 25 ng/ml of activin for 24 to 72 hr significantly inhibited basal secretion of GH (Fig. 6).

Treatments with TGFβ1 and activin did not change the total number of pituitary cells or the incorporation of ^{3}H-labeled thymidine into cells (data not shown). Therefore, TGFβ1 and activin did not inhibit basal secretion of PRL secretion by affecting the proliferation of pituitary cells.

Discussion

This study demonstrated that TGFβ1 and activin inhibit basal secretion of PRL in a pituitary monolayer system and that TGFβ1 is a more potent inhibitor than activin. The incubation of anterior pituitary cells with TGFβ1 or activin resulted not only in the suppression of basal PRL secretion, but also in the decrease of total (secreted and cellular) PRL levels. Therefore, the inhibition of basal PRL secretion may in part be caused by the inhibitory effects of TGFβ1 and activin on PRL synthesis. In addition, treatments with higher doses of TGFβ1 and activin increased intracellular content of PRL. Since total PRL level was inhibited at these doses, it is unlikely that the increase of intracellular PRL is due to the stimulation of biosynthesis. Therefore, it probably reflects attenuated spontaneous secretion of PRL. Thus, TGFβ1 and activin may have a dual role in the regulation of PRL secretion and biosynthesis *in vitro*.

Kitaoka *et al.* (9) reported that preincubation with activin for 48 hr inhibited TRH-mediated PRL secretion, but did not affect basal PRL secretion by the pituitary cells during a short period (2 hr). Present results confirmed this observation and further demonstrated the suppressive effect of TGFβ1 on basal and TRH-mediated PRL secretion. The effect of TRH on

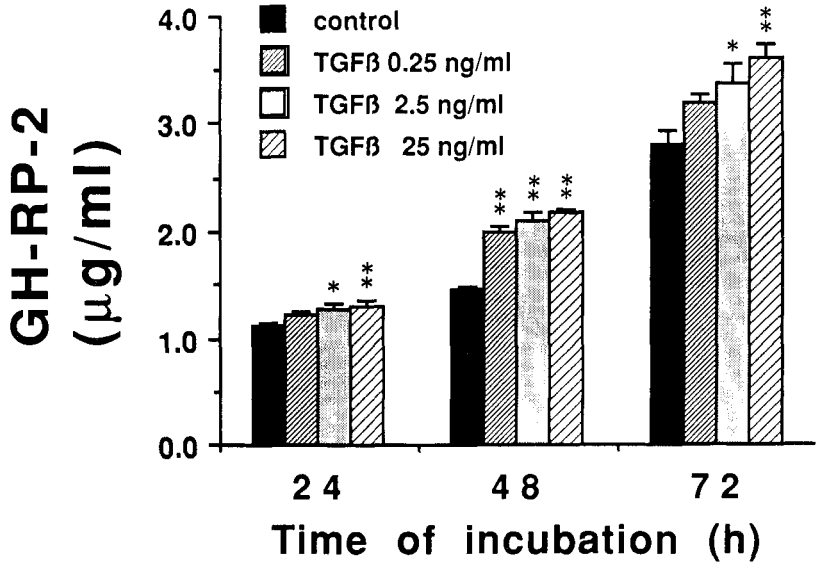

Figure 5. The effects of 24-, 48-, or 72-hr incubation with TGFβ1 (0.25, 2.5, or 25 ng/ml) on basal GH secretion in cultured rat anterior pituitary cells. Data are the mean ± SE from three or more independent experiments performed in triplicate. Asterisks denote significant differences from the corresponding control (*$P < 0.05$, **$P < 0.01$).

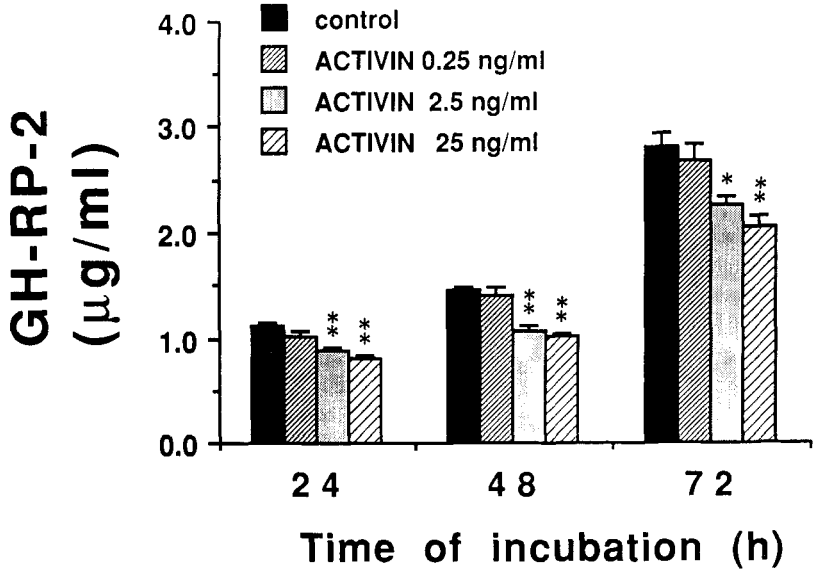

Figure 6. The effects of 24-, 48-, or 72-hr incubation with activin (0.25, 2.5, or 25 ng/ml) on basal GH secretion in cultured rat anterior pituitary cells. Data are the mean ± SE from three or more independent experiments performed in triplicate. Asterisks denote significant differences from the corresponding control (*$P < 0.05$, **$P < 0.01$).

PRL secretion is mediated by a protein kinase C pathway in cultured rat anterior pituitary cells (15) and rat GH_3 cells (16, 17). Therefore, TGFβ1 possibly suppresses the protein kinase C-dependent mechanism and may also affect basal PRL secretion, which is mediated by the protein kinase C-dependent pathway.

This study also demonstrated that TGFβ and activin have multiple effects on basal secretion of pituitary hormones. Both TGFβ1 and activin inhibited PRL secretion, but stimulated FSH secretion (3, 11) by cultured pituitary cells. Interestingly, TGFβ1 and activin showed an opposite effect on GH secretion by the pituitary. Kitaoka *et al.* (9) have reported that growth hormone-releasing factor-mediated GH secretion by cultured pituitary cells was reduced after preincubation with activin for 48 hr. Bilezikjian *et al.* (18) confirmed these observations and further observed that activin suppressed basal secretion of GH by cultured pituitary cells for up to 72 hr. Our results confirmed that activin has an inhibitory effect on basal secretion of GH by cultured pituitary cells.

On the other hand, TGFβ1 stimulated and activin

inhibited basal secretion of GH by cultured pituitary cells, suggesting that there are different mechanisms by which TGFβ1 and activin act at different target cells. Several types of receptors for TGFβ1 and activin have been identified on various target cells. First, Cheifetz *et al.* (19) have demonstrated that, in GH_3 pituitary tumor cell, activin and inhibin interacted with TGFβ1 in a ligand receptor assay, but did not interact in other cells containing TGFβ receptors, such as Buffalo rat liver cells (BRL-3A), mink lung epithelial cells (Mv1Lu), and human skin fibroblast (GM370). Conversely, activin receptors that did not interact with TGFβ1 have been identified on rat granulosa primary cultured cells (20), human leukemia cells (K562) (21), and murine erythroleukemia cells (F5-5) (22). Furthermore, Kondo *et al.* (23) have found activin receptors that interact with TGFβ1 on Friend leukemia cell and embryonal carcinomal cells. Therefore, it is possible that similar receptors on gonadotrophs and lactotrophs recognize both TGFβ1 and activin, consequently resulting in a similar effect on basal secretion of FSH and PRL, whereas different receptors on somatotrophs are responsible for the opposite effect of TGFβ1 and activin on GH secretion.

In a previous study, one of us has observed that human platelet-derived TGFβ1 had no effect on basal PRL secretion (3). But, in the present study, human recombinant TGFβ1 inhibited PRL secretion. This discrepancy in PRL secretion may be due to different preparations of TGFβ1. Certainly, further study is needed to test this possibility. Another possibility is probably the sensitivity of PRL RIA employed. Recently, we have established an improved RIA which routinely measures PRL levels in medium at 1/1000 dilution, whereas previous RIA were not as sensitive. Therefore, samples were measured at PRL concentrations below the sensitivity of the previous RIA procedure. Indeed, a recent concurrent comparison of these two procedures of PRL RIA has provided a different sensitivity for PRL measurement.

Prolactin, an important hormone during pregnancy and lactation (24), also has an effect on immune responses. The hypoprolactinemic condition in mice induced by administration of dopamine agonist, bromocriptine, suppressed T cell-dependent induction of macrophage activation and the production of interferon by T lymphocytes (6). Treatment with antibodies against PRL inhibited mitogen-induced proliferation of both murine- and human-cultured lymphocyte (8). Thus, PRL is an important factor that maintains lymphocyte function. On the other hand, TGFβ1 and activin have suppressive activity in mitogen-induced lymphocyte proliferation (25). Since TGFβ1 is a factor that is secreted by activated macrophages (26), the effect of TGFβ1 on the suppression of PRL secretion by the pituitary may be involved in feedback action on the immune system. Moreover, macrophages have been identified in various endocrine organs, including anterior pituitary lobes, by the immunohistochemical localization of a macrophage-specific antigen (27). It is possible that the suppressive effects of TGFβ1 and activin on PRL secretion by the pituitary are mediated by macrophage. Consequently, TGFβ1 and activin could play an antagonist paracrine/autocrine role in immunomodulation.

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1. Ying S-Y. Inhibins, activins, and follistatins: Gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr Rev **9**:267–293, 1988.
2. Roberts AB, Sporn MB. Transforming growth factor β. In: Klein G, Weinhouse S, Eds. Advances in Cancer Research. New York: Academic Press, Vol **51**: p107, 1988.
3. Ying S-Y, Becker A, Baird A, Ling N, Ueno N, Esch F, Guillemin R. Type beta transforming growth factor (TGFβ) is a potent stimulator of the basal secretion of follicle stimulating hormone (FSH) in a pituitary monolayer system. Biochem Biophys Res Commun **135**:950–956, 1986.
4. Ying S-Y, Becker A, Ling N, Ueno N, Guillemin R. Inhibin and beta type transforming growth factor (TGFβ) have opposite modulating effects on the follicle stimulating hormone (FSH)-induced aromatase activity of cultured rat granulosa cells. Biochem Biophys Res Commun **136**:969–975, 1986.
5. Hutchinson LA, Findlay JK, de Vos FL, Robertson DM. Effects of bovine inhibin, transforming growth factor-β and bovine activin-A on granulosa cell differentiation. Biochem Biophys Res Commun **146**:1405–1412, 1987.
6. Bernton EW, Meltzer MS, Holaday JW. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science **239**:401–404, 1988.
7. Russell DH. New aspects of prolactin and immunity: A lymphocyte-derived prolactin-like product and nuclear protein kinase C activation. Trends Pharmacol Sci **10**:40–44, 1989.
8. Hartmann DP, Holaday JW, Bernton EW. Inhibition of lymphocyte proliferation by antibodies to prolactin. FASEB J **3**:2194–2202, 1989.
9. Kitaoka M, Kojima I, Ogata E. Activin A: A modulator of multipule types of anterior pituitary cells. Biochem Biophys Res Commun **157**:48–54, 1988.
10. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB, Goeddel DV. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature **316**:701–705, 1985.
11. Ling N, Ying S-Y, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. Pituitary FSH is released by a heterodimer of the β-subunits from the two forms of inhibit. Nature **321**:779–782, 1986.
12. Rao IM, Mahesh VB. Role of progesterone in the modulation of the preovulatory surge of gonadotropins and ovulation in the pregnant mare's serum gonadotropin-primed immature rat and the adult rat. Biol Reprod **35**:1154–1161, 1986.
13. Ying S-Y. Radioimmunoassay of peptide hormones using killed *Staphylococcus aureus* as a separating agent. Methods Enzymol **73**:245–253, 1981.

14. Cara JF, Stuart CA, Furlanetto RW. A monoclonal antibody to the type 1 insulin-like growth factor and insulin receptors stimulates deoxyribonucleic acid synthesis in human and murine fibroblasts. Endocrinology **123**:1341–1347, 1988.
15. Martinez de la Escalera G, Guthrie J, Weiner RI. Transient removal of dopamine potentiates the stimulation of prolactin release by TRH by not VIP: Stimulation via Ca^{2+}/protein kinase C pathway. Neuroendocrinology **47**:38–45, 1988.
16. Martin TFJ, Kowalchyk JA. Evidence for the role of calcium and diacylglycerol as dual second messengers in thyrotropin-releasing hormone action: Involvement of diacylglycerol. Endocrinology **115**:1517–1526, 1984.
17. Martin TFJ, Kowalchyk JA. Evidence for the role of calcium and diacylglycerol as dual second messengers in thyrotropin-releasing hormone action: Involvement of Ca^{2+}. Endocrinology **115**:1527–1536, 1984.
18. Bilezikjian LM, Corrigan AZ, Vale W. Activin-A modulates growth hormone secretion from cultures of rat anterior pituitary cells. Endocrinology **126**:2369–2376, 1990.
19. Cheifetz S, Ling N, Guillemin R, Massague J. A surface component on GH_3 pituitary cells that recognizes transforming growth factor-β, activin, and inhibin. J Biol Chem **263**:17225–17228, 1988.
20. Sugino H, Nakamura T, Hasegawa Y, Miyamoto K, Igarashi M, Eto Y, Shibai H, Titani K. Identification of a specific receptor for erythroid differentiation factor on follicular granulosa cell. J Biol Chem **263**:15249–15252, 1988.
21. Campen CA, Vale W. Characterization of activin A binding sites on the human leukemia cell line K562. Biochem Biophys Res Commun **157**:844–849, 1988.
22. Hino M, Tojo A, Miyazono K, Miura Y, Chiba S, Eto Y, Shibai H, Takaku F. Characterization of cellular receptors for erythroid differentiation factor on murine erythroleukemia cells. J Biol Chem **264**:10309–10314, 1989.
23. Kondo S, Hashimoto M, Etoh Y, Murata M, Shibai H, Muramatsu M. Identification of the two types of specific receptor for activin/EDF expressed on friend leukemia and embryonal carcinoma cells. Biochem Biophys Res Commun **161**:1267–1272, 1989.
24. Neill JD. Neuroendocrine regulation of prolactin secretion. In: Martini L, Ganong WF, EDs. Frontiers in Neuroendocrinology. New York: Raven Press, Vol **6**: p129, 1980.
25. Ying S-Y, Becker A. Follistatin and activin are important immunoregulatory gonadal proteins. Endocrinology **124**:A218, 1989.
26. Assoian RK. Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, Ross R, Sporn MB. Expression and secretion of type β transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA **84**:6020–6024, 1987.
27. Hume DA, Halpin D, Charlton H, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: Macrophages of endocrine organs. Proc Natl Acad Sci USA **81**:4174–4177, 1984.