Inhibition of growth hormone secretion by activin A in human growth hormone-secreting tumour cells

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Abstract. Effect of activin A on growth hormone secretion was studied in primary culture of 8 human GH-secreting adenomas, which were responsive to TRH in vivo. When studied in vitro, basal GH secretion was reduced in all cases when cells were pre-incubated for 48 h with activin A at a concentration of 5×10⁻⁹ mol/l or greater. Pretreatment of GH-secreting cells with 1×10⁻⁹ mol/l activin A did not affect either basal secretion or cellular content of GH. These tumour cells also responded to TRH in vitro and the GH response to TRH was completely blocked in cells pretreated with activin A. Activin A slightly reduced the increase in cytoplasmic free calcium concentration induced by TRH. Furthermore, pretreatment of the cells with activin A attenuated GH secretion induced by A23187 or 12-O-tetradecanoylphorbol-4-acetate, agents which bypass receptor-mediated generation of second messengers. These results indicate that activin A inhibits GH secretion by directly acting on human GH-secreting cells and that activin A inhibits the action of TRH by acting on multiple steps in the messenger system.

During the isolation of inhibin from ovarian fluid, two polypeptide stimulators of follicle-stimulating hormone secretion were discovered (1,2). These stimulators resemble inhibin in their structures. One is a homodimer of βA-subunit of inhibin and the other is a heterodimer of βA- and βB-subunits (1,2). These two stimulators, which are now termed activin A and activin AB, respectively, affects not only FSH secretion from gonadotropes, but they also modify secretion of other pituitary hormones (1-4). We reported previously that activin A attenuated release of prolactin and GH in response to TRH and GHRH, respectively (3,4). It is, however, not certain at present whether activin A modifies secretion of prolactin and GH by acting directly on lactotropes and somatotropes because rat pituitary cells consist of various types of cells. This is of particular importance in view of the fact that at least some effects of activin A result from an interaction of multiple types of pituitary cells (5). In this regard, it would be helpful if we could examine the effect of activin A in pure preparations of either lactotropes or somatotropes.

To elucidate whether or not activin A directly modifies the function of somatotropes, we examined the effect of activin A in human GH-secreting tumour cells in primary culture. Using this cell system, we now demonstrate that activin A inhibits basal as well as stimulated GH secretion by acting directly on GH-secreting cells. The present results therefore suggest that the inhibitory action of activin A on GH secretion observed in rat pituitary cells (3,4) is due at least partly to its direct action on somatotropes. We also examined the mechanism by which activin A inhibits GH secretion.

Materials and Methods

Cell culture
GH-secreting tumours from 8 patients with acromegaly were obtained by transsphenoidal surgery. Characteristics of patients are listed in Table 1. Serum concentration of GH was elevated in response to iv administration of TRH in all patients. Resected tumours were dispersed by
using Dsipase (Gohdoh Shisei Co, Tokyo, Japan) and were cultured in Dulbecco’s modified Eagle’s (DME) medium containing 10% fetal calf serum (FCS) for three to five days before the start of experiments (6). Cells were seeded at a density of 5 × 10⁴ cells/24 well dish. When an effect of activin A on cell growth was measured, cells were cultured for 4 days and the cell number was counted by hemacytometer after detaching the cells by use of trypsin.

**Determination of GH secretion**

Pituitary cells were incubated for 48 h with DME medium containing 10% FCS in the presence and absence of activin A. Cells were washed twice with DME medium and were then incubated for 2 h with or without TRH. Furthermore, to examine whether activin A inhibited GH secretion induced by agents which bypass receptor-mediated generation of second messengers, we employed 12-tetradecanoyl phorbol-4-acetate (TPA) and A23187. TRH activates phospholipase C specific to phosphatidylinositol 4,5-bisphosphate and generates diacylglycerol and inositol 1,4,5-trisphosphate (7,8). Diacylglycerol activates protein kinase C and inositol 1,4,5-trisphosphate increases cytoplasmic free calcium concentration \([\text{Ca}^{2+}]\), by causing a mobilization of calcium from an intracellular pool. We employed TPA and A23187 to activate protein C kinase and to increase \([\text{Ca}^{2+}]\), respectively (9,10). The medium was collected and was stored at −20°C until the assay of GH. GH was measured by radioimmunoassay (11). To determine intracellular content of GH, cells were scraped off using a rubber policeman. Then 0.1 mol/l NaOH was added and cells were freeze-thawed for three times followed by an aspiration through a 26 G needle. The broken cell suspension was neutralized by adding 0.1 mol/l HCl and centrifuged. The supernatant was stored at −20°C until the assay. In some experiments, cells were incubated with transforming growth factor-β (TGF-β), since activin A is structurally related with TGF-β.

Statistical analysis was performed with the use of the Wilcoxon two-sample test. Simple and exponential models of regression analysis were also employed. Values were expressed as means ± SEM.

**Measurement of cytoplasmic free calcium concentration**

Changes in \([\text{Ca}^{2+}]\), was monitored in single cell by measuring fluorescence of a calcium-sensitive indicator, fura-2, using a microfluorometer (12). Cells cultured on a glass coverglass coated with poly-L-lysine were incubated with DME medium containing 2 μmol/l fura-2/AM, membrane permeable acetoxyxymethylster of fura-2, for 30 min at 37°C. The coverglass containing fura-2-loaded cells was mounted in a flow-through system and was superfused with Hanks’ solution at a flow rate of 1 ml/min. Each cell was excited alternatively with 340 and 380 nm at 60 Hz. Emissions from each wave length were collected and averaged, and the ratio of two emissions (340/380) was calculated every second. The 340/380 ratio was monitored as \([\text{Ca}^{2+}]\). Since the measurement is qualitative rather than quantitative and since intracellular distribution of fura-2 is not totally even, we did not calibrate the fluorescence in terms of free calcium concentration.

**Materials**

Activin A purified from conditioned medium of human monocytic leukemia cells, TPH-1 cells, was provided by Dr Hiroshi Shibai of Central Laboratory, Ajinomoto Co (Kawasaki, Japan). TRH was provided by Tanabe (Osaka, Japan). Fura-2/AM was purchased from Wako Chemical (Osaka, Japan).

**Results**

**Effect of activin A on basal secretion of GH**

GH-secreting tumour cells were incubated for 48 h with various concentrations of activin A. Cells were washed twice with the medium. Basal GH release during the last 2 h was measured. In all 8 patients, basal GH secretion did not change significantly when the activin A concentration was 1 × 10⁻⁹ mol/l or less. Higher concentration of activin A reduced basal GH secretion. Fig. 1 depicts a dose-response relationship for the activin A-induced inhibition of basal secretion in a representative tumour. The inhibitory effect was maximal at 10⁻⁷ mol/l and, at higher concentrations, activin A was less effective in inhibiting GH secretion. The concentration of activin A which elicited the maximum inhibitory effect differed slightly among the 8 patients, but ranged between 10⁻⁸ and 10⁻⁷ mol/l. It should be noted that TGF-β did not reproduce the effect of activin A (data not shown). In addition, activin A did not affect proliferation of GH-secreting

**Table 1.**

Clinical features of Patients with GH-secreting pituitary adenoma.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Basal GH (μg/l)</th>
<th>TRH responsiveness</th>
<th>Basal PRL (μg/l)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>34</td>
<td>female</td>
<td>44</td>
<td>+</td>
<td>28</td>
</tr>
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<td>50</td>
<td>+</td>
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<td>8</td>
<td>45</td>
<td>female</td>
<td>95</td>
<td>+</td>
<td>17</td>
</tr>
</tbody>
</table>
tumour cells in our experimental condition (data not shown).

**Effect of activin A on TRH-mediated GH secretion**

The tumours under investigation came from TRH-responsive patients and their cells also responded to TRH in vitro. In these tumours, GH secretion was 200±35% of the basal (mean ± SEM, N=8) in the presence of 10 nmol/l TRH. When cells were pretreated with 1 nmol/l activin A for 48 h, a concentration which did not affect basal secretion significantly, GH release induced by TRH was almost completely blocked (Fig. 2). Thus, GH secretion in the presence of TRH was 110% of basal rate in activin A-treated cells.

**Effect of activin A on cellular GH content**

The above results indicate that 1 nmol/l activin A completely inhibited TRH-mediated GH secretion without inhibiting basal release of GH. We then examined whether 1 nmol/l activin A affected cellular content of GH. In activin A-treated cells, the GH content was 95.8±5.2% (N=8), which was not significantly different from that of untreated cells (100±3.9%).

**Effect of activin A on changes in cytoplasmic free calcium concentration induced by TRH**

To obtain insight into the mechanism of the inhibitory action of activin A, we examined whether activin A modified changes in \([Ca^{2+}]_c\) induced by TRH. Activin A by itself did not cause any immediate change in \([Ca^{2+}]_c\) as monitored by measuring the 340/380 ratio in GH-secreting tumour cells (data not shown). TRH caused an immediate rise in \([Ca^{2+}]_c\) within 10 sec and the initial peak of \([Ca^{2+}]_c\) was followed by a sustained plateau of \([Ca^{2+}]_c\). TRH also increased \([Ca^{2+}]_c\) in the absence of extracellular calcium even though the magnitude of the response was smaller. The plateau phase was abolished in the absence of extracellular calcium (data not shown). In an activin A-treated cell, TRH induced a similar increase in \([Ca^{2+}]_c\), however, the magnitude of the response was smaller.

**Effect of activin A on GH secretion induced by A23187 and TPA**

TPA elicited approximately a 4-fold stimulation of GH secretion (Fig. 3). In cells pretreated with activin A, TPA induced only a 2-fold stimulation of GH. A23187 stimulated GH secretion by 320%. Again, activin A pretreatment markedly reduced A23187-mediated GH secretion (Fig. 4).

**Fig. 1.**
Dose-response relationship for activin A action on GH secretion.
GH-secreting tumour cells obtained from a representative tumour were incubated for 48 h with varying concentrations of activin A. Cells were then washed twice with DME medium and were incubated for 2 h in DME medium. GH secreted was measured by radioimmunoassay. Values are the mean ± SEM for four experiments.

**Fig. 2.**
Effect of activin A on TRH-induced GH secretion.
TRH-responsive GH-secreting cells obtained from 8 tumours were incubated for 48 h with 1 nmol/l activin A. Cells were then washed twice and were incubated for 2 h in DME medium in the presence or absence of 10 nmol/l TRH. GH secreted was measured. Results are the mean ± SEM for eight experiments, each done in quadruplicate.
Discussion

Results of the present study clearly demonstrate that, like rodent pituitary cells, activin A inhibits basal GH secretion in human GH-secreting tumour cells. Also, at the concentrations used activin A abolishes GH secretion stimulated by TRH (data not shown). In addition, a lower concentration of activin A, which did not affect GH secretion by itself, attenuated GH secretion induced by TRH. These results are in agreement with previous reports showing that activin A inhibits synthesis and secretion of GH in rat pituitary cells (3,4,14). They further demonstrate that activin A is capable of modulating GH secretion by acting directly on somatotropes. Although the present study was done using tumour cells, these cells may originate from somatotropes and, in fact, share many characteristics with somatotropes: they synthesize GH; they store GH in secretory vesicles; they have many of the transduction systems for hypothalamic releasing hormones; and they secrete GH in response to hypothalamic hormones. The fact that activin A modifies GH secretion in GH-secreting tumour cells in vitro suggests that activin A directly affects normal somatotropes. Hence, inhibitory effects of activin A on GH secretion observed in rat pituitary cells in primary culture (3,4) may be due, at least partly, to a direct influence of activin A on somatotropes.

The present study also examined the mechanism by which activin A affected GH secretion. As mentioned, activin A did not cause any change in $[Ca^{2+}]_c$, an observation indicating that activin A has no effect on phosphoinositide turnover. Hence, in the pituitary somatotrope, activin A may not activate the calcium messenger system. This is in contrast to the mode of action of activin A in erythro-leukemia cells and in isolated hepatocytes, where activin A increases $[Ca^{2+}]_c$ by causing hydrolysis of polyphosphoinositides (15,16). These results indicate that the signal transduction system for activin A is complex: it activates the calcium messenger system in some types of cells, but not in the other.

Our present results further demonstrate that, in pituitary GH-secreting cells, activin A rather inhibits the effect of the calcium mobilizing hormone,
TRH. It should particularly be noted that, at a concentration of 1 nmol/l, activin A almost completely blocked GH secretion in response to TRH without affecting basal secretion of GH. At this concentration, activin A did not decrease cellular content of GH. It is therefore likely that activin A exerts its inhibitory effect by blocking at a certain point(s) the information flow of the signal transduction system. It is now established that TRH induces breakdown of phosphatidylinositol 4,5-bisphosphate, leading to generation of two intracellular messengers: inositol 1,4,5-trisphosphate (Ins-P3) and diacylglycerol (7,8). Ins-P3 causes a release of calcium from an intracellular trigger pool (17) and thereby increases cytoplasmic free calcium. On the other hand, diacylglycerol activates protein kinase C by increasing the sensitivity of the enzyme to calcium (18). Both an increase in [Ca\(^{2+}\)\(_c\)] and an activation of protein kinase C are prerequisites for the TRH action (9,10). The [Ca\(^{2+}\)\(_c\)] response to TRH was slightly inhibited, but not totally abolished in activin A-treated cells. Although we did not quantify the [Ca\(^{2+}\)\(_c\)] response, this observation indicates that TRH-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate and/or the action of Ins-P3 on the intracellular trigger pool may be partially inhibited by pretreatment with activin A. Hence, early events in the TRH action, including binding of TRH to its receptor, activation of phospholipase C via a G protein, hydrolysis of phosphatidylinositol 4,5-bisphosphate, were inhibited to some extent at a certain step(s). Alternately, activin A activates the calcium pump in the plasma membrane. In any event, pretreatment with activin A completely blocked TRH-induced GH secretion, whereas activin A only partially inhibited TRH-induced elevation of [Ca\(^{2+}\)\(_c\)]. Therefore, activin A may affect an additional step(s) to attenuate GH secretion. Consistent with this idea, GH release induced by both A23187 and TPA, agents which bypass the generation of two intracellular messengers, was markedly reduced by pretreatment with activin A. Activin A therefore modifies the action of TRH by acting on multiple steps, presumably generation of second messengers and a step distal to the generation of second messengers, Ins-P3 and diacylglycerol. It is possible that activin A modifies the function of protein kinases activated by these messengers. Alternately, it is also possible that activin A directly affects exocytosis of GH. Further studies are required to determine the mechanism by which activin A inhibits GH secretion.

Acknowledgments

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