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Activin-A: A Modulator of Multiple Types of Anterior Pituitary Cells

Masafumi Kitaoka*, Itaru Kojima and Etsuro Ogata

Fourth Department of Medicine
University of Tokyo School of Medicine,
3-28-6 Mejirodai, Bunkyo-ku,
Tokyo 112, Japan

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When primary culture of rat pituitary cells were incubated with 1 nM activin-A for more than 24 hrs, activin-A significantly increased intracellular content of FSH without affecting the control of LH. Pretreatment of the cells with activin-A also enhanced LHRH-induced FSH release without affecting LH release. Furthermore, pretreatment of the cells with activin-A significantly reduced both GRF-mediated GH release and TRH-mediated PRL release. However, activin-A did not affect the response of ACTH and TSH to their releasing hormones. These results indicate that, in addition to the known action on gonadotrophs, activin-A also modifies the function of somatotrophs and lactotrophs.

Researches on the isolation and characterization of inhibin from overian fluid have led to the discovery of a family of proteins which stimulate FSH secretion, namely activins (see review for 1). Activin-AB, a heterodimer of β_A- and β_B-subunits of inhibin (2), and activin-A, a homodimer of inhibin β_A-subunits (3), are the present members of this family. Independent of these works, Eto et al. (4) have isolated a polypeptide from conditioned medium of phorbol ester-treated human monocytic leukemia cells which causes differentiation of Friend erythroleukemia cells. The primary structure of this peptide, designated as erythroid differentiation factor (EDF), is a homodimer of β_A- subunits

*To whom correspondence should be addressed.

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of inhibin (5). Thus, EDF and activin-A are coded in the same messenger RNA (5). We have extended these observations by showing that EDF actually stimulates FSH secretion in primary cultures of rat pituitary cells (6), an observation suggesting that EDF and activin-A are in fact identical in their tertiary structure.

The present study was conducted to further define the action of activin-A on the secretory response of anterior pituitary cells. The results indicate that activin-A has a unique action on gonadotrophs. Furthermore, activin-A alters the secretion of growth hormone and prolactin.

Materials and Methods

Primary Culture of Anterior Pituitary Cells

Anterior pitutaries from female Wistar rats weighing 180 - 200 g were minced and digested by incubating with Dispase (1000 U/ml). The dispersed cells were seeded at a density of 2×10^5 cells/ml in 24-well culture dish and cultured for 3 to 5 days in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated fetal calf serum (FCS) at 37°C under humidified air containing 5% CO_2 (6).

Measurement of Hormone Content in the Cells

Monolayer cells were incubated for various periods in DMEM containing 10% FCS and 1 nM activin-A. Cells were then washed with DMEM and a 100 µl aliquot of 0.1 N NaOH was added to each well. Cells were freeze-thawed several times. The lysed cell suspension was centrifuged by a microfuge and hormone content in the supernatant was measured.

Measurement of Hormone Release

Monolayer cells were incubated for indicated time in DMEM containing 10% FCS in the presence or absence of 1 nM activin-A. After washing for two times with serum-free DMEM containing 0.1% BSA, cells were incubated for two hours with DMEM in the presence and absence of either of the following peptides: 10 nM luteinizing hormone-releasing hormone (LHRH), 10 nM growth hormone-releasing factor (GRF), 10 nM thyrotropin-releasing hormone (TRH), or 10 nM corticotropin-releasing factor (CRF). After the incubation, the medium was aspirated and stored at -20°C for the measurement of LH, FSH, GH, PRL, TSH and ACTH.

Hormones were assayed by double antibody RIA using antisera provided by NIDDKD. Data were expressed as mean ± S.E. and statistical analysis was done by using Student's t test.

Results

In our previous study, we have shown that activin-A increases secretion of FSH without increasing LH secretion (6). The action of activin-A is unique in that the stimulatory action was evident after 48 hrs. This observation suggests that activin-A increases FSH secretion presumably by stimulating synthesis of FSH. To examine this possibility, we determined the effect of activin-A on FSH content in pituitary cells. Figure 1 depicts the time course of the effect of activin-A on intracellular content of FSH and LH. Percent increments of FSH induced by 1 nM activin-A treatment for 6, 24 and 48 hrs were 119.3 ± 7.6%, 157.2 ± 14% and 162.8 ± 9.6%, respectively. Activin-A significantly increased intracellular FSH content after 24 and 48 hrs incubation. By contrast, activin-A did not affect LH content up to 48 hrs. (Figure 1). In cells not treated with activin-A, LHRH induced an approximately 3-fold stimulation of FSH release in 120 min. When cells were pretreated with 1 nM activin-A, LHRH-induced FSH release increased as a function of preincubation period. As shown in Figure 2, percent increments of LHRH-induced FSH release by activin-A pretreatment for 6, 24 and 48 hrs are 118.9 ± 5.3%, 151.0 ± 8.8% and 179.2 ± 9.2%, respectively. LHRH-induced FSH release was significantly increased by 24 and 48 hrs pre-incubation with 1 nM activin-A. In contrast, activin-A did not alter LHRH-induced LH release.

The effect of pretreatment with activin-A on basal and GRF-induced GH release is shown in Figure 3. Basal GH secretion in activin-A-untreated cells is expressed as 100%. GRF increased in GH release by 290.2 ± 19.0% during 120 min incubation. In activin-A-pretreated cells, basal GH

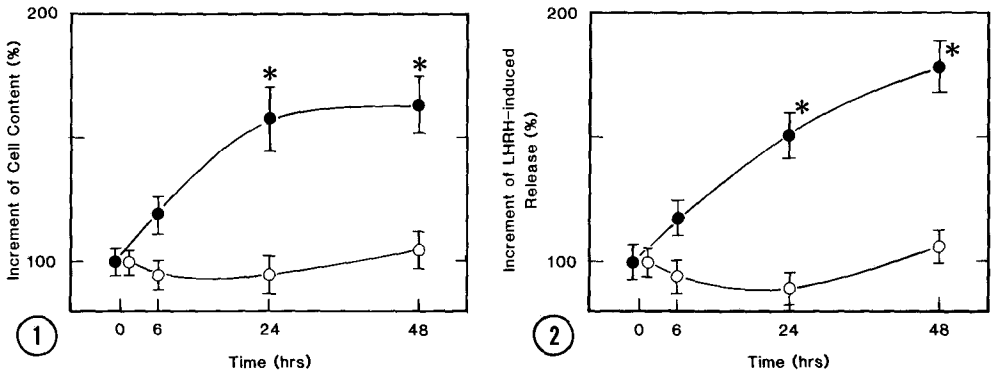

Figure 1. Time course of the effect of activin-A on intracellular content of FSH and LH in cultured rat anterior pituitary cells. Cells were incubated for the indicated time with 1 nM activin-A and then the cellular content of FSH (●) and LH (○) was measured (n = 12). Control is represented as 100% and the each bar represents S.E. *, $P < 0.01$

Figure 2. Time course of the effect of activin-A on LHRH-induced FSH- and LH-release from cultured rat anterior pituitary cells. Cells were incubated for the indicated time with 1 nM activin-A. Cells were washed and then incubated with serum-free DMEM containing 10 nM LHRH for 2 hrs. Release of FSH (●) and LH (○) during 2 hrs was measured (n = 12). *, $P < 0.01$

secretion was not significantly changed. However, GRF-induced GH release was significantly inhibited in cells pretreated with activin-A ($P < 0.02$).

The effect of preteatment with activin-A on basal and TRH-induced PRL release is shown in Figure 4. Basal PRL secretion in activin-A-untreated cells is expressed as 100%. In activin-A-treated cells, basal PRL secretion was not changed significantly while TRH-induced PRL release was significantly reduced ($P < 0.05$). It should be mentioned that activin-A did not affect the cellular content of either GH or PRL (data not shown).

Activin-A did not alter either the basal or CRF- and TRH-stimulated release of ACTH and TSH (data not shown). Moreover, activin-A did not alter the intracellular content of GH, PRL, TSH and ACTH (data not shown).

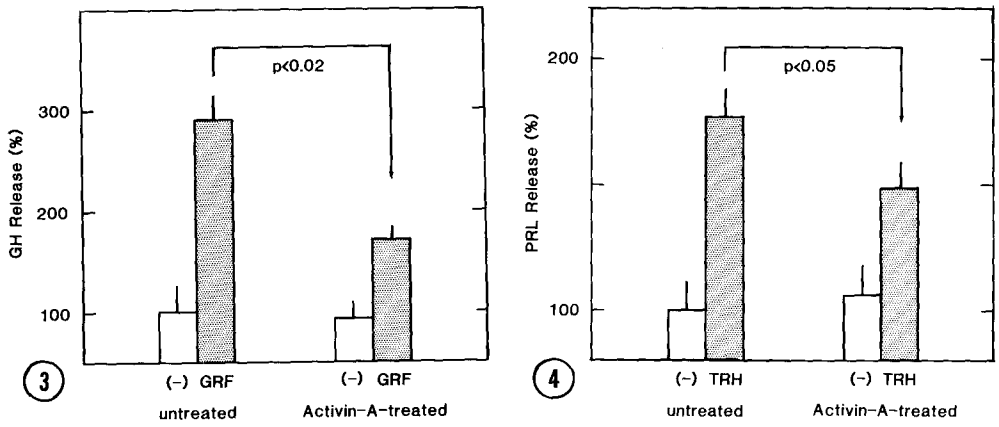

Figure 3. The effect of preteatment with activin-A on basal and GRF-induced GH release. Cells were incubated for 48 hrs with or without 1 nM activin-A. Cells were washed and then further incubated with 10 nM GRF for 2 hrs. GH release during 2 hrs was measured and is expressed as mean ± S.E. (n = 12). Basal GH secretion in activin-A-untreated cells is represented as 100%.

Figure 4. The effect of pretreatment with acvitin-A on basal and TRH-induced PRL release. Cells were incubated for 48 hrs with or without 1nM activin-A. Cells were washed and then further incubated with 10 nM TRH for 2 hrs. PRL release during 2 hrs was measured and is expressed as the mean ± S.E. (n = 12). Basal PRL secretion in activin-A-untreated cells is represented by 100%.

Discussion

The present results confirmed the observation by Vale et al. (3) that activin-A increases the cell content of FSH in pituitary cells. We also found that the actions of activin-A and LHRH are additive. Enhancement by activin-A of LHRH-induced FSH release is due largely to the increase in FSH content of the cells. These results together with the results by Vale et al. (3) indicate that activin-A and LHRH increases FSH secretion by different mechanisms. Several possibilities can be considered for the action of activin-A. First, activin-A selectively increases the synthesis of FSH in gonadotrophs. Second, activin-A increases the number of gonadotrophs containing preferentially FSH by changing the characteristics of ganadotrophs, and third, activin-A

increases the number of FSH-containing gonadotrophs by causing the differentiation of immature or undifferentiated pituitary cells. Since activin-A causes erythroid differentiation (4, 5) and since activin-A is a member of the TGF-β gene family, the latter two possibilities should be tested in the near future.

Another action of activin-A revealed in the present study is the inhibition of GRF-mediated GH secretion and TRH-induced PRL secretion. Since the cell contents of GH and PRL are not changed significantly by preteatment with activin-A, activin-A-induced inhibitory action may not be solely due to the reduction of hormone synthesis. Activin-A may modify either directly or indirectly the transduction systems of the two releasing hormones. The possibility that activin-A modifies both synthesis and release of GH and PRL is also plausible. From an embryological point of view, both lactotrophs and somatotrophs are derived from the common origin, namely acidophil stem cell. Hence, the fact that the inhibitory actions of activin-A are limited on lactotrophs and somatotrophs is an indication of the action of activin-A on this cell lineage.

The present findings that activin-A inhibits both GRF-mediated GH release and TRH-mediated PRL release, in addition to the action on gonadotrophs, suggest that activin-A may be a modulator of multiple types of anterior pituitary cells.

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