Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 413-419

INTERACTION OF ACTIVIN A AND GONADAL STEROIDS ON FSH SECRETION FROM PRIMARY CULTURED RAT ANTERIOR PITUITARY CELLS

Toshikazu Miyake, Minoru Irahara, Keiji Shitukawa, Toshiyuki Yasui and Toshihiro Aono

Department of Obstetrics and Gynecology, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima, 770, Japan

Received May 24, 1993

Summary : To clarify the mechanism of FSH secretion from the pituitary induced by activin A, we studied the interaction between activin A and gonadal steroids in inducing FSH release from primary cultured female rat pituitary cells in serum-free medium. The basal release of FSH was stimulated by activin A, testosterone (T) and progesterone (P), and T and P also facilitated basal FSH release stimulated by activin A. The GnRH-stimulated FSH release was facilitated by activin A, P and 17β-estradiol (E_2), but suppressed by T. The effect of activin A on GnRH-stimulated FSH release was facilitated by P, but not affected by T or E_2. These findings suggested that the interaction between activin A and T or P may be involved in the regulation of FSH secretion during the estrus cycle in rats .

Secretion of gonadotropins from the anterior pituitary is controlled by various factors such as hypothalamic GnRH, gonadal steroids and protein hormones produced by the gonads (1). The mechanism of gonadotropin secretion from the pituitary by GnRH and gonadal steroids have been studied extensively (2-4). The protein hormones, inhibin and activin produced by the gonads, are also reported to have important roles in this mechanism. Inhibins inhibit basal and GnRH-stimulated secretions of FSH from the pituitary, and the profile of change in serum inhibin during the menstrual cycle has been reported (5,6). In contrast, activins stimulate FSH secretion, but details of their role in the mechanism of gonadotropin secretion are not known, because the serum activin level is very difficult to measure (7). Recently inhibin was suggested to interact

0006-291X/93 $4.00

with gonadal steroids on gonadotropin secretion by cultured pituitary cells from male rats (8). To clarify the mechanism of the effect of activin A on FSH secretion from the pituitary, we studied its interaction with gonadal steroids in inducing FSH secretion from primary cultured female rat pituitary cells in serum free medium.

MATERIALS AND METHODS

Materials : Recombinant activin A was provided by Ajinomoto Co, (Tokyo) (9). Testosterone, progesterone, 17β-estradiol and GnRH were obtained from Sigma (St. Louis, MO).

Primary cell culture of rat anterior pituitary : Female Wistar rats weighing 180-250 g were decapitated and their anterior pituitaries were excised. The pituitaries were cut into small pieces and washed in HEPES dissociation buffer, consisting of 25 mM HEPES, 137 mM NaCl, 5 mM KCl, and 0.7 mM Na_2HPO_4, pH 7.3. The cells were dissociated by treatment with 0.4 % collagenase plus DNase at 37℃ for 2h, followed by incubation with pancreatin at 37℃ for 7 min, according to a reported method (8). These cells were seeded at 2 x 10^5 cells / ml in 24-well culture dishes (Falcon Plastics, Los Angels, CA) and cultured for 72 h in phenol red free Dulbecco's modified Eagle medium (DMEM ; Nissui Co Tokyo) containing 10 % fetal calf serum (FCS) at 37℃ under 5 % CO_2 humidified air. The FCS added to cultures was treated with dextran-coated charcoal to remove endogenous steroids before use (8). After culture for 72 h, the cells were washed 3 times with serum-free DMEM containing 0.1% bovine serum albumin and then incubated for 48 h in serum-free DMEM medium in the presence or absence of 1nM activin A and various concentrations of gonadal steroids as indicated. For determination of basal release of FSH, the supernatant were collected for RIA of FSH. The cells were then washed 3 times with serum-free fresh DMEM medium, and activin A and/or gonadal steroids were added at the same concentrations as used for measurement of basal release, followed by 10 nM GnRH. The cultures were then incubated for 4h at 37℃. The supernatant were then aspirated for measurement of FSH by RIA.

Assay of FSH : FSH in supernatants was assayed by double antibody RIA with a kit of NIADDK. The FSH concentrations in different cultures varied, and so were expressed as persentages of the control value. All measurements were made on four samples and data are expressed as means±SE.

Statistical analyses : All data were calculated using the ANOVA program and the significance of differences was examined by Student's t-test.

RESULTS

Individual effects of activin A and gonadal steroids on basal and GnRH-stimulated FSH secretion from primary cultured rat anterior pituitary cells

The effects of activin A, T, P, and E_2 individually on the basal and GnRH-stimulated FSH release from primary cultured rat anterior pituitary cells are shown in Figs.1 and

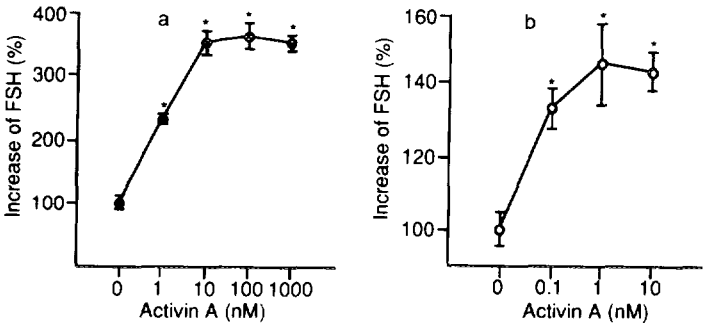

Fig.1(a). Effect of activin A on basal FSH release by cultured rat anterior pituitary cell. Cells were incubated with the indicated concentrations of activin A for 48 h. The control value (0 nM activin A) is taken as 100 %. The value of 100 % was 3.7±0.41 ng / well. Points and bars represent means ± SE. *p < 0.01 vs. control.

Fig.1(b). Effect of activin A on GnRH-stimulated FSH release. The cells were treated for the results in Fig.1 (a). Then the supernatant was removed and the cells were washed 3 times with fresh medium and retreated with the same concentration of activin A, followed by addition of 10 nM GnRH for 4h. The control value (0 nM activin A) is taken as 100 %. The value of 100 % was 4.4±0.21 ng / well. Points and bars represent mean ± SE. *p < 0.01 vs. control.

2. Activin A significantly stimulated both the basal and GnRH-stimulated FSH release in a dose dependent manner (Fig. 1a, Fig. 1b). P and T also significantly stimulated basal FSH release in dose dependent manners, but E_2 did not stimulate FSH release (Fig. 2a). P and E_2 facilitated but T suppressed GnRH-stimulated FSH release (Fig. 2b). These changes were statistically significant.

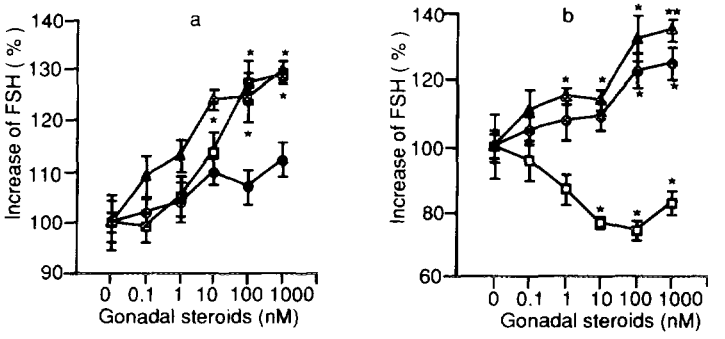

Fig.2(a). Effects of gonadal steroids on basal FSH release. Rat anterior pituitary cells were incubated for 48 h with the indicated concentrations of T(□), P (▲), or E_2 (●). The control value (0 nM steroid) is taken as 100 %. Points and bars represent means ± SE. *p < 0.05 vs. control.

Fig.2(b). Effects of gonadal steroids on GnRH-stimulated FSH release. Rat anterior pituitary cells were incubated with T(□), P (▲), or E_2 (●) as in Fig.2(a). Then the media were removed , and the cells were washed 3 times with fresh medium and retreated with the same concentration of gonadal steroids, followed by addition of 10 nM GnRH for 4h. The control value is taken as100 %. Points and bars represent means ± SE. *p < 0.05 , **p <0.01 vs. the control values.

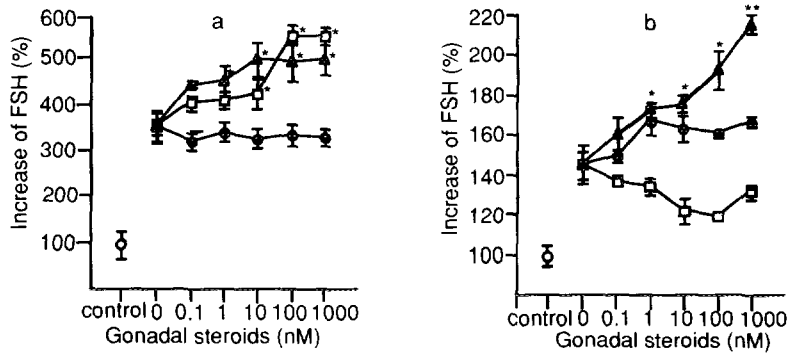

Fig.3(a). Combined effects of activin A and gonadal steroids on basal FSH release. Rat anterior pituitary cells were incubated for 48h with 1nM activin A and the indicated concentrations of T(□), P(▲) or E2(●). The control value is that without activin A or steroids. Values are means ± SE. *p< 0.05 vs. value with 1nM activin A but without steroids.

Fig.3(b). Combined effects of activin A and gonadal steroids on GnRH-stimulated FSH release. Rat pituitary cells were incubated with 1nM activin A and T(□), P(▲) or E2(●) as described for Fig.3(a). Then the media were removed and the cells were washed 3 times and retreated with same concentrations of activin A and gonadal steroids, followed by addition of 10nM GnRH. The control value is that without activin A or steroids. Values are means ± SE. *p<0.02, **p<0.01 vs. value with 1 nM activin A but without steroids.

Combined effects of activin A and gonadal steroids on basal and GnRH-stimulated gonadotropin release from primary cultured rat anterior pituitary cells

The combined effects of activin A and gonadal steroids on basal and GnRH-stimulated FSH release from primary cultured rat anterior pituitary cells are shown in

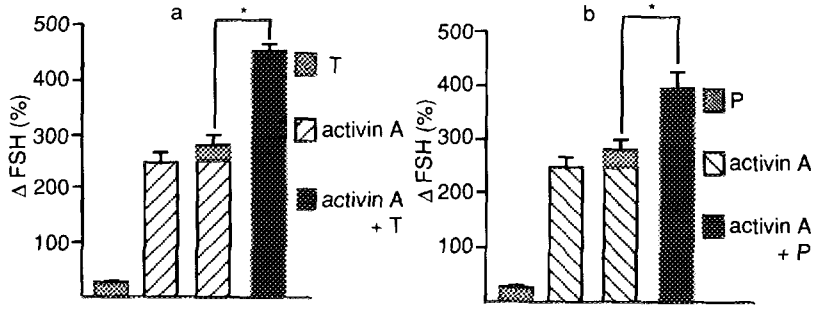

Fig.4 (a). Increases in basal FSH release (Δ FSH) by activin A plus T. The Δ FSH value with activin A plus T was greater than the sum of the Δ FSH values with activin A and T individually. Columns and bars represent means ± SE. *p < 0.05.

Fig.4(b). Increases in basal FSH release (Δ FSH) by activin A plus P. The Δ FSH value with activin A plus P was greater than the sum of Δ FSH values with activin A and P individually. Columns and bars represent means ± SE. *p < 0.05.

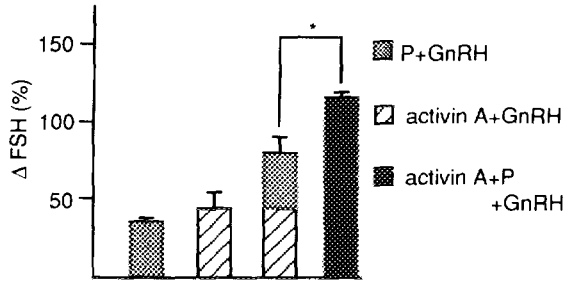

Fig.5. Change of increases in GnRH-stimulated FSH release (Δ FSH) by activin A plus P. The Δ FSH value with activin A plus P was greater than the sum of the Δ FSH values with either alone. Columns and bars represent means ± SE. *p < 0.05.

Fig. 3. On incubation with 1nM activin A, increasing concentrations of P or T facilitated basal FSH release (p <0.05) but E_2 did not (Fig. 3 a). Further more, incubation of 1 nM activin A with increasing concentrations of P facilitated GnRH-stimulated FSH secretion (p<0.01, 0.02), but T and E_2 did not (Fig. 3 b). The values for the increment of FSH release by activin A plus P or T over the basal FSH release (ΔFSH) were significantly greater than the sum of their individual ΔFSH values (Fig. 4). The ΔFSH value for GnRH-stimulated FSH release by activin A plus P was also greater than the sum of the ΔFSH values by each hormone alone (Fig. 5).

DISCUSSION

In this study, we examined the interaction between activin A and gonadal steroids using primary cultured female rat pituitary cells in serum-free medium. Activin A has been reported to stimulate basal and GnRH-stimulated FSH secretions by primary cultures of rat pituitary cells (10). In these earlier studies, the culture medium contained FCS and phenol red. However, recently phenol red was suggested to have estrogen activity (11), and FCS may also have some activin like activity because the species-specifity of activin is low. Therefore, in the present study we used culture medium without FCS or phenol red to examine the interaction of activin A and various gonadal

steroids on FSH secretion. Our results confirmed that activin A strongly enhances both basal and GnRH-stimulated FSH secretion, and that gonadal steroids also have stimulatory or inhibitory effects on basal and GnRH-stimulated FSH release, even in medium without FCS and phenol red. Our results were statistically significant, but observed effects were rather weak, mainly due to use of the serum-free medium, dose and durations of exposure to these steroids (2-4, 12-14). There are reports that T stimulates basal FSH release and inhibites GnRH-stimulated FSH release (2, 3). T has a biphasic effect on FSH secretion. In our study, T also facilitated activin A-stimulated basal FSH secretion. In the estrus cycle of rats, serum T increases toward the time of the second surge of FSH (14, 15) and pulsatile GnRH secretion becomes low in this period (16). Gay et al. (15) suggest that T is implicated in the stimulus for the second surge of FSH during the estrus cycle in rat, but its site of action is unclear. Hasegawa et al. reported that in rats, serum inhibin level becomes low during the second surge of FSH (5,6). However, they also suggested that elevation of serum inhibin reduces the level of FSH but that its decrease is not involved in increase of FSH. Our results suggest that FSH increases in the second surge of FSH in the estrus cycle of rats due to the combined effect of activin A and T. The physiological significance of activin A in the preovulatory FSH surge is unclear because the high level of serum inhibin may mask the activin activity in this period in vivo. However, recently serum P was found to increase in the preovulatory phase, and this preovulatory increase of serum P was suggested to be necessary for ovulation and LH and FSH spikes (17). The mechanism of the elevation of FSH secretion is not yet clear, but our data demonstrating that interaction of activin A and P facilitated FSH secretion supports this mechanism. We did not detect any significant influence

of E_2 on the effect of activin A on FSH secretion, but more detailed studies on its effects are necessary because it is known to be important in gonadotropin secretion.

In conclusion, our results using primary cultures of rat pituitary cells in vitro demonstrate that T and P facilitate FSH secretion from pituitary cells by activin A. These data suggest that the interaction between activin and these gonadal steroids may be involved in regulation of FSH secretion in the preovulatory and ovulatory phases of the estrus cycle in rats. For obtaining further information on this problem, a method is required for measuring the serum level of activin to determine the profile of its change during the menstrual cycle.

REFERENCES

(1) Savoy-Moore R.T., Schwartz, N. (1980) Int. Rev. of Physiol. 22 203-248.
(2) Schally, A. V., Redding, T. W., Arimura, A. (1973) Endocrinol. 93 893-902.
(3) Drouin, J., Labrie, F. (1976) Endocrinol. 98 1528-1534.
(4) Tang, I. K. L., Spies, H, G. (1975) Endocrinol. 96 349-356.
(5) Hasegawa, Y., Miyamoto, k., Igarashi, M., Yanaka, T., Sasaki, K., Iwamura, S. (1987) : Changes in serum concentrations of inhibin during the estrus cycle of the rat, pig and cow. in "Inhibin-Non-Steroidal Regulation of Follicle Stimulating Hormone Secretion" Serono Symposia, (H.G. Burger, D.M., de Kretser., J.K. Findlay., M. Igarashi., Eds.) pp119-133. Raven Press, New York.
(6) Hasegawa, Y., Miyamoto, K., Igarashi, M. (1989) J. Endocrinol. 121 91-100.
(7) Shintani,Y., Tanaka,Y., Yamasaki, R. (1991) J. Immunol.Methods. 137 267-274.
(8) Campen, C.A., Vale, W. (1988) Endocrinol. 123 1320-1328.
(9) Eto, Y., Tsuji, T., Takezawa, M., Yokogawa, Y., Shibai, H. (1987) Biochem. Biophys. Res. Commun. 142 1095-1103.
(10) Kitaoka, M., Kojima, I., Ogata, E., (1988) Biochem. Biophys. Res. Commun. 157 48-54.
(11) Hubert, J. F., Vincent, A., Labrie, F. (1986) Biochem. Biophys. Res. Commun. 141 885-891.
(12) Drouin, J., Labrie, F. (1981) Endocrinol. 108 52-57.
(13) Kamel, F. (1987) Endocrinol. 120 1651-1657.
(14) Butcher, R. L., Collins, W .E., Fugo, N. W. (1974) Endocrinol. 94 1704-1708.
(15) Gay, V. L., Tomacari, R. L. (1974) Science. 184 75 -77.
(16) Norman, R. L., Lindstrom, S. A., Bangsberg, D., Ellinwood, W. E., Gliessman, P., Spies, H. G. (1984) Endocrinol. 115 261-266.
(17) Batista, M. C., Cartledge, T. P., Zellmer, A. W., Nieman, L. K., Merriam, G. R., Loriaux, D.L. (1992) Endocrinol. 74 565-570.