Effect of Activin A and Follistatin on the Release of Pituitary Hormones in the Bullfrog Rana catesbeiana

Authors: Koda, Aya, Yamamoto, Kazutoshi, Uchiyama, Hideho, Vaudry, Hubert, and Kikuyama, Sakae

Source: Zoological Science, 17(7) : 971-975

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.17.971

ZOOLOGICAL SCIENCE **17**: 971–975 (2000)

Effect of Activin A and Follistatin on the Release of Pituitary Hormones in the Bullfrog *Rana catesbeiana*

Aya Koda[1], Kazutoshi Yamamoto[1], Hideho Uchiyama[2], Hubert Vaudry[3] and Sakae Kikuyama[1]*

[1]*Department of Biology, School of Education, Waseda University, Nishiwaseda 1-6-1, Shinjuku-ku, Tokyo 169-8050, Japan*
[2]*Department of Environmental Science, Yokohama City University, Seto 22-2 , Kanazawa-ku, Yokohama, Kanagawa 236-0027, Japan*
[3]*European Institute for Peptide Research, Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U 413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France*

ABSTRACT—The effects of activin A and follistatin on the release of follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH) and prolactin (PRL) from dispersed pituitary cells of the bullfrogs *Rana catesbeiana* were studied. Activin A stimulated the release of FSH, GH, and PRL dose-dependently, but not that of LH. Follistatin suppressed the activin-induced FSH, GH, and PRL release, but did not affect the basal secretion of those hormones. From the results obtained in this experiment, together with the previously obtained findings that activin B enhanced the release of FSH, LH, GH, and PRL, we conclude that activin A, in addition to activin B, influences the function of multiple types of pituitary cells in the bullfrog.

INTRODUCTION

Evidence has accumulated that in mammals, different types of anterior pituitary cells regulate the function of the neighboring cells and/or themselves by secreting paracrine and/or autocrine factors. (Denef *et al*., 1989; Denef and Van Bael, 1998; Schwarts and Cherney, 1992). In amphibians, we have also demonstrated that pituitary hormones or substances present in the pituitary cells act in a paracrine and/or autocrine fashion to regulate the release of pituitary hormones. In the bullfrog (*Rana catesbeiana*), the α-subunit of glycoprotein hormones stimulates prolactin (PRL) release (Oguchi *et al*., 1996), PRL enhances the release of luteinizing hormone (LH) (Oguchi *et al*., 1997), and endothelin-3 existing in gonadotrophs (Suzuki *et al*., 1997a) stimulates the release of growth hormone (GH) and PRL (Suzuki *et al*., 1997b). Proopiomelanocortin (POMC)-derived peptides, namely, the N-terminal peptide of POMC (NPP), and adrenocorticotropic hormone (ACTH) enhance the release of LH (Aida *et al*., 1997), GH and PRL (Aida *et al*., 1999).

Recently, the presence of immunoreactive activin/inhibin β_B in bullfrog gonadotrophs and thyrotrophs has been demonstrated. Investigation of the effects of activin B and inhibin B on the release of gonadotropins from dispersed anterior pituitary cells revealed that activin B enhanced the release of both follicle-stimulating hormone (FSH) and LH, and that inhibin B blocked the activin B-induced enhancement of FSH and LH release without suppressing their basal secretion (Uchiyama *et al*., 2000), suggesting the possibility that activin B is one of the paracrine and/or autocrine factors in the bullfrog pituitary.

Activin and inhibin were first purified from mammalian follicular fluid as factors that regulate the secretion of FSH from pituitary cells in culture (Ling *et al*., 1985; 1986a,b; Mason *et al*., 1985; Rivier *et al*., 1985; Vale *et al*., 1986). Inhibin, a suppressor of FSH secretion, is composed of an a chain covalently linked to a β chain; whereas activin, a stimulator of FSH secretion, is composed of a dimer of the β chain. Since there are two structurally related β chains, activin has three isoforms ($\beta_A\beta_A$ as activin A, $\beta_A\beta_B$ as activin AB, and $\beta_B\beta_B$ as activin B).

In the present experiment, we studied the effect of activin A on the release of FSH, LH, GH and PRL from dispersed pituitary cells of the bullfrog. The effect of follistatin, a potent inhibitor of activin, on the release of the four pituitary hormones in the presence or absence of activin A was also investigated.

* Corresponding author: Tel. +81-3-5286-1517;
FAX. +81-3-3207-9694.
E-mail: kikuyama@mn.waseda.ac.jp

MATERIALS AND METHODS

Animals

Adult male bullfrogs, weighing approximately 600g were supplied from Oouchi (Misato, Japan) during summer and were kept in a laboratory condition for 10–15 days.

Hormones

Human recombinant activin A and follistatin were provided by Ajinomoto Corporation (Kawasaki, Japan). Mammalian gonadotropin-releasing hormone (mGnRH) and thyrotropin-releasing hormone (TRH) were purchased from Peptide Institute (Osaka, Japan) and Sigma-Aldrich Corporation. (Tokyo, Japan), respectively.

Cell incubation and hormone assay

Preparation of anterior pituitary cells of the bullfrog was performed according to the procedures described elsewhere (Oguchi *et al.*, 1996). The enzymatically dispersed anterior pituitary cells were suspended in 70% Medium 199 (M199; Nissui Pharmaceutical, Tokyo, Japan) containing 0.1% BSA (Fraction V; Sigma, St. Louis, MO). Two hundred microliters containing 60,000 pituitary cells were plated in each well of a 96-multiwell plate (Corning Inc., NY) and incubated at 25°C

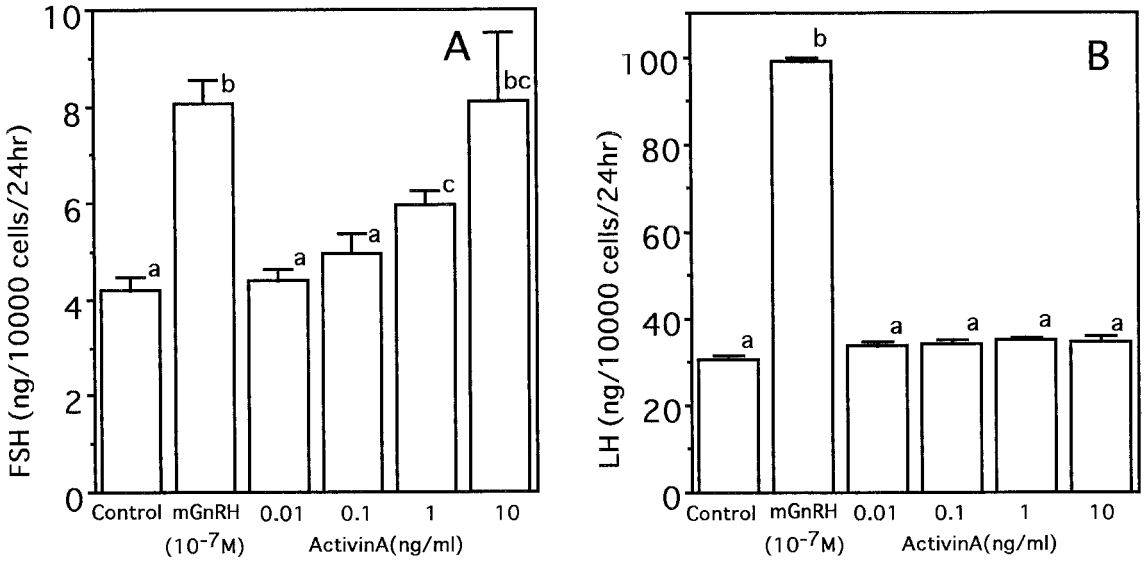

Fig. 1. Effect of activin A on the release of FSH (A) and LH (B) from dispersed pituitary cells of the bullfrog. As a reference secretagogue mGnRH was employed. Each column and vertical bar represent the mean of 6 determinations and SEM, respectively. The values with the same superscript are not significantly different at the 5% level.

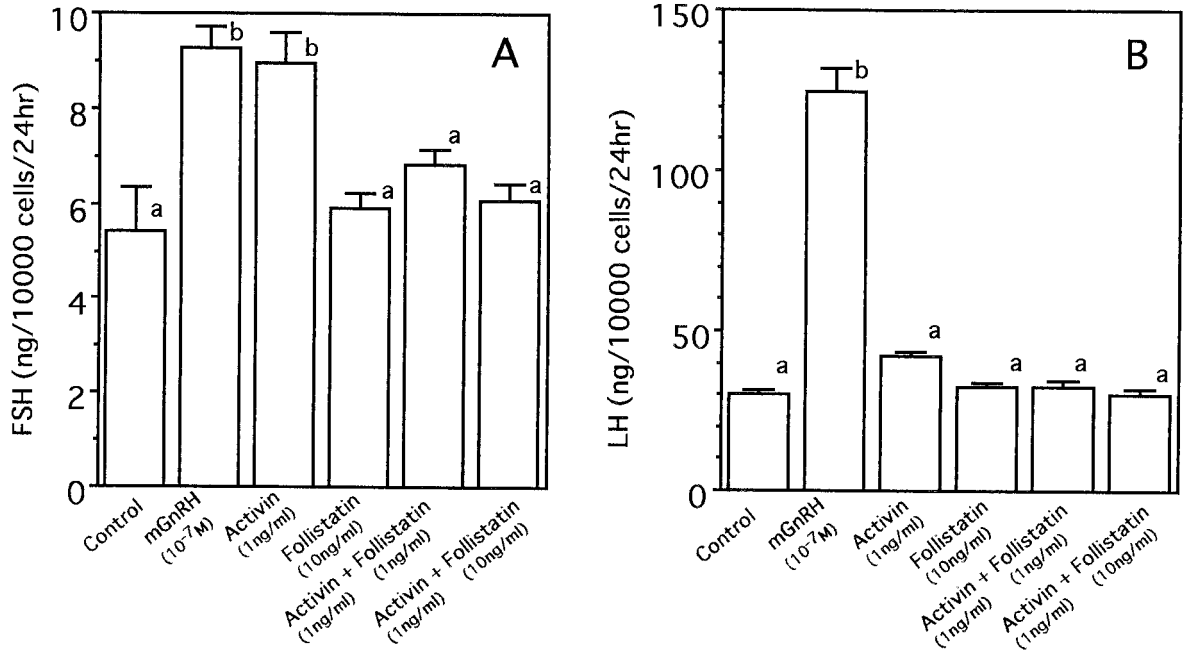

Fig. 2. Effect of follistatin on the activin A-induced release of FSH (A) and LH (B) from dispersed pituitary cells of the bullfrog. The cells were incubated in the presence of mGnRH, activin, follistatin and combination of activin and follistatin. Each column and vertical bar represent the mean of 6 determinations and SEM, respectively. The values with the same superscript are not significantly different at the 5% level.

in a humidified atomosphere of 95% air-5% CO_2. After preincubation for 24 hr, the medium was replaced with 70% M199 containing a test substance i.e. activin A or follistatin either singly or in combination. When necessary, hGnRH or TRH was also used in order to secure the responsiveness of pituitary cells. Incubation was continued for 24 hr, since dispersed pituitary cells of the bullfrog are known to release various pituitary hormones continuously during this period (Oguchi *et al*., 1997; Aida *et al*., 1997). After incubation, the medium was collected from each well and centrifuged, and the supernatant was subjected to radioimmunoassay (RIA) for bullfrog FSH (Polzonetti-Magni *et al*., 1998), LH (Oguchi *et al*., 1997), GH (Kobayashi *et al*., 1991) and PRL (Yamamoto and Kikuyama, 1982).

The values, given as the mean±SEM, were expressed as ng/10^4 cells and analyzed by using analysis of variance and Duncan's multiple range test. Differences at $P<0.05$ were considered significant.

RESULTS

Effect of activin A on the release of FSH and LH.

Activin A stimulated the release of FSH from the dispersed pituitary cells dose-dependently in the range of 0.01–10 ng/ml (Fig. 1A). Activin A at the concentration of 10 ng/ml and 10^{-7}M mGnRH, which was employed as a reference secreta-

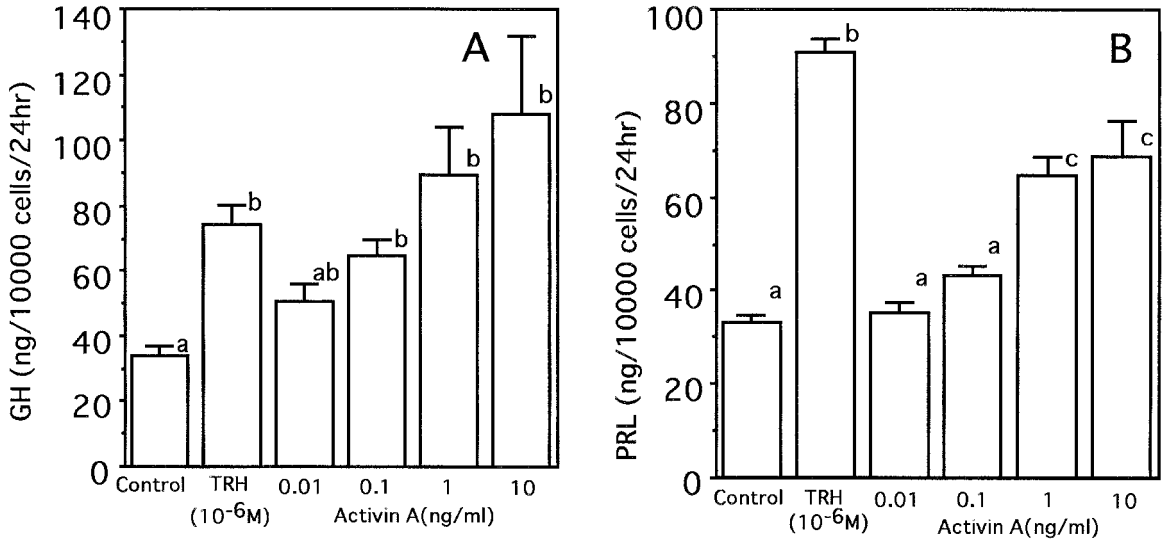

Fig. 3. Effect of activin A on the release of GH (A) and PRL (B) from dispersed pituitary cells of the bullfrog. TRH was employed as a reference secretagogue. Each column and vertical bar represent the mean of 6 determinations and SEM, respectively. The values with the same superscript are not significantly different at the 5% level.

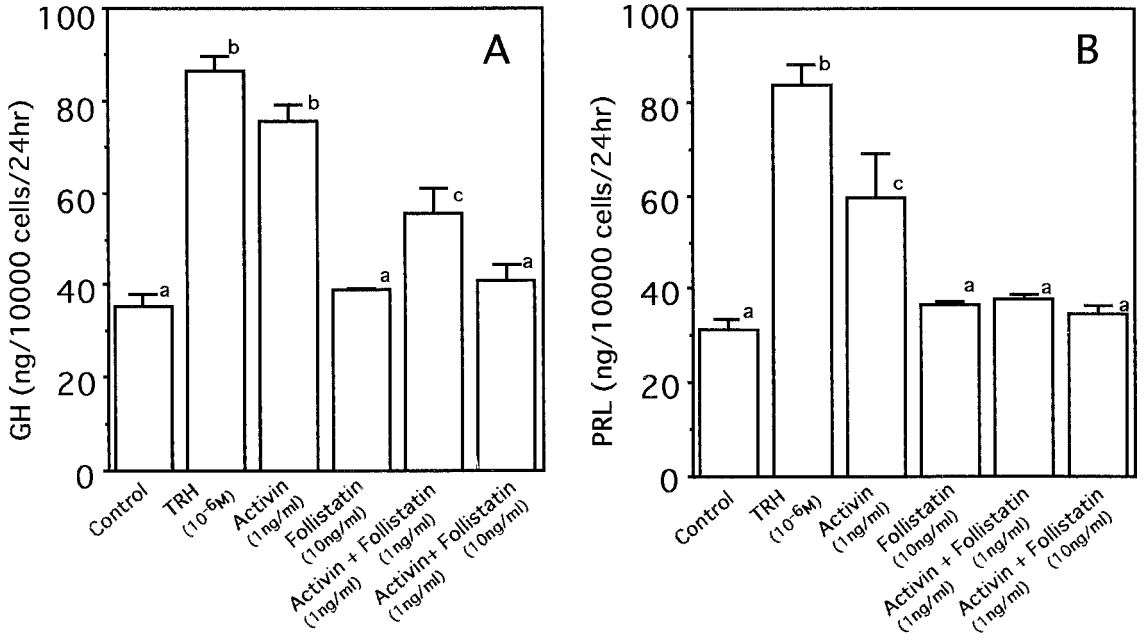

Fig. 4. Effect of follistatin on the activin A-induced release of GH (A) and PRL (B) from dispersed pituitary cells of the bullfrog. The cells were incubated in the presence of TRH, activin, follistatin, and the combination of activin and follistatin. Each column and vertical bar represent the mean of 6 determinations and SEM, respectively. The values with the same superscript are not significantly different at the 5% level.

gogue, were equipotent in terms of stimulating the release of FSH during the 24-hr incubation. On the other hand, activin A in the same dose range did not exert any influence upon the release of LH (Fig. 1B). As shown in Fig. 2, follistatin alone (10ng/ml) did not affect the basal release of either FSH or LH. However, it completely suppressed the activin (1 ng/ml)-induced FSH release at the concentration of 1–10 ng/ml. (Fig. 2A); whereas the combination of activin A and follistatin did not affect the release of LH (Fig. 2B).

Effect of activin A on the release of GH and PRL.

TRH used as a reference secretagogue significantly stimulated the release of both GH and PRL during the 24-hr incubation period. Activin A stimulated the release of GH markedly (Fig. 3A); and PRL, rather moderately (Fig. 3B). In either case, the response to activin A was dose-dependent in the range of 0.01–10 ng/ml. The effect of follistatin on the activin-induced GH and PRL release was then studied. As shown in Fig. 4, follistatin completely suppressed the activin (1 ng/ml)-induced release of both GH and PRL at the dose of 10 ng/ml. When the follistatin concentration was reduced to 1 ng/ml, the inhibitor blocked the release of GH partially but the release of PRL completely. Follistatin did not affect the basal secretion of either GH or PRL at the concentration of 10 ng/ml.

DISCUSSION

The present experiment indicated that activin A has a stimulatory effect on the release of hormones from multiple types of pituitary cells. We focused on the release of LH, FSH, GH, and PRL because RIA systems for these four hormones of the bullfrog are available in our laboratory. Activin A stimulated the release of FSH but not that of LH. This is in accord with the results obtained in mammals. Activin selectively stimulates FSH secretion but has little or only a slight effect on LH secretion in cultured and perifused rat pituitary cells (Ling *et al*., 1986b; Vale *et al*., 1986; Ying *et al*., 1988; Carroll *et al*., 1991), although there are reports that activin enhances GnRH-induced LH as well as FSH release from rat (Weiss *et al*., 1993) and macaque (Zhengwei *et al*., 1998) pituitaries. In the bullfrog, we observed that activin B stimulates both FSH and LH from dispersed pituitary cells (Uchiyama *et al*., in press). In the goldfish, Ge *et al*. (1992) reported that activin A promotes GTH-II secretion. More recently, they found that recombinant goldfish activin B stimulated the expression of GTH-Iβ subunit mRNA but suppressed that of GTH-IIβ mRNA (Yan *et al*., 1999).

In this experiment, we found that activin A stimulated not only FSH but also GH and PRL release, GH cells being the most sensitive to activin A among LH, GH, and PRL cells. In mammals, there are some reports on the effects of activin on the release of GH and PRL. Incubation of rat anterior pituitary cells with activin A results in a reduction in basal GH secretion (Billestrup *et al*., 1990). Also, activin A suppresses GH-releasing hormone-induced GH release (Billestrup *et al*., 1990; Kitaoka *et al*., 1988). On the other hand, activin A has no effect on basal PRL secretion but restrains TRH-induced PRL release (Kitaoka *et al*., 1988). In lower vertebrates, only one report on the effect of activin on GH and PRL cell function has been published. In goldfish, activin A stimulated GH release from perifused pituitary fragments (Ge and Peter, 1994). We observed that activin B enhanced the release of GH and PRL from the bullfrog pituitary *in vitro* (unpublished data). Thus, we can conclude that activins exert diverse effects on the pituitary function, according to the cell types and species.

In lower vertebrates, information about the localization of activins in the pituitary is rather scanty, although genes for the activin/inhibin β_A and β_B chains have been cloned in *Xenopus* (Thomsen *et al*., 1990; Dohrmann *et al*., 1993) and the goldfish (Ge *et al*., 1993). According to Ge and Peter (1994), activin/inhibin subunits (β_A and β_B) do not occur in gonadotrophs but are confined to somatotrophs in the goldfish pituitary. Since the inhibin α-subunit is not expressed in somatotrophs but in other cells, they concluded that somatotrophs may contain activin(s). More recently, we demonstrated that immunoreactive β_B exists in thyrotrophs, somatotrophs and gonadotrophs of the *Xenopus* pituitary (Uchiyama *et al*., 1996) and in thyrotrophs and gonadotrophs of the bullfrog pituitary (Uchiyama *et al*., in press). However, further studies are needed to determine which isoforms of activins and inhibins are actually contained in these immunoreactive cells.

ACKNOWLEDGMENT

We thank Dr. Y. Eto of Ajinomoto Corporation for the gifts of human recombinant activin A and follistatin and Prof. K. Wakabayashi of Gunma University for supplying the goat anti-rabbit IgG serum (HAC-RBA2-05GTP91). Thanks are also extended to Drs. A. Oguchi and T. Aida for their help during the course of the experiments. This work was supported by INSERM-JSPS exchange program to H.V. and S.R. and research grants from Waseda University, Asahi Glass Research Foundation, Uehara Memorial Foundation, and the Ministry of Education, Science, and Culture of Japan to S.K.

REFERENCES

Aida T, Oguchi A, Iwamuro S, Yamamoto K, Kikuyama S (1997) Stimulation by proopiomelanocortin-derived peptides of LH release by bullfrog dispersed anterior pituitary cells. Zool Sci 14: 827–831

Aida T, Yamamoto K, Kikuyama S (1999) Enhancement by proopiomelanocortin-derived peptides of growth hormone and prolactin secretion by bullfrog pituitary cells. Gen Comp Endocrinol 115: 101–109

Billestrup N, Gonzalez-MC, Potter E, Vale W (1990) Inhibition of somatotroph growth and growth hormone biosynthesis by activin *in vitro*. Mol Endocrinol 4: 356–362

Carrolle R, Corrigan AZ, Vale W, Chin W (1991) Activin stabilizes follicle-stimulating hormone-beta messenger ribonucleic acid levels. Endocrinology 129: 1721–1726

Denef C, Maertens P, Allaerts W, Mignon A (1989) Cell–to-cell communication in peptide target cells of the anterior pituitary. Methods Enzymol 168: 47–71

Denef C, Van Bael A (1998) A new family of growth and differentiation factors derived from the N-terminal domain of proopiomelanocortin (N-POMC). Comp Biochem Physiol C 119: 317–

324
Dohrmann CE, Hemmati-Brivanlou A, Thomsen GH, Fields A, Woolf TM, Melton DA (1993) Expression of activin mRNA during early development in *Xenopus laevis.* Dev Biol 157: 474–483
Ge W, Chang JP, Peter RE, Vaughan J, Rivier J, Vale W (1992) Effects of porcine follicular fluid, inhibin-A, and activin-A on goldfish gonadotropin release *in vitro.* Endocrinology 131: 1922–1929
Ge W, Gallin WJ, Strobeck C, Peter RE (1993) Cloning and sequencing of goldfish activin subunit genes: strong structural conservation during vertebrate evolution. Biochem Biophys Res Commun 193: 711–717
Ge W, Peter RE (1994) Activin-like peptides in somatotrophs and activin stimulation of growth hormone release in goldfish. Gen Comp Endocrinol 95: 213–221
Kitaoka M, Kojima I, Ogata E (1988) Activin-A: modulator of multiple types of anterior pituitary cells. Biochem Biophys Res Commun 157: 48–54
Kobayashi T, Kikuyama S (1991) Homologous radioimmunoassay for bullfrog growth hormone. Gen Comp Endocrinol 82: 14–22
Ling N, Ying S-Y, Ueno N, Esch F, Denoroy L, Guillemin R (1985) Isolation and partial characterization of a Mr 32,000 protein with inhibin activity from porcine follicular fluid. Proc Natl Acad Sci USA 82: 7217–7221
Ling N, Ying S-Y, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R (1986a) Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature 321: 779– 782
Ling N, Ying S-Y, Ueno N, Shimasaki S, Esch F, Hotta F, Hotta M, Guillemin, R (1986b) A homodimer of the beta-subunits of inhibin A stimulates the secretion of pituitary follicle stimulating hormone. Biochem Biophys Res Commun 138: 1129–1137
Mason AJ, Hayflick JS, Ling N, Esch F, Ueno N, Ying S-Y, Guillemin R, Niall H, Seeburg PH (1985) Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-beta. Nature 318: 659–663
Oguchi A, Tanaka S, Yamamoto K, Kikuyama S (1996) Release of α-subunit of glycoprotein hormones from the bullfrog pituitary: possible effect of a-subunit on prolactin cell function. Gen Comp Endocrinol 102: 141–146
Oguchi A, Tanaka S, Aida T, Yamamoto K, Kikuyama S (1997) Enhancement by prolactin of the GnRH-induced release of LH from dispersed anterior pituitary cells of the bullfrog (*Rana catesbeiana*). Gen Comp Endocrinol 107: 128–135
Polzonetti-Magni AM, Mosconi G, Carnevali O, Yamamoto K, Hanaoka Y, Kikuyama S (1998) Gonadotropoins and reproductive function in the anura amphibian, (*Rana esculenta*). Biol Reprod 58: 88–93
Rivier J, Spiess J, McClintock R, Vaughan J, Vale W (1985) Purification and partial characterization of inhibin from porcine follicular fluid. Biochem Biophys Res Commun 133: 120–127
Schwartz J, Cherney R (1992) Intercellular communication within the anterior pituitary influencing the secretion of hypophysial hormones. Endocr Rev 13: 453–475
Suzuki H, Yamamoto H, Kikuyama S, Uemura H (1997a) Distribution of endothelin 3-like immunoreactivity in gonadotrophs of the bullfrog (*Rana catesbeiana*) pituitary. Gen Comp Endocrinol 107: 12–22
Suzuki H, Yamamoto H, Kikuyama S, Oguchi A, Uemura H (1997b) Distribution of endothelin 3-like immunoreactivity in bullfrog and soft-shelled turtle pituitaries. In “Advances in Comparative Endocrinology” (S. Kawashima and S. Kikuyama, Eds.), Monduzzi Editore, Bologna Vol.2, pp 1033–1037
Thomsen G, Woolf T, Whitman M, Sokol S, Vaughan J, Vale W, Melton DA (1990) Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. Cell 63: 485–493
Uchiyama H, Komazaki S, Asashima M, Kikuyama S (1996) Occurrence of immunoreactive activin/inhibin β_B in gonadotrophs, thyrotrophs and somatotrophs of the *Xenopus* pituitary. Gen Comp Endocrinol 102: 1–10
Uchiyama H, Koda A, Komazaki S, Oyama M, Kikuyama S (2000) Occurrence of immunoreactive activin/inhibin bB in thyrotropes and gonadotropes in the bullfrog pituitary: Possible paracrine/autocrine effects of activin B on gonadotropin secretion. Gen Comp Endocrinol 118: 68–76
Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 321: 776–779
Weiss J, Crowley WF, Jr, Halvorson LM, Jameson JL (1993) Perifusion of rat pituitary cells with gonadotropin-releasing hormone, activin, and inhibin reveals distinct effects on gonadotropin gene expression and secretion. Endocrinology 132: 2307–2311
Yamamoto K, Kikuyama S (1982) Radioimmunoassay of prolactin in plasma of bullfrog tadpoles. Endocrinol Jpn 29: 159–167
Yan K, Yoshiura Y, Kobayashi M, Ge W (1999) Recombinant goldfish activin B stimulates gonadotropin-Iβ but inhibits gonadotropin-IIβ expression in the goldfish, Carassius auratus. Gen Comp Endocrinol 116: 81–89
Ying SY (1988) Inhibins, activins and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr Rev 9: 267–293
Zhengwei Y, Wreford NG, Schlatt S, Weinbauer GF, Nieschlag E, McLachlan RI (1998) Acute and specific impairment of spermatogonial development by GnRH antagonist-induced gonadotrophin withdrawal in the adult macaque (*Macaca fascicularis*). J Reprod Fertil 112: 139–147

(Received February 21, 2000 / Accepted April 12, 2000)