Endocrine, vol. 5, no. 1, 41–46, August 1996 1046-3976/5/1:41–46/$6.50 © 1996 by Humana Press Inc. All rights of any nature whatsoever reserved.

Effects of Inhibin on Activin A-Induced Glucose Metabolism in Rat Hepatocytes

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This study was conducted to investigate the effects of inhibin on hepatic glucose metabolism. We have previously reported that activin A induced a dose-dependent glycogenolytic action on hepatocytes, and that $10^{-9}M$ activin A induced a maximum glycogenolytic effect. Inhibin itself induced no increase or decrease in glucose output at any dose tested. At a concentration of $10^{-10}M$, inhibin was seen to inhibit $10^{-9}M$ activin A-induced glucose output by 30% as compared to the control. In contrast to its inhibitory effect on the action of activin A, $10^{-10}M$ and higher concentrations of inhibin did not inhibit angiotensin II- or vasopressin-induced glycogenolysis. We further investigated the mechanism of the inhibitory effect of inhibin on activin A-induced glycogenolysis, and found that $10^{-10}M$ inhibin did inhibit the increase in cytoplasmic-free calcium concentration that was seen with $10^{-9}M$ activin A.

We also investigated the effects of inhibin on the activin A-induced production of inositol trisphosphates, and the results showed that $10^{-10}M$ inhibin inhibited the activin A-induced production of inositol trisphosphates by 30% compared to the control. Furthermore, it was demonstrated that inhibin did not affect the binding of activin A to isolated hepatocytes. These data demonstrated that inhibin inhibited the activin A-induced glycogenolysis by inhibiting the increases of inositol trisphosphates and cytoplasmic free calcium concentrations.

Key Words: Activin A; inhibin; hepatocytes.

Introduction

Activin A is a homodimer with a molecular weight of 25,000 and an NH_2 terminal sequence identical to that of the β subunit of inhibin (Ling et al., 1985, 1986). This peptide has been proven to be erythroid differentiation factor (EDF) (Murata et al., 1988) which acts on the murine Friend leukemia cell line (THP-1) (Eto et al., 1987). Subsequently, many kinds of physiological activities of activin A have been discovered, for example, activin A was reported to stimulate FSH secretion (Ying, 1988; Vale et al., 1986; Kitaoka et al., 1987) and the number of gonadotrophs in anterior pituitary cultures (Katayama et al., 1990). In addition to its action on the pituitary-gonadal axis, activin A also induces differentiation of erythroid cells (Murata et al., 1988; Yu et al., 1987) and megakaryocytes (Nishimura et al., 1991), modifies growth and differentiation of neuronal cells (Hashimoto et al., 1990; Schubert et al., 1990), and induces mesodermal development (Smith et al., 1990; Sokol and Melton et al., 1991). We demonstrated that activin A induced hepatic glycogenolysis (Mine et al., 1989b). We further demonstrated that activin A exists in A- and D-cells of rat pancreatic tissue (Yasuda et al., 1993). From these data, it is assumed that activin A has some action on the liver as a humoral factor (Yasuda et al., 1993a). We further reported that activin A inhibited DNA synthesis in rat hepatocytes (Yasuda et al., 1993b) and that hepatocytes by themselves produced activin A, and other researchers reported that activin A induced apoptosis of hepatocytes (Schwall et al., 1993). It is now considered to act not only as a humoral factor, but also as a local cytokine-like regulator. However, it is assumed that activin A acts as a local cytokine-like regulator only with regard to cell proliferation and differentiation. Inhibin has been purified from porcine follicular fluids (Ling et al., 1985, 1986) and it is characterized as a heterodimeric protein composed of α and β polypeptides linked by disulfide bonds (Esch et al., 1987). We also reported that inhibin also exists diffusely in rat pancreatic endocrine tissue (Yasuda et al., 1993a), and from these data, it is assumed that inhibin has some action on the liver, similar to activin A. In the pituitary-gonadal axis, inhibin- and activin-mediated feedback control of FSH secretion has been established (Ying, 1988; Hillier, 1991). However, no system except pituitary-gonadal axis system has been shown to have inhibin- and activin-mediated interaction. As described earlier (Mine et al., 1989b), hepatocytes are targets for activin A, and it might be likely that there is an inhibin- and activin-mediated feedback control system in the liver.

Received November 27, 1995; Revised April 18, 1996; Accepted May 7, 1996.
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In the present study, therefore, we demonstrated that, in hepatocytes, inhibin inhibited activin A-induced glycogenolysis as inhibin inhibits activin A induced FSH release in the pituitary cells.

Materials and Methods

Preparation of Parenchyma Liver Cells

Parenchymal liver cells were prepared by the method of Berry and Friend (1969). Cells were suspended in modified Hanks' solution containing (in m*M*) NaCl 137, KCl 3.5, KH_2PO_4 0.44, $NaHCO_3$ 4.2, $NaHPO_4$ 0.33, $CaCl_2$ 1.0, and HEPES/NaOH, pH 7.4, 20, equilibrated with O_2 gas.

Determination of Glycogenolysis

Isolated hepatocytes were prepared from fed rats weighing about 200 g using collagenase, as described previously (Mine et al., 1988, 1989a, 1990). Cells were suspended in Krebs-Ringer bicarbonate buffer equilibrated with 95% O_2 and 5% CO_2 at 37°C. More than 95% of cells excluded trypan blue. Aliquots of cell suspensions containing 4×10^6 cells/mL were incubated for 11 min, and the glucose concentration in the incubation medium was determined as described by Corvera et al. (1986). In some experiments, modified Hanks' solution was used and the results were essentially the same. The glucose output, measured under these conditions, mainly reflects glycogen breakdown. Results were been expressed as glucose production per 10^6 parenchymal cells.

Measurement of Changes in the Cytoplasmic Free Calcium Concentration ($[Ca^{2+}]_c$) by Aequorin

Changes in $[Ca^{2+}]_c$ were measured by monitoring aequorin bioluminescence. Aequorin was loaded into hepatocytes by making the plasma membrane reversibly permeable, according to the method of Borle et al. (1986). In brief, after a 2-min incubation, the supernatant was discarded. The aliquot of packed cells was then suspended and incubated for 10 min at 4°C in 0.5 mL of buffered solution containing 10 μg aequorin: (in m*M*) NaCl 140 and HEPES 3, at pH 7.4. Finally, the cells were centrifuged at 200*g* for 30 s and the supernatant was discarded. Aequorin-loaded cells were incubated in modified Hanks' solution and aequorin luminescence was measured as described previously (Mine et al., 1988, 1989a, 1990). A portion of the suspension containing 10^6 aequorin-loaded cells/mL was put into a cuvet and incubated at 37°C with constant stirring. $[Ca^{2+}]c$ in stimulated and unstimulated cells was estimated as described by Snowdowne and Borle (1984). Aequorin-loaded hepatocytes showed a normal response to glucagon in terms of glycogenolysis.

Iodination of Activin A and Binding of [^{125}I] Activin A to Hepatocytes

Activin A (10 μg) was iodinated with Na[^{125}I] by the method of Volton-Hunter, to a specific activity of 50–90 μCi/mg. [^{125}I] activin A was purified on a Sephadex G-25 column in 50 m*M*: phosphate buffer, pH 7.4, containing 1% bovine serum albumin (BSA). For the binding study, parenchymal cells (2×10^5 cells/mL) were incubated for 30 min at 37°C with buffer containing 50 m*M* HEPES, pH 7.4, 128 m*M* NaCl, 5 m*M* KCl, 1.2 m*M* $CaCl_2$, 5 m*M* $MgSO_4$, and 0.5% BSA. Then, [^{125}I]activin A was added, followed by incubation for 60 min in the presence or absence of unlabeled agents.

Also, inhibition of the binding of activin A to hepatocytes by inhibin was investigated. [^{125}I]activin A was incubated with parenchymal cells for 30 min. Then inhibin was added, followed by incubation for 60 min. At the end of incubation, the cells were washed four times with ice-cold binding buffer, and the radioactivity was then counted.

Measurement of Inositol Trisphosphate Production

Hepatocytes (10^7 cells/mL) were labeled with [^{3}H]inositol by incubating the cells with 10 μCi/ mL [^{3}H]inositol for 120 min. After the labeling period, cells were washed and incubated at 37°C in modified Hank's solution containing 10 m*M* LiCl, for 10 min. The cells were stimulated for 20 s with activin A in the pretreatment of inhibin, and this reaction was stopped by adding perchloric acid (final concentration, 10%). The cells were homogenized by repetitive aspirations through a 26-gage needle and centrifuged at 800*g* for 5 min. The supernatant was taken, neutralized with 5*M* KOH, and applied to an anion exchange column. Inositol phosphates were separated as described by Berridge and Irvine (1983). The isomers of inositol polyphosphates were not determined.

Materials

Activin A was kindly donated by Y. Eto and H. Shibai of Ajinomoto Co., Inc. (Kawasaki, Japan). Briefly, recombinant human, activin A/EDF was purified from the culture supernatant of Chinese hamster ovary cells (CHO cells) (Eto et al., 1987; Shiozaki et al., 1992). Inhibin (bovine inhibin, 32 kDa) was purified as previously described Miyamoto et al., 1985; Fukuda, 1986). Briefly, inhibin was purified using chromatographies under protein-dissociating condition from bovine follicular fluid (Fukuda, 1986). Aequorin was purchased from J. R. Blinks of the Mayo Foundation (Rochester, MN).

Results

The Effects of Inhibin on Activin A-Induced Glycogenolysis

As earlier described, activin A induced glucose production in hepatocytes of fed rats (Fig. 1). A concentration of $10^{-10}M$ inhibin by itself induced no glucose production (Fig. 1) and it did not inhibit basal glucose output. However, at concentrations of $10^{-11}M$ and $10^{-10}M$, inhibin inhibited $10^{-9}M$ activin A-induced glucose output by 50 and 30%,

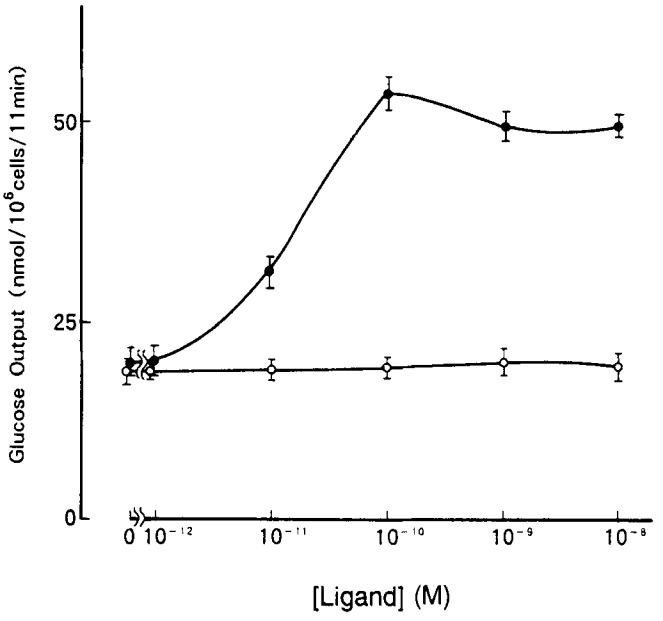

Fig. 1. Dose response relationship for activin A- and inhibin-induced glucose output in isolated hepatocytes. Cells were stimulated by various concentrations of activin A (●) or inhibin (○), and glucose output was measured. Basal glucose output was 22.1 ± 0.3 nmol/10^6 cells/11 min. Values are means ±SE for seven determinations.

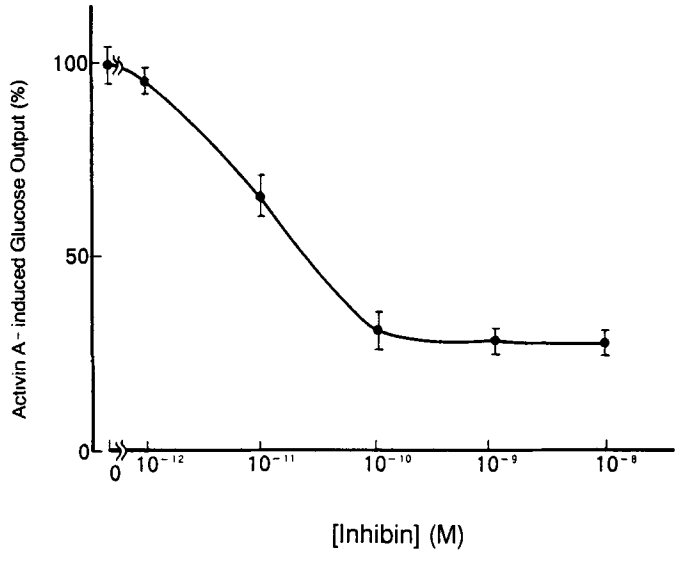

Fig. 2. Inhibitory effect of inhibin on activin A-induced glucose output. Cells were pretreated by various concentrations of inhibin, and $10^{-9}M$ activin A was added, and glucose output was measured. Values are means ±SE for seven determinations.

respectively (Fig. 2). Higher concentrations of inhibin also inhibited the activin A-induced glucose output, but the maximum inhibitory effect was not attained to 100%; rather the effect was only 70% (data not shown).

Effects of Inhibin on Angiotensin II- and Vasopressin-Induced Glycogenolysis

Angiotensin II and vasopressin induced glycogenolysis via a Ca^{2+}-dependent pathway, as did activin A (Mine et al., 1992). Both peptides are believed to induce activation of Gq protein (Taylor et al., 1991), with subsequent activation of phospholipase $C_{\beta 1}$. The activation of the latter induces the production of inositol- 1,4,5,-trisphosphate, thereby promoting an increase in $[Ca^{2+}]_c$. However, activin A does not appear to be connected with Gq protein. In contrast to its action on activin A, no concentration of inhibin inhibited angiotensin II- or vasopressin-induced glucose output (Fig. 3). Furthermore, we confirmed that inhibin did not inhibit the production of inositol—1,4,5-trisphosphate or the increase in $[Ca^{2+}]_c$ induced by angiotensin II or vasopressin (data not shown).

Effects of Inhibin on the Increase of $[Ca^{2+}]_e$-Induced by Activin A

Activin A induced a transient increase in $[Ca^{2+}]_c$ in rat hepatocytes (Mine et al., 1989b). As reported in our previous paper (Mine et al., 1989b), we demonstrated that activin A induced the production of inositol 1,4,5–trisphosphate and Ca^{2+} release from the putative internal Ca pool. Inhibin

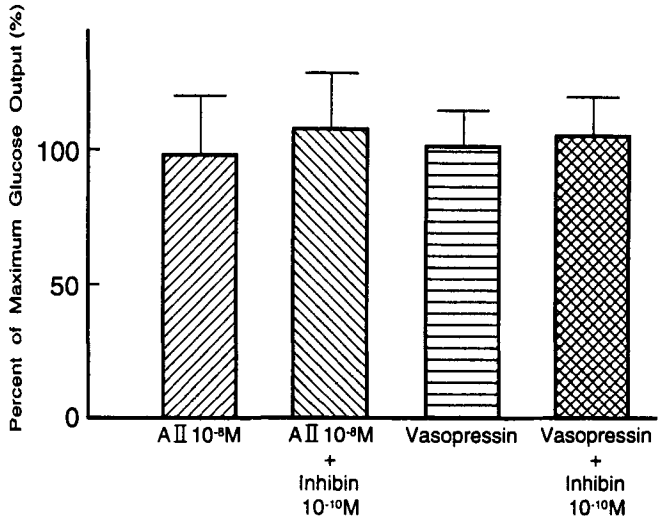

Fig. 3. Effect of inhibin on angiotension II- or vasopressin-induced glucose output. Cells were pretreated with $10^{-10}M$ inhibin, and after the addition of inhibin, $10^{-8}M$ angiotensin II and $10^{-8}M$ vasopressin were added, and then glucose output was measured. Results were expressed as percent of maximum glucose output. Net maximum glucose output induced by Angiotension II or vasopressin was 26.3 + 0.5 nmol/10^6 cells/11 min or 22.1 ± 0.4 nmol/10^6 cells/11 min, respectively. Values are means ±SE for nine determinations.

alone did not affect $[Ca^{2+}]_c$ in rat hepatocytes (data not shown). A concentration of $10^{-10}M$ inhibin inhibited the increase in $[Ca^{2+}]_c$ induced by activin A (Fig. 4).

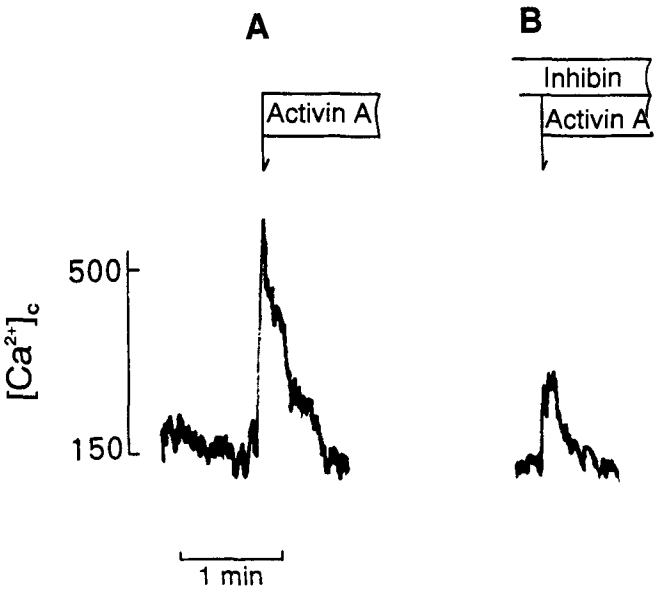

Fig. 4. Effect of inhibin on the increase in $[Ca^{2+}]_c$ induced by activin A Aequorin-loaded hepatocytes were incubated with modified Hanks' solution, and aequorin luminescence was measured. **(A)** $10^{-9}M$ activin A alone was added. **(B)** $10^{-10}M$ inhibin was pretreated, and subsequently $10^{-9}M$ activin A was added. Results are representative of four experiments with similar results.

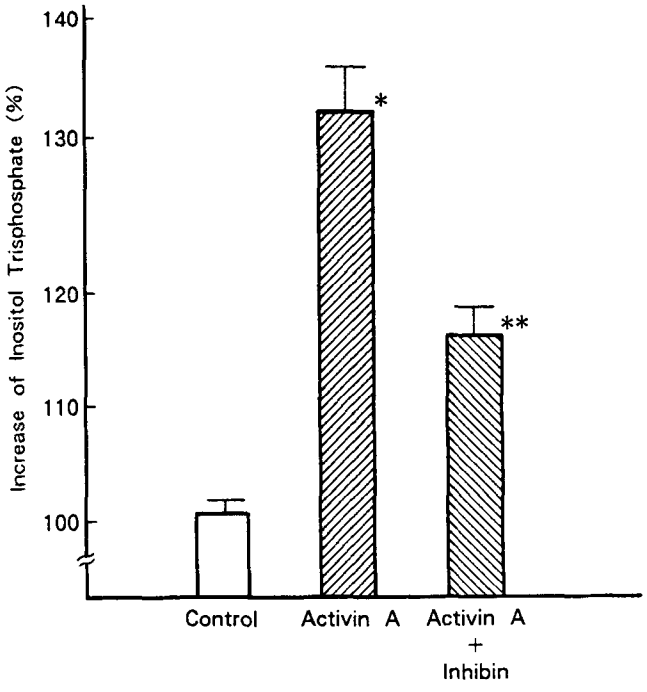

Fig. 5. Effect of inhibin on the production of inositol trisphosphates induced by activin A. Hepatocytes (10^7 cells/mL) were labeled with [^{3}H]inositol by incubating cells with 10 μCi/mL [^{3}H]inositol for 120 min. Cells were pretreated with or without $10^{-10}M$ inhibin, and subsequently stimulated for 20 s with $10^{-9}M$ activin A. Values are means ±SE for six determinations. $*p < 0.05$ vs control; $**p < 0.05$ activin A plus inhibin vs activin A only.

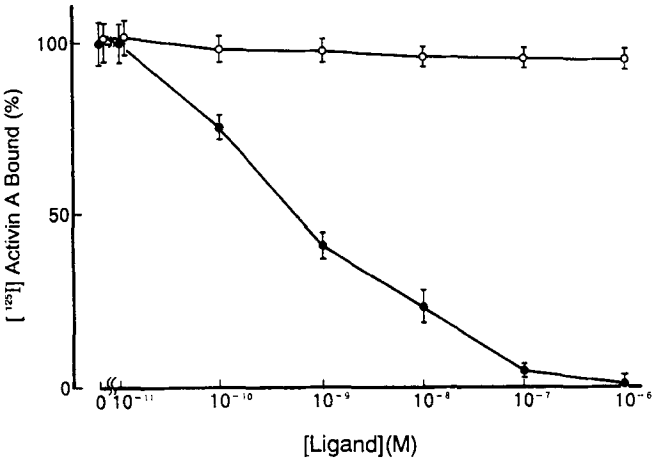

Fig. 6. Effect of inhibin on the binding of [^{125}I]activin A. As described in Methods, the specific binding of activin A was measured after, respectively, each of unlabeled activin A and inhibin was added.

Effects of Inhibin on the Production of Inositol Trisphosphates Induced by Activin A

As described earlier (Mine et al., 1989b), activin A induced the production of inositol trisphosphates and increased $[Ca^{2+}]_c$. Therefore, we investigated the effects of inhibin on the production of inositol trisphosphates. Inositol trisphosphates appeared to induce Ca^{2+} release from the endoplasmic reticulum, and to be a vital factor for the increase of the cytoplasmic-free calcium concentration. Inhibin alone did not affect the production of inositol trisphosphates (data not shown). $10^{-10}M$ inhibin showed significantly inhibition of $10^{-9}M$ activin A-induced production of inositol trisphosphates, by 55% (Fig. 5), and also inhibition of $10^{-8}M$ activin A-induced production of inositol trisphosphates (data not shown).

Effects of Inhibin on the Binding of [^{125}I]Activin A

Our data suggested that inhibin inhibited the activin A-induced increase in $[Ca^{2+}]_c$ and also inhibited the glycogenolytic action of activin A. It is important to examine whether inhibin inhibits the binding of activin A to hepatocytes. We have previously demonstrated that activin A binds to specific binding receptors on hepatocytes, which receptors are different from TGF receptors (Yasuda et al., 1993b). We have also investigated the effect of inhibin on the binding of activin A to hepatocytes. As shown in Fig. 6, inhibin did not inhibit the binding of [^{125}I]activin A to hepatocytes, although activin A could replace the [^{125}I]activin A bound to the hepatocytes in this system.

Discussion

Gonadal tissue can produce bioassayable inhibin, which specifically binds to rat pituitary gland homogenates. However, the biosynthetic pathway of inhibin has not been established. As described earlier, we demonstrated that inhibin also exists diffusely in rat pancreatic endocrine tissue

(Yasuda et al., 1993a), and these data suggested that rat pancreatic endocrine cells can also produce inhibin as gonadal tissue. In males, inhibin, which selectively suppresses FSH secretion, subsequently plays a putative role on FSH-mediated cellular components of the tests. Also, it is assumed that inhibin has a humoral action on the liver, as well as the pituitary gland. The discovery of activins was related to the isolation and characterization of inhibin. Activin A is composed of a homodimer of two β-subunits of inhibin A (Ling et al., 1985, 1986). It has also been isolated and characterized from the supernatant medium of THP-1 cells (human leukemic cell line) treated with 4β-phorbol 12-myristate 13 acetate. This peptide induces the differentiation of erythroleukemic cells, and it is called erythroid differentiation factor (EDF). Muramatsu and his colleagues (Murata et al., 1988) showed that activin A is the same as EDF, and its action as activin A has been intensively investigated. Activin A stimulates FSH secretion (Vale et al., 1986; Kitaoka et al., 1987; Ying, 1988) and it also induces insulin secretion from β cells of the pancreas (Totsuka et al., 1988). It reportedly has a dual action on the growth of fibroblasts (Kojima and Ogata, 1989). Also, we reported that activin A also stimulates hepatic glycogenolysis via a Ca^{2+}-dependent pathway (Mine et al., 1989b); i.e., activin A induces the production of inositol trisphosphates and increases the cytoplasmic-free calcium concentration. The relationship between activin A and inhibin has been demonstrated only in pituitary tissue (Ying, 1988; Hillier, 1989), where inhibin inhibits FSH secretion, in contrast to activin A, which promotes FSH secretion from the pituitary gland. In the present study, the relationship between inhibin and activin A has been demonstrated in rat hepatocytes. Our data have revealed that inhibin inhibits the activin A-induced glucose output, and the mechanism of the inhibitory effect of inhibin has been investigated. It was clearly demonstrated that inhibin has its own receptor and binds to the receptor in rat hepatocytes (Yu et al., 1987). In the present study, inhibin did not affect the binding of activin A to hepatocytes, but it inhibited the activin A-induced increase in $[Ca^{2+}]_c$. However, inhibin did not inhibit the increase in $[Ca^{2+}]_c$ induced by angiotensin II or vasopressin. Also, both angiotensin II and vasopressin induce the production of inositol trisphosphates, by activating Gq and phospholipase $C_{\beta 1}$ (Taylor et al., 1991). Activin A also induces the production of inositol trisphosphates, but probably by a different pathway from angiotensin II or vasopressin, because the action of activin A is blocked by pretreatment with pertussis toxin, in contrast to the action of angiotension II or vasopressin that is not blocked by pretreatment with the pertussis toxin (Mine et al., 1992). These data suggested that the mechanism by which activin A induces the activation of phospholipase C is different from that of angiotension II or vasopressin. The site of the action of inhibin on the hepatocyte in inhibiting the action of activin A, is yet to be investigated. In the present study, we have obtained three facts. First, inhibin did not affect the binding of activin A to hepatocytes. Second, inhibin did inhibit the signal transduction pathway of activin A after the activation of phospholipase C. Third, inhibin did not inhibit the signal transduction pathway of angiotensin II or vasopressin.

Further, we have reached the assumption that activin A, angiotensin II, and vasopressin have a common pathway after the production of inositol trisphosphates. Based on our three facts and the assumption, it can be concluded that inhibin probably interferes at the step between the attachment of activin A to the receptor and the activation of phospholipase C.

It is well recognized that inhibins and activins have a diverse action on the pituitary-gonadal axis; that is, inhibin inhibits the effect of activin on the release of FSH (Rivier et al., 1986; Ying, 1988; Hillier, 1989). The manner of inhibition of activin A activity by inhibin in the pituitary cells seems to be similar to that in hepatocytes. That is, in the pituitary cells, $10^{-11}M$ and $10^{-10}M$ inhibin inhibit $10^{-9}M$ activin-induced FSH release (Ying, 1988; Hillier, 1989; Rivier et al., 1986). However, the mechanism of this action is uncertain. Recently, it was reported that in HepG2 cells, inhibin reversed activin A-induced inhibition of proliferation of HepG2 cells (Xu et al., 1995). The investigators assumed that inhibin has properties of a natural dominant-negative inhibitor of the heterodimeric type II type I receptor kinase complex. Their result is compatible with our data. Therefore, it can be firmly suggested that inhibin might well interfere at the step between the site after the attachment to the receptor and the activation of phospholipase C induced by activin A in rat hepatocytes.

References

Berridge, M. J. R., Dawson, M. C., Downes, C. P., Heslop, J. P., and Irvine, R. F. (1983). *Biochem. J.* **212,** 473–482.

Berry, M. N. and Friend, D. S. (1969). *J. Cell Biol.* **43,** 506–520.

Borle, A. B., Freudenrich, C. C., and Snowdowne, K. W. (1986). *Am. J. Physiol.* **251,** C323–C326.

Corvera, S., Huerta-Bahena, J., Pelton, J. T., Hruby, V. J., Rivedi, D., and Garcia-Sainz, J. A. (1986). *Biochim. Biophys. Acta* **4304,** 434–441.

Esch, F. S., Shimasaki, S., Cooksey, K., Mercado, M., Mason, A. J., Ying, S.-Y., Ueno, N., and Ling, N. (1987). *Mol. Endocrinol.* **1,** 388–396.

Eto, Y., Tsuji, T., Takezawa, M., Takano, S., Yokogawa, Y., and Shibai, H. (1987). *Biochem. Biophys. Res. Commun.* **42,** 1095–1103.

Fukuda, M., Miyamoto, K., Hasegawa, Y., Nomura, M., Igarashi, M., Kangawa, K., and Matsuo, H. (1986). *Mol. Cell Endocrinol.* **44,** 55–60.

Hashimoto, M., Kondo, S., Sakurai, Y., Eto, Y., Shibai, H., and Muramatsu, M. (1990). *Biochem. Biophys. Res. Commun.* **173,** 193–200.

Hillier, S. G. (1991). *J. Endocrinol.* **131,** 171–175.

Katayama, T., Shiota, K., and Takahashi, M. (1990). *Mol. Cell Endocrinol.* **69,** 179–185.

Kitaoka, M., Yamashita, N., Eto, Y., Shibai, H., and Ogata, E. (1987). *Biochem. Biophys. Res. Commun.* **146,** 1382–1385.

Kojima, I. and Ogata, E. (1989). *Biochem. Biophys. Res. Commun.* **159,** 1107–1113.

Ling, N., Ying, S.-Y., Ueno, N., Esch, F., Denoroy, L., and Guillemin, R. (1985). *Proc. Natl. Acad. Sci. USA* **82,** 7217–7221.

Ling, N., Ying, S.-Y., Ueno, N., Schimasaki, S., Esch, F., Hotta, M., and Guillemin, R. (1986). *Nature (Lond.)* **321,** 179–782.

Mine, T., Kojima, I., and Ogata, E. (1988). *Biochim. Biophys. Acta* **970,** 166–171.

Mine, T., Kojima, I., and Ogata, E. (1989a). *Biochem. J.* **258,** 889–894.

Mine, T., Kojima, I., and Ogata, E. (1989b). *Endocrinology* **125,** 586–591.

Mine, T., Kojima, I., and Ogata, E. (1990). *Endocrinology* **126,** 2831–2836.

Mine, T., Kojima, I., and Ogata, E. (1992). *Biochem. Biophys. Res. Commun.* **186,** 205–210.

Miyamoto, K., Hasegawa, Y., Fukuda, M., Nomura, M., Igarashi, M., Kangawa, K., and Matsuo, H. (1985). *Biochem. Biophys. Res. Commun.* **129,** 396–403.

Murata, M., Eto, Y., Shibai, H., Sakai, M., and Muramatsu, M. (1988). *Proc. Natl. Acad. Sci. USA* **85,** 2434–2438.

Nishimura, M., Kaku, K., Azuno, Y., Okafuji, K., Eto, Y., Shiozaki, M., Sasaki, M., Inoue, T., and Kaneko, T. (1991). *Biochem. Biophys. Res. Commun.* **181,** 1042–1047.

Rivier, C., Rivier, J., and Vale, W. (1986). *Science* **234,** 205–208

Schubert, D., Kimura, H., La Corbiere, M., Vaughan, J., Karr, D., and Fisher, W. H. (1990). *Nature (Lond.)* **344,** 868–870.

Schwall, R. H., Robbins, K., Jardieu, P., Chang, G., Lai, L., and Terrell, T. G. (1993). *Hepatology* **18,** 347–356.

Shiozaki, M., Sakui, R., Tabuchi, M., Nakamura, T., Sugino, K., Sugino, H., and Eto, Y. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 1653–1556.

Smith, J. C., Price, B. M. J., Van Nimmen, K., and Huylebroeck, D. (1990). *Nature (Lond.)* **345,** 729–731.

Snowdowne, K. W. and Borle, A. B. (1984). *Am. J. Physiol.* **246,** E198–E201.

Sokol, S. and Melton, D. A. (1991). *Nature (Lond.)* **351,** 409–411.

Taylor, S. J., Chae, H. Z., Phee, S. G., and Exton, J. H. (1991). *Nature (Lond.)* **350,** 516–518.

Totsuka, Y., Tabuchi, M., Kojima, I., Shibai, H., and Ogata, E. (1988). *Biochem. Biophys. Res. Commun.* **156,** 335–339.

Vale, W., Rivier, J., Vaughan, J., McClintock, R., Corrigan, A., Woo, W., Karr, D., and Spiess, J. (1986). *Nature (Lond.)* **321,** 776–779.

Xu, J., McKeehan, K., Matsuzaki, K., and McKeehan, W. L. (1995). *J. Biol. Chem.* **270,** 6308–6313.

Yasuda, H., Inoue, K., Shibata, H., Takeuchi, T., Eto, Y., Hasegawa, Y., Sekine, N., Totsuka, Y., Mine, T., Ogata, E., and Kojima, I. (1993a). *Endocrinology* **133,** 624–630.

Yasuda, H., Mine, T., Shibata, H., Eto, Y., Hasegawa, Y., Takeuchi, T., Asano, S., and Kojima, I. (1993b). *J. Clin. Invest.* **92,** 1491–1496.

Ying, S.-Y. (1988). *Endocr. Rev.* **9,** 267–293.

Yu, J., Shao, L., Leman, V., Yu, L., Vaughan, J., Rivier, J., and Vale, W. (1987). *Nature (Lond.)* **310,** 765–767.