Volume 312, number 1, 53–56 FEBS 11691 November 1992

Cloning and sequencing of a rat type II activin receptor

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Received 1 September 1992

A full-length cDNA for a rat type II activin receptor was cloned by hybridization from a rat ovary cDNA library. The deduced amino acid sequence (513 residues) containing a single membrane-spanning domain and an intracellular kinase domain with predicted serine/threonine specificity. The amino acid sequence is 99.8% and 99.4% identical in the coding region with the previously cloned mouse and human type II activin receptor, and only 66.7% identical in the coding region with the previously cloned rat type IIB activin receptor. We examined the effect of PMSG-hCG on the mRNA level of type II activin receptor in immature rat ovaries. Northern blot analysis of ovarian RNA revealed two mRNAs (3.0 kb and 6.0 kb).

Ovary; Activin receptor; mRNA

1. INTRODUCTION

Activins, dimers of the β subunits of inhibin (β_A or β_B), which stimulate FSH secretion in the anterior pituitary gland have been isolated from the mammalian follicular fluids [1,2]. They belong to a member of TGF-β superfamily which includes TGF-βs, Müllerian inhibiting substance, bone morphogenetic proteins, the product of decapentaplegic gene complex of *Drosophila* and Vg1 gene product of *Xenopus* [3]. Activins have an extensive anatomical distribution and are implicated in the regulation of many biological processes, including the proliferation of many cell lines [4–6], control of the secretion and expression of the anterior pituitary hormones FSH, GH, and ACTH [1], neuronal survival [7,8], hypothalamic oxytocin secretion [9], erythropoiesis [10,11], and early embryonic development [12–14]. In the ovary, the LH-stimulated androstenedione production was suppressed, and FSH-enhanced aromatase activity was stimulated by activin [15,16]. A previous study reported that administration of PMSG led to a increase in the expression of all three inhibin subunits (α, β_A, and β_B) whereas injection of hCG cause a decreased in the level of the respective mRNA in vivo system [17]. Therefore, activin has been suggested to play important local roles in the modulation of gonadal function. However, an understanding of the molecular mechanism of activin action has to be determined. Recently, activin receptors were cloned in several species, and subtypes were reported [18–22].

We report here the cloning of rat type II activin receptor and the effect of PMSG-hCG on the mRNA levels of this receptor in immature rat ovaries.

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2. MATERIALS AND METHODS

2.1. *Isolation and sequence determination of rat activin receptor*

A size-selected (1.5–5 kb) randomly primed rat ovarian cDNA was prepared by reverse transcription of poly(A)$^+$ RNA. The end of the cDNA were blunted with T4 DNA polymerase and *Eco*RI adaptors were added. The cDNA was ligated to the vector λgt10 and packed with Stratagene Gigapack Gold. The library contains 1.5×10^6 independent recombinants, and was amplified. This library (5×10^5 clones) was screened with nick translated ^{32}P-labeled 1.4-kilobase (kb) *Acc*I fragment (780–2194) from the mouse type II activin receptor clone [18]. Nylon membranes were hybridized and washed successively in 1 × SSPE (0.18 M NaCl, 0.01 M sodium phosphate, 1 mM EDTA, pH 7.7), 0.1% SDS for 15 min at 42°C and 0.1 × SSPE, 0.1% SDS for 10 min at 65°C. Several clones were identified, and one clone was selected for sequence analysis. These clones were subcloned into M13mp and single-stranded DNA templates were prepared for sequence determination. Sequencing of strands of DNA was done by the dideoxy chain termination method [23].

2.2. *Extraction of RNA and Northern blot analysis*

Twenty-five-day-old immature female rats of Wistar stain were primed with 30IU PMSG alone or 30IU PMSG plus 20IU hCG 60 h later. After each rat was sacrificed at selected intervals, ovaries were removed and immediately stored in liquid nitrogen until extracting RNA for Northern blot analysis. Rat type II activin receptor mRNA probe was prepared using mRNA probe synthesis kit. Total RNA (15 μg) extracted from the ovaries by the acid guanidinium thiocyanate/phenol/chloroform extraction method was fractionated by electropho-

resis through a 1% agarose/formaldehyde gels [24]. The RNAs were transferred to a nylon membrane (Biodyne, !CN) [25]. Blots were prehybridized for 6 h at 68°C and then hybridized overnight at 68°C with a digoxigenin-labeled RNA probe. Under the standard protocol of nucleic acid detection kit (Boehringer-Mannheim), autoradiographic bands were quantitated by densitometric scanning to be standerlized against the corresponding amount of β-actin mRNA.

2.3. *Synthesis of RNA probe*

Rat activin receptor cDNA was subcloned into Bluescript and linearized by *Bam*HI. Digoxigenin-labeled activin receptor RNA probe corresponding to −21 to 2256 was produced by in vitro transcription with T3 RNA polymerase and RNA labeling kit (Boehringer-Mannheim). Digoxigenin-labeled β-actin RNA probe was obtained by the same method.

3. RESULTS AND DISCUSSION

Fig. 1 shows the 2277-nucleotides sequence of the cloned cDNA and amino acid sequence deduced for the rat type II activin receptor. The first methionine in the sequence was considered the initiator codon. This was followed by a amino acid sequence having the characteristics of a signal peptide with a cleavage site as defined by von Heijne [26]. As in mouse type II activin receptor, the 19 amino acid stretch at the amino-terminus is assumed to be a single peptide and a single putative 26 residue membrane-spanning region occurs between amino acids 136 and 161 [18]. Therefore, the

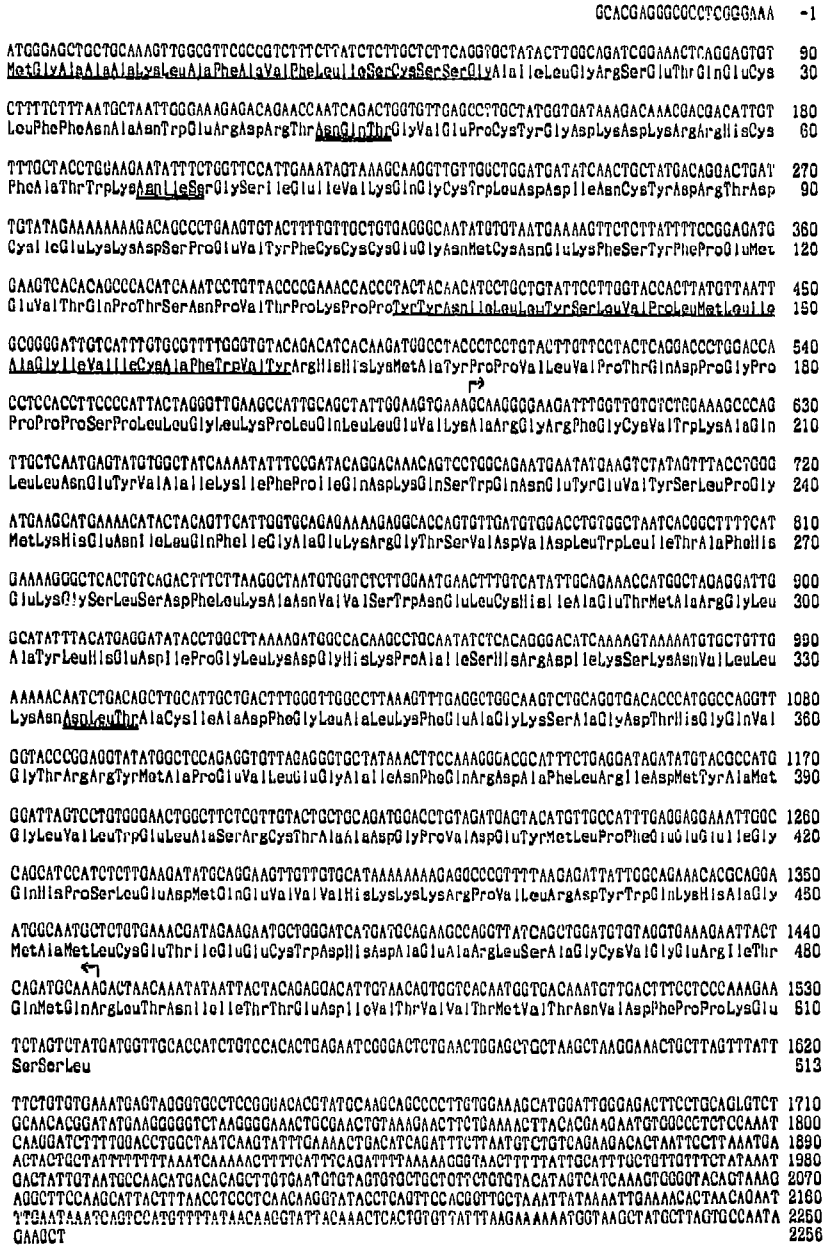

Fig. 1. Nucleotide and deduced amino acid sequence of rat type II activin receptor. The single peptide and transmembrane domain are indicated by a single underline. The kinase domain is indicated by arrows. Potential sites of *N*-glycosylation are indicated by double underlines.

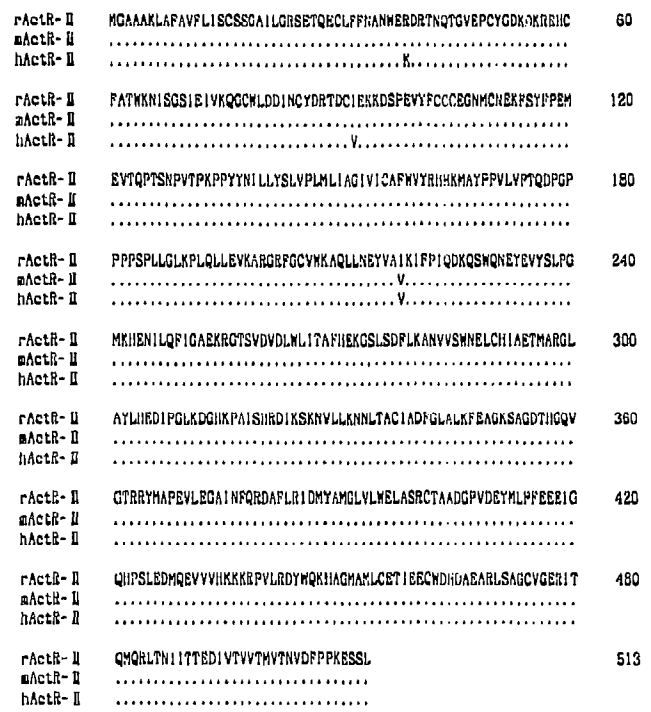

Fig. 2. Detailed comparison of the amino acid sequence of the rat, mouse and human type II receptors: identical residues are represented by a dot. The numbering of the amino acids is indicated on the right.

mature rat type II activin receptor is predicted to be a 494 amino acid membrane protein of M_r 56,000 with a 116 amino acid N-terminal extracellular ligand binding domain, and a 352 amino acid intracellular signalling domain with predicted serine/threonine specificity. The position of all cysteines, as well as sites of *N*-linked glycosylation are conserved. There are two potential sites of *N*-linked glycosylation in the N-terminal extracellular domain.

The amino acid sequence is 99.8% and 99.4% identical in the coding region with the previously cloned mouse and human type II activin receptors (Fig. 2), and is only 66.7% identical in the coding region with the previously cloned rat type IIB activin receptor (Fig. 3). The existence of heterogeneity in the activin receptor population has been suggested by the widespread actions of activin. In addition, two types of activin receptor were detected on Friend leukemia and embryonal carcinoma cells [27]. According to our data, this degree of sequence divergence indicate the difference between the rat type II and the rat type IIB receptor. However, the serine/threonine kinase show a high degree of homology within the two receptors than the ligand binding or transmembrane domain. The availability of a cDNA for type II activin receptor might allow us to study structure–function analysis of this receptor. Moreover, the existence of distinct activin receptors may contribute significantly to the varied biological effects of activin.

In granulosa cell culture, addition of FSH induced a 7–8-fold rise in the number of activin binding sites without alternation of the binding affinity [28]. Moreover, it has been reported that activin increased FSH receptor content [29] and augmented the FSH stimulation of progesterone production and LH receptor induction. Therefore, the specific activin receptors in the granulosa cells may mediate the observed effects of activin on granulosa cell differentiation. On the other hand, activin-binding protein was purified from rat ovary [30] and bovine pituitary [31] and was identical to follistatin. It was reported that FSH induced the production of follistatin in granulosa cell culture [32]. In order to investigate the regulation of activin activity, it might be essential to know the dynamic relationship between production of follistatin and activin receptor in the ovary. Therefore it is possible that activin may exert local effects such as the modulation of the production of gonadotropin receptor through their own receptor which might be affected by the existence of follistatin. As shown in Fig. 4, Northern blot analysis of ovarian RNA revealed a major mRNA of 6.0 kb nucleotides and a minor that of 3.0 kb nucleotides. Although the intensity of 6.0 kb band did not change significantly after PMSG administration, hCG administration caused an increase in activin receptor messenger level and 5-fold increase of control level by day 3. Thus, the mRNA levels of activin receptor in immature rat ovary has been induced according to the maturation of follicles and even after ovulation it continued to increase. The physiological roles of accumulation of activin receptor levels after PMSG-hCG priming in the female rat are not known, and we believe that our results are of significance in ascertaining the existence of a specific receptor in rat ovary, and contribute further insight into the mechanism of regulation of ovarian function.

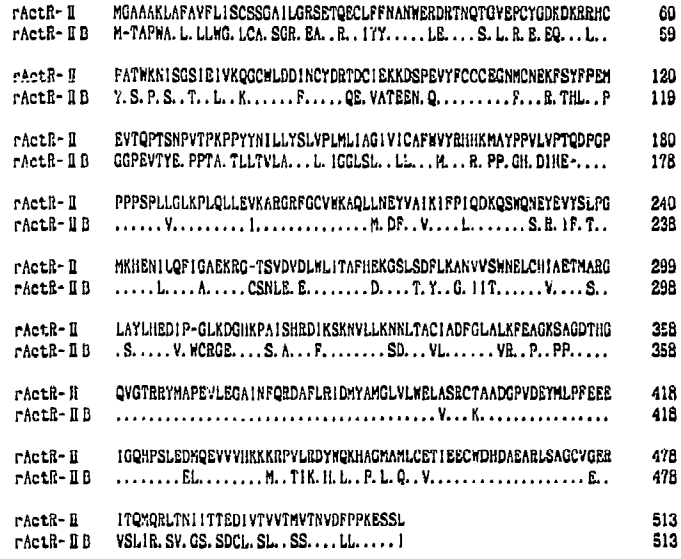

Fig. 3. Detailed comparison of the amino acid sequence of the rat type II and the rat type IIB activin receptors: identical residues are represented by a dot. The numbering of the amino acids is indicated on the right.

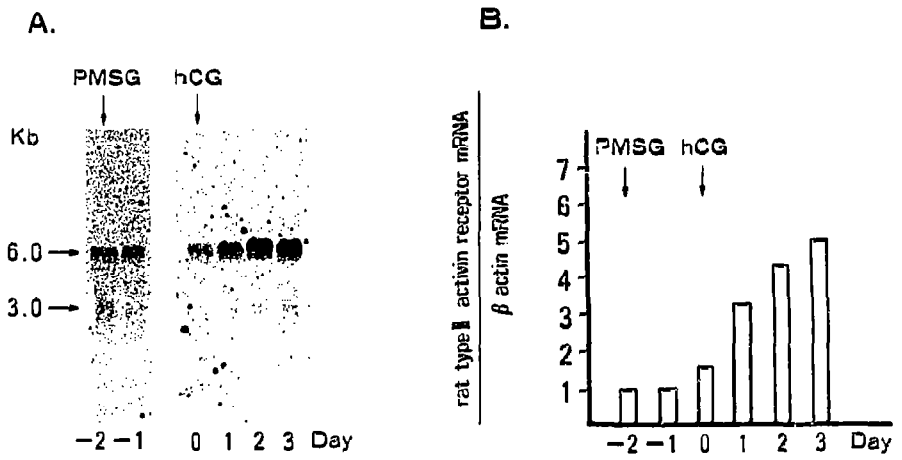

Fig. 4. The effect of PMSG and hCG treatment on rat type II activin receptor mRNA levels in immature rat ovary. From each point 15 μg of total RNA was prepared and fractionated through a 1% agarose gel and blotted as described in Materials and Methods. Blots were probed with digoxigenin-labeled rat type II activin receptor RNA. (A) The filters were exposed to Kodak XRP film. (B) Autoradiographs were quantitated by densitometric scanning, and the increase in activin receptor mRNA (6.0 kb band) relative to β-actin is expressed as arbitrary units. The mRNA level at each time point is expressed as the fold increase over day −2. The results shown are representative of three experiments.

Acknowledgements: This study was supported by a grant from the Ministry of Education, Science and Culture of Japan (04670988).

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