# Blockage of Ovulation by an Angiotensin Antagonist

ANTONIO PELLICER,* ANGELA PALUMBO, ALAN H. DECHERNEY, FREDERICK NAFTOLIN†

**Angiotensin II (Ang II) is present in high concentrations in preovulatory follicular fluid, and ovarian follicular cells have specific Ang II receptors. To investigate the possible direct involvement of Ang II in ovulation the specific receptor antagonist of Ang II, saralasin, was administered by intraperitoneal injection to immature rats in which follicle development and ovulation had been induced with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG), respectively. Saralasin halved the number of oocytes found in the fallopian tubes 17 to 20 hours after administration of hCG. The antiovulatory effect was observed when saralasin was given 1 hour before hCG or 1 or 3 hours after hCG but not when given 5 hours after hCG. Simultaneous administration of Ang II reversed the saralasin blockage of ovulation. These results indicate a direct, obligate role for Ang II in ovulation and raise the possibility of contraceptive and profertility applications for agonists or antagonists of the renin-angiotensin system that are aimed at the ovulatory process.**

THE RENIN-ANGIOTENSIN SYSTEM IS well known for its role as a systemic regulator of blood pressure and fluid homeostasis. It operates through formation of the active octapeptide, angiotensin II (Ang II), via sequential cleavage of circulating angiotensinogen and angiotensin I by kidney renin and angiotensin-converting enzyme, respectively. Recently, several organs have been shown to contain local renin-angiotensin systems and research is in progress to outline their functions. Ang II has been linked to the ovulatory process. High concentrations of Ang II are present in preovulatory follicular fluid from both natural and stimulated cycles in women (*1, 2*) and cows (*3*). Granulosa cells contain specific Ang II receptors and Ang II increases estrogen secretion in vitro by pregnant mare's serum gonadotrophin (PMSG)–stimulated rat ovaries (*4, 5*). Immunoreactive Ang II is present in theca and luteal cells of rat and human ovaries (*6, 7*). However, no clear evidence of a direct Ang II effect on ovarian function in vivo has yet been reported. Therefore, we investigated the role of endogenous Ang II in ovulation by blockage of Ang II receptors with the specific antagonist saralasin (*8*).

Immature, 25-day-old, female Sprague-Dawley rats (Charles River Farms, Wilmington, Massachusetts) were primed with 10 IU of PMSG to achieve follicular development. Ovulation was induced with a subcutaneous injection of 5 IU of human chorionic gonadotrophin (hCG) 48 hours after PMSG administration. To test the requirement for Ang II in ovulation, 100 μl of a 1 m*M* solution of saralasin [(Sar[1],Val[5],Ala[8])–Ang II, Sigma Chemical Company, St. Louis, Missouri)] was injected intraperitoneally 1 hour before or 1, 3, or 5 hours after hCG. Control rats were injected with the same volume of saline solution at the same time intervals. The animals were killed, and the ovaries and oviducts were removed en bloc 17 to 20 hours after hCG administration. The oviducts were separated from the ovaries, and the oocytes were freed from the mass of cumulus cells and counted under a dissecting microscope. The operator did not know which group was being studied, and recorded the number of oocytes found in each animal's paired oviducts. In addition, a representative number of ovaries from each group were carefully examined via dissecting microscope by slitting the ovarian follicles and expressing the retained ovum-cumulus complexes. In this way it was seen that animals with low numbers of oocytes in the oviducts had retained oocytes in their follicles. Analysis of variance (ANOVA) and Duncan's multiple comparisons test were employed for the statistical analysis of the results. Three-way ANOVA revealed a significant effect with respect to saralasin treatment ($F = 17.98$; df = 2.48; $P < 0.001$). When injected 1 hour before or 1 or 3 hours after hCG, saralasin halved the number of oocytes released in the oviducts relative to that in controls ($P < 0.05$, Table 1). Animals injected with saralasin 5 hours after hCG administration released fewer ova compared to controls injected with saline, but the differences did not reach statistical significance. A lower dose of saralasin, 100 μl of a 1 μ*M* solution, had no effect when injected 1 hour before or 1 hour after hCG administration.

To more directly assess the role of angiotensin in ovulation, two additional groups of rats were injected intraperitoneally with 100 μl of 1 m*M* saralasin and then immediately injected with the same volume and dose of Ang II, 1 ($n = 11$) or 3 ($n = 5$) hours after hCG. This tandem intraperitoneal administration of Ang II blocked the saralasin effect; no difference was found between the number of oocytes recovered from the oviducts of control and saralasin + Ang II–treated rats (Table 1).

These experiments, taken together with our data showing cyclic variation of follicular Ang II receptors (*6*) and markedly increased Ang II in preovulatory follicular fluid (*1, 2*), support the existence in the ovary of an intrinsic renin-angiotensin system which, being sensitive to gonadotrophins (*2*), is particularly active around the time of ovulation. This work suggests that Ang II may play a direct role in ovulation. Steele *et al.* (*9*), using intraventricular brain administration of saralasin to study the role of Ang II in pituitary hormone secretion in cycling adult rats, produced inhibition of the proestrous luteinizing hormone (LH) surge and secondarily blocked ovulation. In our experiments, the saralasin must have acted directly on the ovary, because ovulation was not dependent on pituitary gonadotrophins but was initiated with an injection of hCG. Further, since the saralasin blockade of hCG-induced ovulation was reversed by the simultaneous administration of Ang II, it may be inferred that Ang II plays an obligate role in ovulation, possibly as a mediator of hCG action. However, further experiments are necessary to test this hypothesis.

**Table 1.** Number of oocytes recovered from the oviducts after intraperitoneal injection of 100 μl of saline solution or saralasin (1 m*M*) or saralasin (1 m*M*) + Ang II (1 m*M*). *n*, number of rats, each with both adnexae uteri studied.

| Time of injection | Control | | Saralasin | | Saralasin + Ang II | |
|---|---|---|---|---|---|---|
| | *n** | ($\bar{X}$ ± SD) | *n* | ($\bar{X}$ ± SD) | *n* | ($\bar{X}$ ± SD) |
| 1 hour before hCG | 5 | 25.8 ± 5 | 6 | 13.8 ± 3.5* | | |
| 1 hour after hCG | 10 | 28.7 ± 9.1 | 12 | 14.4 ± 6.3* | 11 | 28.6 ± 9.8† |
| 3 hours after hCG | 7 | 25.6 ± 7.9 | 9 | 10.7 ± 6.1* | 5 | 25.0 ± 7† |
| 5 hours after hCG | 4 | 19.8 ± 2.7 | 5 | 17.8 ± 2.9† | | |

*$P < 0.05$. †$P > 0.05$ compared to controls (Duncan's test).

Department of Obstetrics and Gynecology, Yale University School of Medicine, and Yale University Center for Research in Reproductive Biology, New Haven, CT 06510.

*Present address: Department of Obstetrics and Gynecology, University of Valencia, Avda. Blasco Ibanez, 17, 46010 Spain.
†To whom all correspondence should be addressed.

The mechanism by which angiotensin directly affects ovulation remains to be determined. In preliminary experiments Ang II added to ovarian follicles that were being cultured in vitro increased the level in the culture medium of prostaglandin F2 α and plasminogen activator, both of which have been associated with LH action and specifically with ovulation (*10*).

Thus, this phylogenetically ancient regulatory system, which had previously been considered important only in maintaining fluid and electrolyte balance and vascular tone, also plays a direct role in reproduction (*11, 12*). Furthermore, our findings raise the possibility that among the several groups of compounds active on the renin-angiotensin system there may be some with potential as novel forms of contraception or as profertility drugs.


REFERENCES AND NOTES

1. M. D. Culler *et al.*, *J. Clin. Endocrinol. Metab.* **62**, 613 (1986).
2. A. Lightman *et al.*, *Am. J. Obstet. Gynecol.* **156**, 808 (1987).
3. Estrous cycle and gonadotrophin-related changes of follicular Ang II immunoreactivity and renin-like immunoreactivity were detected in follicular fluid from naturally cycling cows and from gonadotrophin-stimulated calves. In natural cycles, a significant positive correlation was found between renin-like activity in the follicular fluid and estradiol, and this is especially true when the estrogen to progesterone ratio was high; as occurs in dominant follicles (A. Palumbo, J. J. Ireland, R. T. Duby, F. Naftolin, in preparation).
4. A. G. Pucell, F. Bumpus, A. Husain, *J. Biol. Chem.* **262**, 70764 (1987).
5. A. Husain, F. Bumpus, P. DeSilva, R. C. Speth, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2489 (1986).
6. A. Lightman *et al.*, *Am. J. Obstet. Gynecol.*, in press.
7. Renin and Ang II were localized by immunohistochemistry to luteal and theca cells of ovaries from women with regular cycles (A. Palumbo, M. L. Carcangiu, C. Jones, F. Naftolin, in preparation).
8. D. T. Pals *et al.*, *Circulation Res.* **29**, 673 (1971).
9. M. K. Steele, R. V. Gallo, W. F. Ganong, *Am. J. Physiol.* **14**, R805 (1983).
10. A. Pellicer *et al.*, unpublished data.
11. G. Lavy, A. H. DeCherney, F. Naftolin, in *Reproductive Biology*, A. Negro-Vilar, Ed. (Raven, New York, 1988), pp. 153–172.
12. F. Naftolin, G. Lavy, A. Palumbo, A. H. DeCherney, in *Proceedings of the First International Congress of Gynecological Endocrinology*, A. Genazzani, Ed. (Parthenon, Lancaster, U.K., in press).


13. A. Pellicer was a Fulbright Fellow. A. Palumbo is a Lalor Foundation Fellow. Supported by NIH-HD22970 (F.N.) and the Andrew Mellon Foundation. We appreciate the assistance of N. J. MacLusky in the statistical analysis and numerous helpful discussions with H. Behrman and C. Markert.

29 December 1987; accepted 3 May 1988


# In Situ Transcription: Specific Synthesis of Complementary DNA in Fixed Tissue Sections


LAURENCE H. TECOTT, JACK D. BARCHAS, JAMES H. EBERWINE*



**A technique, in situ transcription, is described, in which reverse transcription of mRNAs is achieved within fixed tissue sections. An oligonucleotide complementary to proopiomelanocortin (POMC) mRNA was used as a primer for the specific synthesis of radiolabeled POMC cDNA in fixed sections of rat pituitary, thus permitting the rapid anatomical localization of POMC mRNA by autoradiography. Intermediate lobe signal intensities were sensitive to dopaminergic drugs, demonstrating that the method can be used for studies of mRNA regulation. The transcripts may also be eluted from tissue sections for a variety of uses, including the identification and cloning of autoradiographically localized cDNAs from small amounts of tissue.**


THE SYNTHESIS OF CDNA BY THE enzyme reverse transcriptase is typically performed in a solution reaction with extracted RNA serving as a template. We report here a method whereby mRNA can serve as a template for this enzyme in situ, within a fixed tissue section. The in situ transcription (IST) procedure (Fig. 1) is initiated by in situ hybridization of an oligonucleotide complementary to a specific mRNA, providing a primer that is required for the polymerase activity of reverse transcriptase (*1*). This priming step allows one to select the individual mRNA to be transcribed. The enzyme then extends the primer with the mRNA acting as a template. During the polymerase reaction, radiolabeled deoxynucleotides may be incorporated into transcripts, resulting in high specific activity cDNA.


*To whom all correspondence should be addressed.

Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.


The IST method was developed by examining POMC gene expression in the rat pituitary. The POMC gene gives rise to a family of biologically active peptides including adrenocorticotropin (ACTH), β-endorphin, and α-melanocyte–stimulating hormones (α-MSH) (*2*). An oligonucleotide 36 nucleotides (nt) in length, complementary to the sequence encoding amino acids 100 to 111 of rat POMC, was used as the primer (*3*). After in situ hybridization to fresh frozen paraformaldehyde-fixed sections of rat pituitary (11 μm thick), reverse transcription was performed in the presence of $^{35}$S-labeled deoxycytidine with the other three deoxynucleotides unlabeled. A strong signal was observed in the intermediate lobe (Fig. 2, a and c), consistent with the known localization of POMC mRNA (*4–8*). When the primer was omitted from the hybridization mix, this signal was not observed (Fig. 2b). Omission or heat inactivation of the reverse transcriptase also eliminated the signal. As a negative control, reverse transcription was performed after a hybridization step with a 36-nt probe complementary to tyrosine hydroxylase mRNA (*9*), which has not been detected in the rat pituitary (*10*). Accordingly, no signal above background was pro-

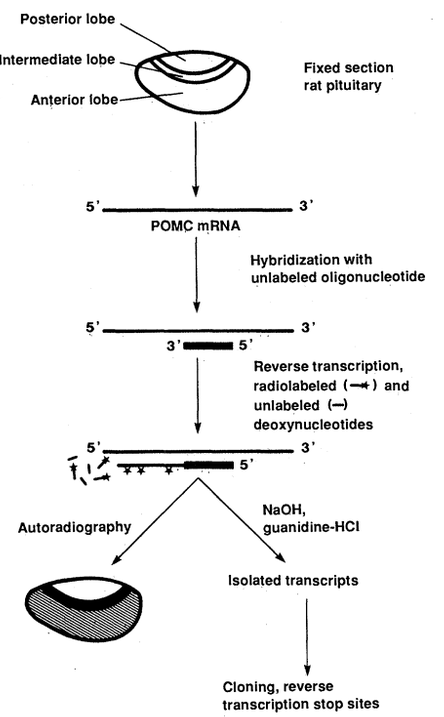


**Fig. 1.** Schematic outline of the in situ transcription (IST) procedure for the detection of POMC mRNA. An unlabeled oligonucleotide is hybridized to POMC mRNA, followed by reverse transcription within the tissue section in the presence of radiolabeled deoxynucleotides. Autoradiography is then performed for the localization of radiolabeled POMC cDNA. After autoradiography, the transcripts may be denatured and eluted by NaOH or guanidine-HCl treatments, and the transcripts may be either cloned or separated by electrophoresis for subsequent analysis.