

Angiotensin II: Does It Have a Direct Obligate Role in Ovulation?

By demonstrating that the angiotensin II (Ang II) receptor antagonist (Sar¹,Val⁵,Ala⁸)-Ang II blocks ovulation in immature rats treated with pregnant mares' serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), Pellicer *et al.* (1) propose "a direct, obligate role for Ang II in ovulation." This conclusion presents an interesting conceptual problem regarding the site of action of Ang II within the ovary. Since our studies on the adult cycling female rat have shown the lack of Ang II receptors on preovulatory follicles (2), we felt that either (i) the PMSG + hCG-treated immature rat model of ovulation differed significantly from that for the adult female rat with respect to the occurrence of Ang II receptors in the preovulatory follicle; (ii) the Ang II receptor antagonist (Sar¹,Val⁵,Ala⁸)-Ang II displayed nonspecific effects on the process of ovulation; or (iii) the effects of endogenous Ang II on the preovulatory follicle were indirect.

To examine whether Ang II receptors are present in preovulatory follicles in the PMSG-treated or in the PMSG + hCG-treated immature rat ovary, we removed ovaries for receptor autoradiography from three rats each, 48 hours after PMSG treatment and 3 hours after hCG treatment in rats primed 51 hours earlier with PMSG. We chose these two time points such that they were within the period of maximum sensitivity for ovulation inhibition by (Sar¹,Val⁵,Ala⁸)-Ang II as reported by Pellicer *et al.* (1). Preovulatory follicular granulosa cells characteristically express luteinizing hormone (LH) receptors (3). In both sets of ovaries, we observed LH receptors, identified by specific ¹²⁵I-labeled hCG binding (4), on the granulosa cell layer of certain large follicles (≥400-μm diameter); these were therefore characterized as preovulatory follicles. LH receptors were also present on the theca cell layers of most follicles. In ovarian sections adjacent to those used for the localization of LH receptors, we localized Ang II receptors using the radiolabeled Ang II receptor antagonist ¹²⁵I-labeled (Sar¹,Ile⁸)-Ang II (2). We examined more than 600 follicles. We found that, in ovaries from both the PMSG-primed and the PMSG + hCG-treated rats, follicles containing LH receptors on their granulosa cell layer did not contain Ang II receptors. Figures 1 and 2 show a representative set of adjacent sections from a PMSG + hCG-

treated immature rat ovary, demonstrating the lack of Ang II receptors in follicles containing LH receptors on their granulosa cell layer. These findings illustrate the similarity of the PMSG + hCG-treated rat model to that for the normal cycling adult female rat, where Ang II receptors do not exist in preovulatory ovarian follicles.

To examine the possibility that (Sar¹,Val⁵,Ala⁸)-Ang II may nonspecifically or indirectly affect ovulation, we repeated the ovulation inhibition experiment of Pellicer *et al.* (1) using the Ang II receptor antagonists (Sar¹,Ile⁸)-Ang II and (Sar¹,Val⁵,Ala⁸)-Ang II. Antagonists were administered intraperitoneally 1 hour before the hCG treatment. In contrast to the ~50% reduction in

the number of ova reported by Pellicer *et al.* (1), we observed no significant differences ($P > 0.05$; analysis of variance) between the number of oviductal ova of the vehicle injected control rats [22.7 ± 2.0 ova (mean ± SEM), $n = 23$] and the oviductal ova of the (Sar¹,Ile⁸)-Ang II-treated rats [18.6 ± 1.9 ova (mean ± SEM), $n = 10$] or the (Sar¹,Val⁵,Ala⁸)-Ang II-treated rats [20.5 ± 2.5 ova (mean ± SEM), $n = 14$]. Since we used the same strain (Sprague-Dawley) and age of rat (25 days old at the time of PMSG treatment), the same dosage (100 μl of a 1 mM solution of antagonist), route (intraperitoneal), and time (1 hour before hCG injection) of administration of Ang II receptor antagonist and the same dose and intervals of gonadotropin treatment in this study as those reported by Pellicer *et al.* (1), our inability to reproduce their experiment cannot be accounted for by these factors.

Although we followed the experimental protocol reported by Pellicer *et al.* (1), it is possible that in the rat ovary the "window of

Fig. 1. Distribution of Ang II receptors and LH receptors in adjacent ovarian sections. Twenty-five-day-old female Sprague-Dawley rats were treated with 10 IU of PMSG followed 48 hours later by 5 IU of hCG. Three hours after hCG injection the rats were killed, and LH and Ang II receptors were localized by autoradiography with the use of ¹²⁵I-labeled hCG (4) and ¹²⁵I-labeled (Sar¹,Ile⁸)-Ang II (2), respectively. (A) Autoradiogram showing the distribution of LH receptor binding sites in an ovarian section. LH receptor binding sites are present on the granulosa cell layer of preovulatory follicles (open arrows). LH receptor binding sites are also present on the theca cell layer of most follicles. Filled arrows show the positions of follicles containing Ang II receptor binding sites, as determined in the adjacent autoradiogram shown in (C); Ang II receptor-containing follicles lack LH receptors in their granulosa cell layer. (B) Nonspecific ¹²⁵I-labeled hCG binding to a section adjacent to that used in (A). (C) Autoradiogram showing the distribution of Ang II receptor binding sites in an ovarian section. Ang II receptor-containing follicles are indicated by filled arrows. The open arrows show preovulatory follicles (identified by the presence of granulosa cell LH receptors, as described in (A)). (D) Nonspecific ¹²⁵I-labeled (Sar¹,Ile⁸)-Ang II binding to a section adjacent to that used in (C); nonspecific ¹²⁵I-labeled (Sar¹,Ile⁸)-Ang II binding is virtually undetectable. Bar = 1 mm.

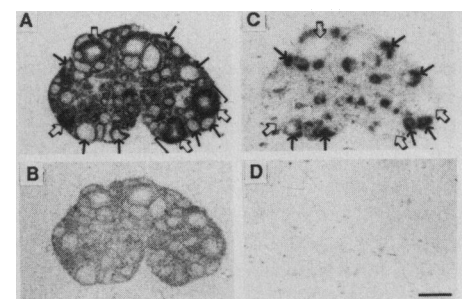
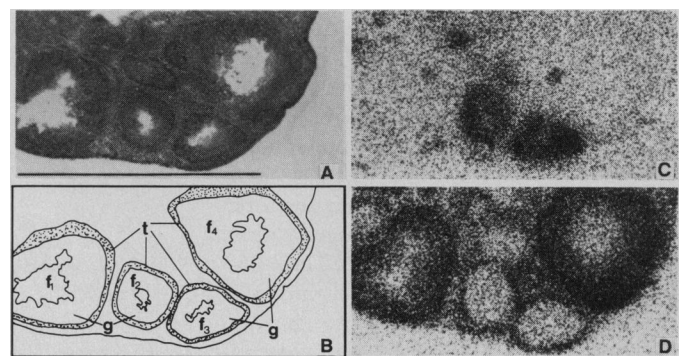


Fig. 2. Enlargement of the bracketed area in Fig. 1A. (A) Hematoxylin- and eosin-stained ovarian section. (B) Schematic illustration of ovarian section in (A) showing theca (t) and granulosa (g) cell layers of follicles f₁, f₂, f₃, and f₄. (C) High-power view of an autoradiogram showing the distribution of ovarian Ang II receptor binding sites in a section adjacent to that shown in (A). Ang II receptors are present on the granulosa cell layer of follicles f₂ and f₃, but not on the preovulatory follicles f₁ and f₄. (D) High-power view of an autoradiogram showing the distribution of ovarian LH receptor binding sites in a section adjacent to that shown in (A). LH receptors are present on the granulosa and on theca cell layers of preovulatory follicles f₁ and f₄; in follicles f₂ and f₃, LH receptors are present only on the theca cell layers. Bar = 1 mm.



maximum sensitivity" to Ang II is variable. Therefore, in further studies we extended the duration of Ang II receptor blockade by infusing (Sar¹,Ile⁸)-Ang II (10 nmol/hour) intraperitoneally using an osmotic minipump (Alza Corporation, Palo Alto, California) over a period of 6 days in 23-day-old immature female rats in which ovulation was induced 65 hours before the end of the infusion period by sequential injections of PMSG and hCG. The infusion rate of (Sar¹,Ile⁸)-Ang II was such that 24 hours after the beginning of infusion, pressor responses to bolus intravenous injections of Ang II were almost completely inhibited. Again, however, we observed no significant differences between the number of oviductal ova in (Sar¹,Ile⁸)-Ang II-treated rats [28.5 ± 3.9 ova (mean ± SEM), n = 10] and the vehicle-infused control rats [22.2 ± 2.4 ova (mean ± SEM), n = 9].

Because our autoradiographic studies show that preovulatory follicles in the PMSG + hCG-treated immature rat lack Ang II receptors, they provide no morphological basis for concluding that there is a direct role for Ang II in ovulation. Since we observed no effects of peripheral Ang II receptor blockade on ovulation, our studies do not support the suggestion that Ang II receptor antagonists have a role in contraception, as was proposed by Pellicer *et al.* (1).

ADIL I. DAUD
F. MERLIN BUMPUS
AHSAN HUSAIN
*Department of Heart and
Hypertension Research,
Research Institute,
Cleveland Clinic Foundation,
9500 Euclid Avenue,
Cleveland, OH 44195-5071*

REFERENCES AND NOTES

1. A. Pellicer *et al.*, *Science* **240**, 1660 (1988).
2. A. I. Daud, F. M. Bumpus, A. Husain, *Endocrinology* **122**, 2727 (1988).
3. A. Amsterdam, Y. Koch, M. E. Lieberman, H. R. Lindner, *J. Cell Biol.* **67**, 894 (1975); J. S. Richards and A. R. Midgley, Jr., *Biol. Reprod.* **14**, 82 (1976); A. J. W. Hsueh *et al.*, *Endocrin. Rev.* **5**, 78 (1984).
4. A. R. Midgley, Jr., *Adv. Exp. Med. Biol.* **36**, 365 (1973).
5. Supported in part by grants from the Reinberger Foundation, by National Institutes of Health grant HD-23925, and by a postdoctoral fellowship to A.I.D. from the American Heart Association, Northeast Ohio Affiliate.

25 January 1989; accepted 2 May 1989

Response: We read the comment by Daud *et al.* with keen interest. The original work reported by us (1) was the result of 12 separate experiments performed in August

through October of 1987, using 100 µl of 1 mM saralasin [(Sar¹,Val⁵,Ala⁸)-Ang II]. We had also performed a similar group of experiments using 200 µl of 1 mM saralasin, but since the results were the same and we had not done full time curves, these experiments were not included in the report. In all cases we found a diminished number of tubal oocytes when saralasin was administered around the time of injection of human chorionic gonadotropin (hCG) in immature rats primed with pregnant mare serum gonadotropin (PMSG). Only in those rats given saralasin 5 hours after hCG was the decreased number of tubal oocytes not statistically significant. In a separate group of experiments we found that when angiotensin II was administered simultaneously the effect of saralasin did not occur.

Since reading Daud's comment, we have repeated our studies, using 100 or 200 µl of 1 mM saralasin [(Sar¹,Val⁵,Ala⁸)-Ang II, Sigma Chemical Co., St. Louis, Missouri]. Originally we accepted the breeder's (Charles River Farms, Wilmington, Massachusetts) age dating, but when we found that there was as much as 100% discrepancy in weights of equal-aged animals, we began controlling for both age and weight. We have now done ten replications of the previous work, usually testing seven saline controls and seven saralasin-treated animals in each experiment. Saralasin treatment was given 1 or 3 hours after hCG. In eight experiments there was no statistically significant difference between the groups. In two experiments in which we used 25-day weight-controlled females and 200 µl of 1 mM saralasin, the number of tubal oocytes in saralasin-treated animals was lower (Table 1).

Although we have been able to reproduce our previous findings, we cannot explain the difference between our previous experience, when saralasin regularly diminished the number of hCG-induced tubal oocytes, and our present findings. We believe that some of the difficulty stems from the difference in maturity of the test animals, and we are exploring this variable. We are also assessing the precision of responses with each of the reagents, especially the biologically derived hormone preparations (PMSG and hCG). At present we are investigating different preparations of hCG. Our preliminary results indicate that the variability of this biologically derived hormone may be responsible for the discrepancies present in this work. Using another preparation of hCG, we have observed a statistically significant difference ($P < 0.05$) between the

Table 1. Number of oocytes recovered from the oviducts after intraperitoneal injection of 200 µl of saline solution or saralasin (1 mM).

Time of injection (hours after hCG)	Control ($\bar{x} \pm SD$)	Saralasin ($\bar{x} \pm SD$)	<i>P</i> (<i>t</i> test)
1	45.2 ± 5.5	32.3 ± 3.9	<0.05
3	42.0 ± 5.0	27.3 ± 4.2	<0.05

number of oviductal oocytes of control animals [26.8 ± 2.8 ova (mean ± SEM)] and saralasin-treated rats [18.3 ± 1.5 ova (mean ± SEM)].

With regard to the general issue of angiotensin's role in ovulation, it is of interest that another laboratory has confirmed the action of saralasin in blocking PMSG-induced ovulation, using an *in vitro* perfusion system. Peterson *et al.* (2) have employed perfusion with luteinizing hormone and isobutyl methyl xanthine of ovaries from 27-day-old female rats which had 48 hours previously received 30 IU of PMSG. Under these conditions the addition of 1 nM of saralasin [(Sar¹,Val⁵,Ala⁸)-AII], to the perfusion fluid inhibited ovulations by approximately two-thirds. In further studies they completely abolished the saralasin effect by adding angiotensin II to the perfusion medium (3). Their success with luteinizing hormone again focuses interest on the possibility that hCG is the source of the irregularity in the *in vivo* studies. These independently performed *in vitro* studies support our original contention that the role of luteinizing hormone in ovulation may require the action of angiotensin.

FREDERICK NAFTOLIN
PATRICIA ANDRADE-GORDON
ANTONIO PELLICER
ANGELA PALUMBO
ROSANNA APA
TONY ZREIK
TAE KI YOON
ALAN DECHERNEY
*Department of Obstetrics and Gynecology,
Center for Research in Reproductive Biology,
Yale University,
New Haven, CT 06510-8063*

REFERENCES

1. A. Pellicer *et al.*, *Science* **240**, 1660 (1988).
2. C. M. Peterson, C. Zhu, T. Mukaita, W. J. LeMaire, Abstract 25 presented at the 22nd Annual Meeting of the Society for the Study of Reproduction, Columbia, MO, 7 August 1989.
3. W. J. LeMaire, personal communication.

9 August 1989; accepted 10 August 1989

Angiotensin II: does it have a direct obligate role in ovulation?

ADIL I DAUD, F. MERLIN BUMPUS and AHSAN HUSAIN

Science **245** (4920), 870-871.
DOI: 10.1126/science.2772639

ARTICLE TOOLS

<http://science.sciencemag.org/content/245/4920/870>

REFERENCES

This article cites 6 articles, 2 of which you can access for free
<http://science.sciencemag.org/content/245/4920/870#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 1989 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works.