maximum sensitivity" to Ang II is variable. Therefore, in further studies we extended the duration of Ang II receptor blockade by infusing (Sar^1,Ile^8)–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 (Sar$_1$, Ile^8)–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 (Sar^1,Ile^8)–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

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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 m*M* saralasin [(Sar^1,Val^5,Ala^8)–Ang II)]. We had also performed a similar group of experiments using 200 μl of 1 m*M* 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 m*M* saralasin [(Sar^1,Val^5,Ala^8)–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 m*M* saralasin, the number of tubal oocytes in saralasin-treated animals was lower (Table 1).

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

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

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 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 PMS-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 n*M* of saralasin [(Sar^1,Val^5,Ala^8)–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

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