BIOLOGY OF REPRODUCTION 25, 989–996 (1981)

Effect of Hypophysectomy on Atresia of Rat Preovulatory Follicles[1]

R. H. BRAW, S. BAR-AMI and A. TSAFRIRI[2]

Department of Hormone Research,
The Weizmann Institute of Science,
Rehovot, 76100, Israel

ABSTRACT

Atresia of Graafian follicles was induced by hypophysectomy on the morning of the day of proestrus. As early as 6 h after hypophysectomy, follicles showed a reduced ability to respond with ovulation to administration of hCG, and 12 h after hypophysectomy all the follicles failed to ovulate. At 24 h after hypophysectomy, the follicles exhibited morphological changes characteristic of early atresia followed by advanced atresia at 48 h after the operation. Steroidogenesis in vitro of follicles from hypophysectomized rats was compared with that of sham-operated controls. The mean ± SEM rate of accumulation (ng/follicle/24 h) of progesterone, testosterone, and estradiol-17β was 5.1 ± 0.9, 6.3 ± 0.5, and 20.2 ± 3.7, respectively, in control follicles; 12 h after hypophysectomy progesterone accumulation was 22.7 ± 4.9, testosterone 4.5 ± 0.4, and estradiol-17β 11.7 ± 0.9; and 48 h after the operation accumulation was 59.3 ± 8.3, 1.0 ± 0.3, and 0.10 ± 0.02, respectively. Thus atretic follicles from hypophysectomized rats are characterized by increased progesterone and decreased androgen and estradiol-17β production. Addition of LH to the culture medium stimulated progesterone accumulation in follicles from rats up to 24 h after hypophysectomy but not 48 h after the operation. It appears, therefore, that follicles gradually lose their responsiveness to LH during the atretic process.

INTRODUCTION

In a previous report (Braw and Tsafriri, 1980a), the effect of repeated treatment with pentobarbitone on preovulatory follicles of rats has been described. These atretic follicles exhibited a progressive decrease in their ovulatory response to hCG in vivo and a change in their pattern of steroidogenesis in vitro. Similar atretic changes resulting from pentobarbitone treatment of rats (Uilenbroek et al., 1980) and hamsters (Terranova, 1980) have been described. Pentobarbitone treatment prevents the preovulatory surge of gonadotropins but does not affect their tonic release (Daane and Parlow, 1971; Ashiru and Blake, 1978). The present study was undertaken to examine the effect of total gonadotropin deprivation by hypophysectomy on the preovulatory follicles and to compare the atretogenic action of this operation with that of pentobarbitone treatment.

MATERIALS AND METHODS

Animals

Rats of Wistar origin from the departmental colony were housed in air-conditioned quarters with lights on between 0500 and 1900 h. Pelleted food and water were offered ad libitum. The animals used were 4 months old and showed at least two normal 4-day estrous cycles immediately before the experiment, as determined by vaginal smears obtained daily. Animals were hypophysectomized under ether anesthesia, between 0800 and 1200 h on the day of proestrus, utilizing an intra-aural approach (Zarrow et al., 1964). Following sacrifice of the rats, each sella turcica was examined and only those rats having no traces of the hypophysis were included in the experiments. Control rats were unoperated or sham-operated by inserting a needle into the external auricular opening and puncturing the eardrum.

Induction of Ovulation by hCG

Human chorionic gonadotropin (hCG; Pregnyl, Organon, 4 IU) was injected i.p. 6, 12, and 24 h after hypophysectomy or 6 h after sham-operation (5–14 rats in each group). Ovulation was checked by expressing and counting tubal ova on the day following hCG administration.

Accepted July 27, 1981.
Received March 30, 1981.

[1] A preliminary report of this study was presented at the 62nd Annual Meeting of the Endocrine Society, Washington, DC, June 18–20, 1980, Abstr. 715.

[2] Reprint requests.

Culture of Follicles

Animals were killed by cervical dislocation 6, 12, 24, and 48 h after hypophysectomy or 6 h after sham-operation. Graafian follicles were explanted from the ovaries and cultured as described previously (Tsafriri et al., 1972). Three follicles were cultured in each dish. The follicles were randomly chosen from a pool of follicles explanted from 5–10 rats. Those experiments in which substrates of steroidogenesis were added were performed twice, whereas all other experiments were repeated at least three times. Ovine LH (NIH-LH-S-18; 5 μg/ml), testosterone, or 17α-hydroxyprogesterone (Ikapharm; 1 μg/ml) was added to the medium as indicated. The gonadotropins were dissolved in 0.9% NaCl, and the steroid hormones in absolute ethanol. The final concentration of ethanol (0.1%) had no effect on steroid accumulation.

Medium was collected after 6 h, replaced by fresh medium, and collected again after an additional 18 h of incubation. The pattern of follicular steroidogenesis was similar during the first 6 and 24 h of culture (the data for 24 h were obtained by combining the amounts of steroids obtained after 6 and 18 h of culture), and therefore only the latter results are presented. In experiments where testosterone or 17α-hydroxyprogesterone were added, medium was collected only after 6 h, and results were expressed as nanograms per follicle per 6 h.

RIA of Steroid Hormones

The amounts of progesterone, androstenedione, testosterone, and estradiol-17β in the medium were determined by RIA of unfractionated lipid extracts as described earlier (Lieberman et al., 1975). Well characterized and specific antisera prepared against progesterone, androstenedione, testosterone, and estradiol-17β were used (Bauminger et al., 1974). The minimum sensitivities and intraassay and interassay coefficients of variation were as follows: progesterone, 15 pg/ml, 2% and 20%; androstenedione, 30 pg/ml, 3% and 16%; testosterone, 30 pg/ml, 4% and 18%; and estradiol, 20 pg/ml, 3% and 20%, respectively. Steroid concentrations are expressed as nanograms per follicle. Statistical differences between groups were calculated using Student's t test or one-way analysis of variance.

Histological Procedures

Ovaries used for morphological examination were fixed in Bouin's solution, dehydrated, embedded in paraffin, and cut serially at 7 μm. Sections were stained with hematoxylin-eosin.

RESULTS

Morphology of Graafian Follicles Following Hypophysectomy

Preovulatory follicles from intact or sham-operated rats contained an oocyte at the dictyate stage, a few dividing granulosa cells, and no pyknotic granulosa cell nuclei. Follicles from rats killed 6 and 12 h after hypophysectomy were similar in their morphological appearance to the preovulatory follicles though pyknotic granulosa cell nuclei were occasionally seen (Fig. 1A). At 24 h after hypophysectomy, ∼10% of the granulosa cells were pyknotic but mitotic figures could also still be seen. At 48 h after hypophysectomy, most oocytes showed meiosis-like changes. In the granulosa layer, ∼20–30% of the nuclei were pyknotic and follicle fluid contained much cell debris and atretic bodies (Fig. 1B). Thus, according to our classification (Braw and Tsafriri, 1980b), preovulatory follicles had reached stage I of atresia at 24 h after hypophysectomy and stage II at 48 h.

Induction of Ovulation by hCG

Administration of hCG 6 h after hypophysectomy induced ovulation in 91% of animals but the number of ova shed was reduced to 7.4 ± 0.9 (mean ± SEM) as compared with 10.2 ± 0.9 in sham-operated controls ($P<0.05$; Table 1). When hCG was administered 12 h or 24 h after hypophysectomy, no ovulation was observed.

Follicular Steroidogenesis

Preovulatory follicles from sham-operated controls incubated in LH-free medium accumulated predominantly estradiol-17β (20.2 ± 3.7 ng/follicle/24 h, mean ± SEM), while production of progesterone (5.1 ± 0.9), androstenedione (4.3 ± 0.4), and testosterone (6.3 ± 0.5) was low (Fig. 2). Follicles explanted from hypophysectomized rats accumulated more progesterone, as compared with sham-operated controls ($P<0.05$), while production of androstenedione ($P<0.01$) and estradiol-17β decreased ($P<0.01$). When the follicles were explanted 6 h after hypophysectomy, the rate of progesterone production increased to 15.9 ± 1.8 ng/follicle/24 h. Further increases in progesterone accumulation occurred when the follicles were isolated 12 to 48 h after hypophysectomy, and this was accompanied by a gradual decrease in androstenedione, testosterone, and estradiol-17β production (Fig. 2). At 48 h following hypophysectomy, progesterone secretion was 59.3 ± 8.9 ng/follicle/24 h, i.e., 12-fold greater than that of preovulatory follicles, while production of androstenedione, testosterone, and estradiol-17β was negligible (<1.0 ng/follicle/24 h).

Addition of LH to the culture medium increased progesterone production in all types of

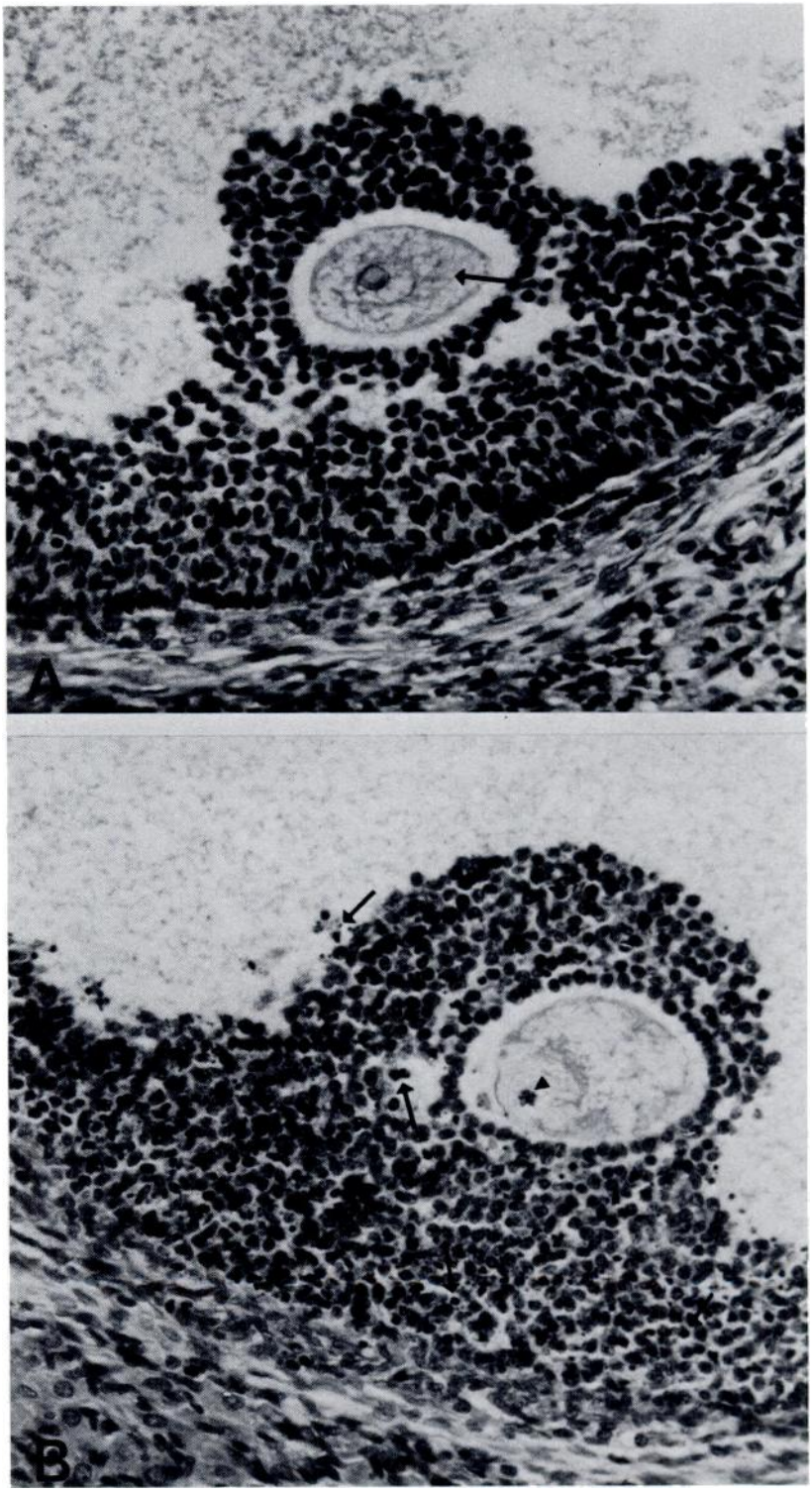

FIG. 1. A) Nonatretic antral follicle 12 h after hypophysectomy. Note germinal vesicle (arrow) in the oocyte and normal appearing granulosa cells.

B) Follicle at stage II atresia 48 h after hypophysectomy. Note meiosis-like changes in the oocyte (arrowhead) and numerous pyknotic cells scattered throughout granulosa layer (arrows). Hematoxylin and eosin. X 350.

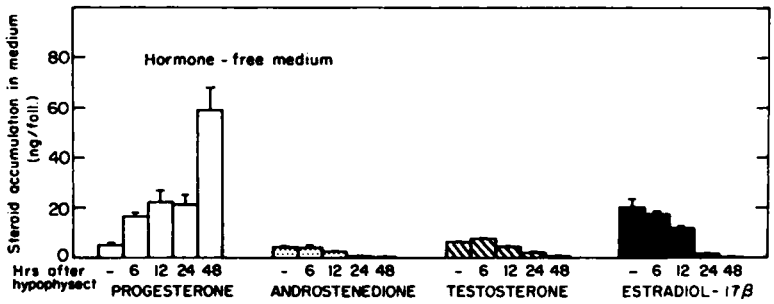

FIG. 2. Accumulation of steroids by follicles from sham-operated (—) or hypophysectomized rats during 24 h of culture in hormone-free medium. Each bar represents the mean ± SEM of 5—10 determinations.

follicles, except those explanted 48 h after hypophysectomy (Fig. 3). In the presence of LH, follicles explanted from hypophysectomized rats accumulated more progesterone than did follicles from sham-operated controls ($P<0.01$). Follicles from sham-operated controls accumulated 89.1 ± 5.0 ng progesterone/follicle/24 h in the presence of LH, while follicles obtained 6, 12, and 24 h after hypophysectomy secreted 111.5 ± 7.7, 108.2 ± 10.9, and 81.4 ± 5.4, respectively. Follicles explanted from hypophysectomized rats secreted much less androstenedione ($P<0.001$), testosterone ($P<0.001$), and estradiol-17β ($P<0.001$) in the presence of LH than did control follicles. A significant increase ($P<0.01$) in androstenedione accumulation, following addition of LH, occurred in the culture of follicles from controls and from rats killed 6 h and 12 h after hypophysectomy, whereas no stimulatory effect of LH was seen 24 and 48 h after the operation. Addition of LH to the culture medium had no effect on accumulation of estradiol-17β by follicles from hypophysectomized rats, but increased the production of estradiol-17β by follicles from sham-operated controls ($P<0.01$).

Effect of Substrate Supplementation on Steroidogenesis of Atretic Follicles

Addition of 17α-hydroxyprogesterone (1 μg/ml) to follicles from intact rats increased testosterone accumulation ($P<0.01$). Follicles explanted 6 h after hypophysectomy secreted as much testosterone as control follicles, but addition of substrate did not further increase testosterone accumulation. Addition of 17α-hydroxyprogesterone resulted in an approximately twofold increase in accumulation of testosterone (Fig. 4, lower panel) by follicles from rats killed 12 h ($P<0.01$) and 24 h ($P<0.02$) after hypophysectomy. Nevertheless, the addition of 17α-hydroxyprogesterone restored testosterone accumulation to the amount observed in control follicles only 12 h after hypo-

TABLE 1. Induction of ovulation by hCG (4 IU/rat) in hypophysectomized rats.

	Time between operation and hCG (h)	Rats ovulating/ rats treated (%)	Ova shed/ treated rat (mean ± SEM)	Ova shed/ ovulating rat (mean ± SEM)
Untreated control	...	12/12 (100)	11.0 ± 0.6	11.0 ± 0.6
Sham-operated	6	13/14 (93)	9.5 ± 1.1	10.2 ± 0.9
Hypophysectomized	6	10/11 (91)	6.7 ± 1.1*	7.4 ± 0.9*
	12	0/11 (0)	0	0
	24	0/5 (0)	0	0

*$P<0.01$ compared with untreated control.

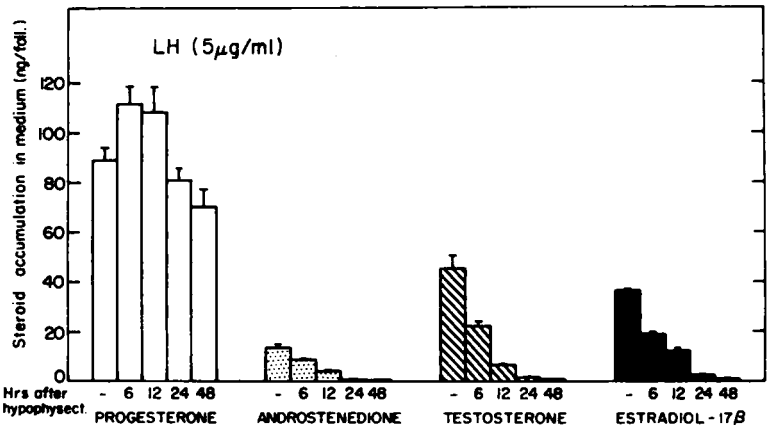

FIG. 3. Accumulation of steroids by follicles from sham-operated (−) or hypophysectomized rats during 24 h of culture in medium supplemented with LH (5 μg/ml). Each bar represents the mean ± SEM of 5−10 determinations.

physectomy and not after 24 h. When 17α-hydroxyprogesterone was added in the presence of LH (5 μg/ml), accumulation of testosterone increased in follicles explanted 6 and 12 h after hypophysectomy to levels not significantly different from those in control follicles cultured without the substrate (Fig. 4, upper panel).

The addition of testosterone (1 μg/ml), an aromatase substrate, increased the basal ($P < 0.01$) production of estradiol-17β in all types of follicles (Fig. 5; lower panel). However, only in follicles explanted 6 and 12 h after hypophysectomy did additional testosterone restore estradiol production to control levels accumulated by follicles cultured without the substrate. When testosterone was added in the presence of LH, it increased estradiol-17β to the control level only in follicles explanted 6 h after hypophysectomy.

DISCUSSION

Hypophysectomy of rats on the morning of the day of proestrus resulted in an early morphological stage of follicular atresia within 24 h of hypophysectomy and in an advanced stage of atresia within 48 h. These atretic changes were similar to those described for intact mice (Byskov, 1974) and rats (Braw and Tsafriri, 1980b). When challenged with hCG at 12 h after hypophysectomy, all the follicles failed to ovulate. These results are in agreement with those of Talbert et al. (1951) who found that only one of eight rats ovulated 12 h after hypophysectomy. Similarly, follicles in hamsters did not ovulate in response to exogenous LH 8 h after hypophysectomy (Greenwald, 1978). Our results show that 48 h of total gonadotropin deprivation are adequate for preovulatory follicles to reach advanced stages of atresia in rats. On the other hand, it has been shown previously that 3 to 4 days of pentobarbitone treatment are needed for preovulatory follicles to become atretic. About 30% of the latter follicles remained capable of ovulating in response to hCG even on Day 4 of pentobarbitone treatment (Braw and Tsafriri, 1980a). This difference can be explained by the fact that, in contrast to hypophysectomy, pentobarbitone blocks only the preovulatory surge of gonadotropins, while basal levels of serum gonadotropins remain unaffected (Daane and Parlow, 1971; Ashiru and Blake, 1978). This continuing availability of gonadotropins may delay atresia of preovulatory follicles in pentobarbitone-treated rats. The action of PMSG in preventing atresia of rat and mouse follicles has been demonstrated by Peters (1979) and Braw and Tsafriri (1980b).

The present study shows that the steroidogenic activity of follicles induced to undergo atresia by hypophysectomy is markedly different from that of preovulatory ones. Follicles explanted 6 h and 48 h after hypophysectomy secreted 3- and 12-fold more progesterone, respectively, as compared with follicles from sham-operated controls. By contrast, accumula-

tion of androgen and estradiol was reduced 12 h or later after hypophysectomy, and both of these steroids reached their minima at 48 h. Similarly, Taya and Greenwald (1980) showed that ovaries from hamsters, cultured in vitro 1–8 days following hypophysectomy, are capable of producing large amounts of progesterone but only small amounts of estradiol and testosterone. Similar changes in follicular steroidogenesis resulted after repeated treatment of proestrous rats with pentobarbitone (Braw and Tsafriri, 1980; Uilenbroek et al., 1980). Thus, atresia induced by hypophysectomy, i.e., total gonadotropin deprivation,

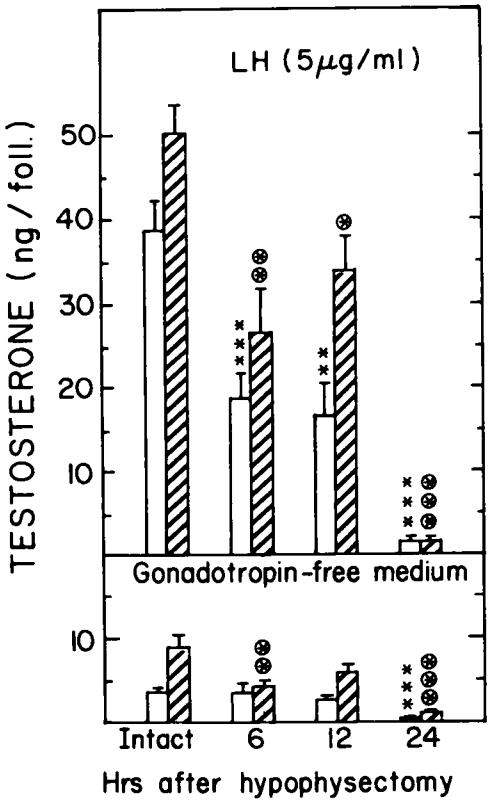

FIG. 4. Accumulation of testosterone in the medium during 6 h culture of the follicles from intact or hypophysectomized rats. Follicles were cultured without (open columns) or with (hatched columns) addition of 17α-hydroxyprogesterone (1 μg/ml) to the medium. Lower panel: no added LH; upper panel: LH (5 μg/ml) added to medium. Each bar represents the mean ± SEM of 4–8 determinations. *P<0.01, **P<0.001 vs follicles from intact rats. ⊛P<0.05, ⊛⊛P<0.01, ⊛⊛⊛P<0.001 vs follicles from intact rats cultured in the presence of the substrate.

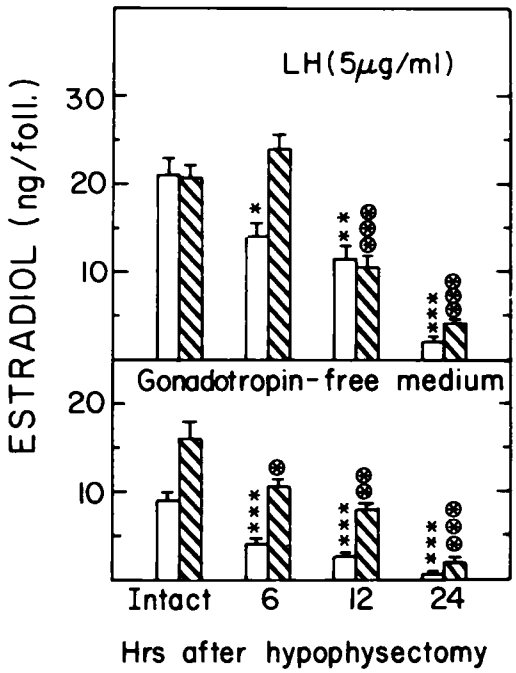

FIG. 5. Accumulation of estradiol-17β in the medium during 6 h culture of the follicles from intact or hypophysectomized rats. Follicles were cultured without (open columns) or with (hatched columns) addition of testosterone (1 μg/ml) to the medium. Lower panel: no added LH; upper panel: LH (5 μg/ml) added to medium. Each bar represents the mean ± SEM of 4–8 determinations. *P<0.05, **P<0.01, ***P<0.001 vs follicles from intact rats. ⊛P<0.05, ⊛⊛P<0.01, ⊛⊛⊛P<0.001 vs follicles from intact rats cultured in the presence of the substrate.

causes changes in synthesis of follicular steroids which are similar to those that follow the blockage of the preovulatory surge of gonadotropins by repeated injections of pentobarbitone. Regardless of the similarity of results, it appears that the kinetics of changes in enzymatic activity differ somewhat in these two experimental circumstances. In atresia induced by pentobarbitone treatment, the enzymes involved in the conversion of progesterone to androgen appear to be affected earlier than are aromatizing enzymes, as the addition of testosterone to the medium restored estradiol-17β production (Braw and Tsafriri, 1980a; Uilenbroek et al., 1980) whereas the addition of 17α-hydroxyprogesterone failed to restore testosterone accumulation (our unpublished data). By contrast, the present results indicate that in atretic follicles induced by hypophysectomy,

aromatizing enzymes, as well as enzymes involved in androgen production, were affected within 12 to 24 h after the operation.

These two approaches, namely pentobarbitone-treatment and hypophysectomy, may allow the detailed study of biochemical changes associated with atresia before morphological changes can be detected. It should be emphasized, however, that atretic changes in only mature Graafian follicles have been examined thus far in these model systems. Atresia at earlier stages of follicular differentiation does not necessarily progress through the same biochemical changes. By using immature PMSG-treated rats, which ovulate only after administration of exogenous hCG, Peluso et al. (1980a) studied atretic changes resulting when such follicles were not exposed to hCG. In these follicles, the appearance of early atretic changes coincided with a decrease in ovarian estradiol and testosterone concentrations. In other species, studies with follicles from mare (Kenney et al., 1979) and sheep (Moor et al., 1978) also revealed that reduced estrogen formation was a consequence of atresia. In sheep, however, the reduction in estrogen secretion by atretic follicles was accompanied by enhanced androgen secretion. It was concluded that in ovine follicles aromatizing enzymes were affected by atresia (Moor et al., 1978). Whether this difference in enzymatic steps associated with atresia is due to species difference or to the stage of follicular development at which atresia was studied remains to be determined.

Follicles explanted from rats up to 24 h after hypophysectomy responded to LH with increased progesterone production. Similarly atretic follicles explanted after 4 days of pentobarbital treatment showed marked stimulation of progesterone accumulation by LH, suggesting that these follicles had not lost their responsiveness to LH (Braw and Tsafriri, 1980a). However, follicles explanted 48 h after hypophysectomy did not show an increase in steroidogenesis when LH was added to the medium. It appears therefore that follicles lose their responsiveness to gonadotropins at only later stages of atresia. Uilenbroek et al. (1980) examined the changes in LH and FSH binding to granulosa cells of atretic follicles resulting from repeated administration of pentobarbitone, and found that gonadotropin binding remained high during the first 2 days of treatment. By contrast, production of estrogen and androgen was significantly reduced after only 1 day of treatment. Similarly, Carson et al. (1979) reported decreased gonadotropin binding by ovine granulosa cells expressed from follicles at advanced stage of atresia but not from follicles in early atresia. These direct measurements of gonadotropin binding to granulosa cells appear to support the notion that loss of gonadotropin binding is the result, rather than the cause of follicular atresia.

Androgens supplied exogenously (Hillier and Ross, 1979), or induced locally by LH (or hCG) in thecal or interstitial cells (Louvet et al., 1975) may act on the follicle to bring about atresia. The finding that androgen synergizes with FSH to stimulate progesterone formation in granulosa cells (Armstrong and Dorrington, 1976; Nimrod and Lindner, 1976; Hillier et al., 1980b) is somewhat surprising in view of the proposed atretogenic action of androgens. Furthermore, a role of androgen in the maintenance of preovulatory follicles was demonstrated by Peluso et al. (1979, 1980b). Hillier et al. (1980a) speculated that prior exposure of granulosa cells to FSH may be the major factor which determines follicular response to androgen. Thus, when exposure to FSH has been inadequate, androgen may provoke an atretic response while appropriate stimulation with FSH may facilitate androgen-responsive steroidogenesis. Data demonstrating that progesterone synthesis is augmented in atretic follicles (Terranova, 1980; Braw and Tsafriri, 1980a; present results) indicate that enhanced steroidogenesis and atresia should not be considered mutually exclusive. It is possible that a similar mechanism(s) underlies these apparently disparate actions of androgens. Androgens may promote atresia by inducing premature differentiation and quasiluteinization of granulosa cells while abrogating the estrogen-mediated proliferative response to FSH. If this is true, the ultimate result of exposure to androgen (i.e., atresia or further development and ovulation) may very well depend upon previous exposure of granulosa cells to FSH.

ACKNOWLEDGMENTS

We thank the NIAMDD for generous supply of gonadotropin preparations; Mrs. A. Tsafriri for skillful technical assistance; and Mr. S. Yosef for devoted care of the animals. We are indebted to Drs. D. J. Dierschke and A. Nimrod for their constructive criticism during the preparation of the manuscript. The

contribution of R.H.B. constitutes part of the requirements for the Ph.D. degree of the Feinberg Graduate School of the Weizmann Institute of Science. The work was generously supported by the Ford Foundation and the Rockefeller Foundation.

REFERENCES

Armstrong, D. T. and Dorrington, J. H. (1976). Androgens augment FSH-induced progesterone secretion by cultured rat granulosa cells. Endocrinology 99, 1411–1414.

Ashiru, O. A. and Blake, C. A. (1978). Restoration of the periovulatory follicle-stimulating hormone surges in sera by luteinizing hormone releasing hormone in phenobarbital-blocked rats. Life Sci. 23, 1507–1514.

Bauminger, S., Kohen, F. and Lindner, H. R. (1974). Steroids as haptens: Optimal design of antigens for the formation of antibodies to steroid hormones. J. Steroid Biochem. 5, 739–747.

Braw, R. H. and Tsafriri, A. (1980a). Follicles explanted from pentobarbitone-treated rats provide a model for atresia. J. Reprod. Fertil. 59, 259–265.

Braw, R. H. and Tsafriri, A. (1980b). Effect of PMSG on follicular atresia in the immature rat ovary. J. Reprod. Fertil. 59, 267–272.

Byskov, A.G.S. (1974). Cell kinetic studies of follicular atresia in the mouse ovary. J. Reprod. Fertil. 37, 277–285.

Carson, R. S., Findlay, J. K., Burger, H. G. and Trounson, A. O. (1979). Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. Biol. Reprod. 21, 75–87.

Daane, T. A. and Parlow, A. I. (1971). Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle: Effect of pentobarbital. Endocrinology 88, 653–663.

Greenwald, G. S. (1978). Ovulatory response of hypophysectomized hamsters to deferred injection of LH. J. Reprod. Fertil. 54, 323–324.

Hillier, S. G. and Ross, G. T. (1979). Effect of exogenous testosterone on ovarian weight, follicular morphology and intraovarian progesterone concentration in estrogen-primed hypophysectomized immature female rats. Biol. Reprod. 20, 261–268.

Hillier, S. G., van den Boogaard, A.M.J., Reichert, L. E., Jr. and van Hall, E. V. (1980a). Alteration in granulosa cell aromatase activity accompanying preovulatory follicular development in the rat ovary with evidence that 5α-reduced C_{19} steroids inhibit the aromatase reaction in vitro. J. Endocrinol. 84, 409–419.

Hillier, S. G., Zeleznik, A. J., Knazek, R. A. and Ross, G. T. (1980b). Hormonal regulation of preovulatory follicle maturation in the rat. J. Reprod. Fertil. 60, 219–229.

Kenney, R. M., Condon, W., Ganjam, V. K. and Channing, C. (1979). Morphological and biochemical correlates of equine ovarian follicles as a function of their state of viability or atresia. J. Reprod. Fertil. Suppl. 27, 163–171.

Lieberman, M. E., Barnea, A., Bauminger, S., Tsafriri, A., Collins, W. P. and Lindner, H. R. (1975). LH effect on the pattern of steroidogenesis in cultured Graafian follicles of the rat: Dependence on macromolecular synthesis. Endocrinology 96, 1533–1542.

Louvet, J.-P., Harman, S. M., Schreiber, J. R. and Ross, G. T. (1975). Evidence for a role of androgens in follicular maturation. Endocrinology 97, 366–372.

Moor, R. M., Hay, M. F., Dott, H. M. and Cran, D. G. (1978). Macroscopic identification and steroidogenic function of atretic follicles in sheep. J. Endocrinol. 77, 309–318.

Nimrod, A. and Lindner, H. R. (1976). A synergistic effect of androgen on the stimulation of progesterone secretion by FSH in cultured rat granulosa cells. Mol. Cell. Endocr. 5, 315–320.

Peluso, J. J., Brown, I. and Steger, R. W. (1979). Effect of cyproterone acetate, a potent antiandrogen, on the preovulatory follicle. Biol. Reprod. 21, 929–936.

Peluso, J. J., England-Charlesworth, C., Bolender, D. L. and Steger, R. W. (1980a). Ultrastructural alterations associated with the initiation of follicular atresia. Cell Tissue Res. 211, 105–115.

Peluso, J. J., Stude, D. and Steger, R. W. (1980b). Role of androgens in hCG-induced ovulation in PMSG-primed immature rats. Acta Endocrinol. 93, 505–512.

Peters, H. (1979). Some aspects of early follicular development. In: Ovarian Follicular Development and Function. (A. R. Midgley and W. A. Sadler, eds.). Raven Press, New York. pp. 1–13.

Talbert, G. B., Meyer, R. K. and McShan, W. H. (1951). Effect of hypophysectomy at the beginning of proestrus on maturing follicles in the ovary of the rat. Endocrinology 49, 687–694.

Taya, K. and Greenwald, G. S. (1980). *In vitro* and *in vivo* ovarian steroidogenesis in the long term hypophysectomized hamster. Endocrinology 106, 1093–1098.

Terranova, P. F. (1980). Effect of phenobarbital-induced ovulatory delay on the follicular population and serum levels of steroids and gonadotropins in the hamster: A model for atresia. Biol. Reprod. 23, 92–99.

Tsafriri, A., Lindner, H. R., Zor, U. and Lamprecht, S. A. (1972). *In vitro* induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. J. Reprod. Fertil, 31, 39–50.

Uilenbroek, J. Th. J., Woutersen, P.J.A. and van der Schoot, P. (1980). Atresia of preovulatory follicles: Gonadotropin binding and steroidogenic activity. Biol. Reprod. 23, 219–229.

Zarrow, M. X., Yochim, J. M. and McCarthy, J. L. (1964). Hypophysectomy, intra-aural method. In: Experimental Endocrinology. A Sourcebook of Basic Techniques. Academic Press, New York and London, pp. 310–312.