Prolactin Stimulation of Testicular Steroid Biosynthesis in the Male Rat

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The effect of prolactin on testicular steroidogenesis was studied in intact adult male rats and in animals treated for 12 days with the LHRH-agonist [D-Ala^6, des-Gly-NH$_2$^{10}]LHRH ethylamide (LHRH-agonist, 1 μg every third day). Testicular LH and prolactin receptors are decreased to 20 and 50% of control, respectively, 26 hours after the last injection of LHRH-agonist. This loss of testicular receptors is accompanied by an increase in the concentration of testicular pregnenolone (500%) and progesterone (700%), whereas 17-OH-progesterone, androstenedione, testosterone, and dihydrotestosterone are decreased to 50, 25, 10, and 60% of control levels, respectively. The injection of 2 mg of ovine prolactin in intact rats 2 hours before sacrifice leads to an increase in testicular 17-OH-progesterone (300%) and androgen (100%) levels. However, in animals treated with the LHRH-agonist, prolactin injection leads to an increase in only progesterone and pregnenolone levels, whereas the concentration of the other steroids remains low. The present data indicate that the stimulatory effect of prolactin at an early stage(s) of the testicular steroidogenic pathway remains relatively intact in the desensitized testis and leads to an apparent accentuation of the LHRH-agonist-induced enzymatic blockage at the level of 17-hydroxylase and 17,20-desmolase activities.

Key words: prolactin, LHRH, 17-hydroxylase, 17,20-desmolase, desensitization, steroidogenesis, LH receptor, prolactin receptor.

Specific binding of prolactin has been demonstrated in Leydig cells (Aragona and Friesen, 1975; Charreau et al, 1977; Costlow and McGuire, 1977). Moreover, many observations indicate that prolactin could play an important role in the control of testicular steroidogenesis. Prolactin increases testicular LH binding in dwarf mice (Bohnet and Friesen, 1976), in light-deprived hypogonadal hamsters (Bex and Bartke, 1977), as well as in hypophysectomized immature (Zipf et al, 1978) or adult (McNeilly et al, 1979) rats. There is also good evidence to indicate that prolactin increases the effects of LH on steroidogenesis and spermatogenesis in hypophysectomized rats and mice (Bartke et al, 1977; Hafiez et al, 1971; Hafiez et al, 1972; Bartke et al, 1977). It has also been suggested that prolactin influences testicular function in the golden hamster by regulating the seasonal cycles of regression and recrudescence of the testis (Bartke et al, 1978).

Recently, we have found that testicular and plasma concentrations of progesterone increase following the administration of an LHRH-agonist to adult male rats. The levels of progestins are further increased in animals having high circulating prolactin concentrations induced by pituitary implants (Bélanger et al, 1979). This effect

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of pituitary transplants was completely prevented by the administration of 2α-bromoergocryptine, a dopamine-agonist. In order to gain a better understanding of the role of prolactin in testicular steroidogenesis, we have studied the acute effect of prolactin on steroidogenesis as reflected by the levels of pregnenolone, progesterone, 17-OH-progesterone, androstenedione, testosterone, and dihydrotestosterone in control adult rats as well as in animals treated with the LHRH-agonist [D-Ala^6, des-Gly-NH$_2^{10}$]LHRH ethylamide.

Materials and Methods

Animals

Adult male Sprague-Dawley rats weighing 250 to 300 g each upon arrival were obtained from Canadian Breeding Farms, St. Constant, Quebec. Animals were housed two per cage in a temperature-controlled (20–22 C) and light-controlled (14 hours light–10 hours darkness, lights on at 05:00 hours) room and given food and water *ad libitum*.

Hormones

Purified hCG (CR119, 11,600 IU/mg) was generously supplied by the Center for Population Research of the NICHHD, NIH. Human FSH (hFSH) (LER 1801-3, 4019 IU/mg) and ovine prolactin (NIH-P-S12, 35 IU/mg) were gifts of the National Pituitary Agency, NIH. As measured by radioreceptor assay (Auclair et al, 1977), the contamination of the ovine prolactin preparation by ovine LH is less than 0.01% (Fig. 1). Highly purified ovine LH (SR 1397-A), kindly provided by Dr. M. R. Sairam, Montreal, was used as standard. [D-Ala^6, des-Gly-NH$_2^{10}$]LHRH ethylamide was generously supplied by Drs. R. Deghenghi and M. Götz, Ayerst Research Laboratories, Montreal.

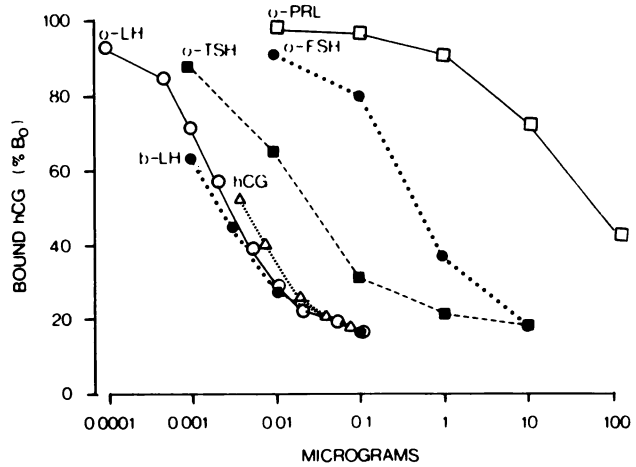

Fig. 1. Binding inhibition curves for peptide hormones incubated with testis homogenate and ^{125}I-hCG for 16 hours at 25 C.

Treatment

Animals (eight per group) were injected once every third day at 08:00 to 09:00 hours for 12 days with a 1-μg dose of [D-Ala^6, des-Gly-NH$_2^{10}$]LHRH ethylamide (LHRH-agonist) or of the vehicle alone (1% gelatin in 0.15 M NaCl). Two milligrams of ovine prolactin was administered 24 hours after the last injection of LHRH-agonist, and the animals were sacrificed 2 hours or 24 hours later. The testes were immediately removed; one was used for measurement of steroid hormone content and the other was processed for LH, FSH, and prolactin receptor assays. Plasma was collected for steroid and pituitary hormone measurements.

LH, FSH, and Prolactin Receptor Assays

Decapsulated testes were homogenized individually in 10 ml of Tris-BSA buffer (0.1 M Tris-HCl, pH 7.4, 0.1% bovine serum albumin, 5 mM $MgCl_2$) containing 0.25 M sucrose, using a Sorvall homogenizer at a speed setting of 10 for 15 seconds. The homogenate was centrifuged at 20,000 *g* for 15 minutes and the pellet resuspended in 10 ml of buffer and recentrifuged. The pellet then was resuspended (using a glass-Teflon homogenizer) in 3 ml of Tris-BSA buffer, and appropriate dilutions were made so that each assay tube received 10-mg equivalents of tissue (wet weight).

Low concentrations of chloramine-T were used to iodinate the hormones (600–800 ng of chloramine-T and 5 μg of peptide). As calculated by isotope recovery, the specific activities were 24.1 (hCG), 22.3 (hFSH), and 70.0 (ovine prolactin) μCi/μg. Hormone binding was measured by duplicate incubation for 14 to 16 hours at 23 C of 100 μl of testicular suspension, 200 μl of buffer, 100 μl of [^{125}I]-labeled hormone (a saturating quantity equivalent to approximately 100,000 to 150,000 cpm representing 83, 99, and 50 fmol, for hCG, hFSH, and ovine prolactin, respectively) in the presence or absence of 100 μl of unlabeled hormone (2 μg of oLH or oFSH and 1 μg of ovine prolactin). The reaction was stopped by the addition of 3 ml of cold Tris-BSA buffer, and the tubes were centrifuged at 2300 *g* for 15 minutes for measurement of bound radioactivity. Specific binding was expressed as femtomoles of bound hormone (means ± SEM).

Steroid Assays

Plasma and testicular steroids were first extracted with benzene and methanol, respectively, before separation on LH-20 columns, and were measured as previously described (Bélanger et al, 1980a).

Calculations

Radioimmunoassay and radioreceptor data were analyzed using a program based on Model II of Rodbard and Lewald (1970; Drouin and Labrie, 1976). Statistical significance was calculated according to the multiple-range test of Duncan-Kramer (Kramer, 1956). All radioimmunoassay and radioreceptor assay data are presented as means ± SEM of triplicate determinations.

Results

As illustrated in Fig. 2, treatment with LHRH-agonist (1 μg every third day) leads to an 80% decrease in testicular LH receptor levels, whereas prolactin receptors are decreased by 50%. No significant effect is observed on the concentration of FSH receptors. Single administration of 2 mg of ovine prolactin 24 hours after the last injection of LHRH-agonist has no effect on the concentrations of LH, FSH, or prolactin receptors 2 or 24 hours later, except for a decrease in prolactin receptors at the 2-hour time interval. This apparent decrease is probably due to the presence of unlabeled ovine prolactin on the prolactin receptors, which prevents binding of the iodinated hormone.

Administration of LHRH-agonist once every third day causes a marked increase in testicular pregnenolone and progesterone levels, from 12 ± 1 and 6 ± 0.5 to 62 ± 5 and 42 ± 6 ng/g testis, respectively (Fig. 3). Although the slight stimulatory effect of prolactin is not significant in control animals, the injection of prolactin in LHRH-agonist-treated rats produces an increase, 2 hours later, in the testicular concentration of these two progestins ($P < 0.01$). The stimulatory effect of prolactin on pregnenolone and progesterone levels remains significant 24 hours after injection of the hormone.

Treatment with the LHRH-agonist lowers 17-OH-progesterone levels by 50%, and no further change is observed after injection of prolactin. However, an approximately three-fold increase in testicular 17-OH-progesterone levels is observed 2 hours after injection of prolactin in control animals. The marked reduction of basal as well as prolactin-induced testicular levels of 17-OH-

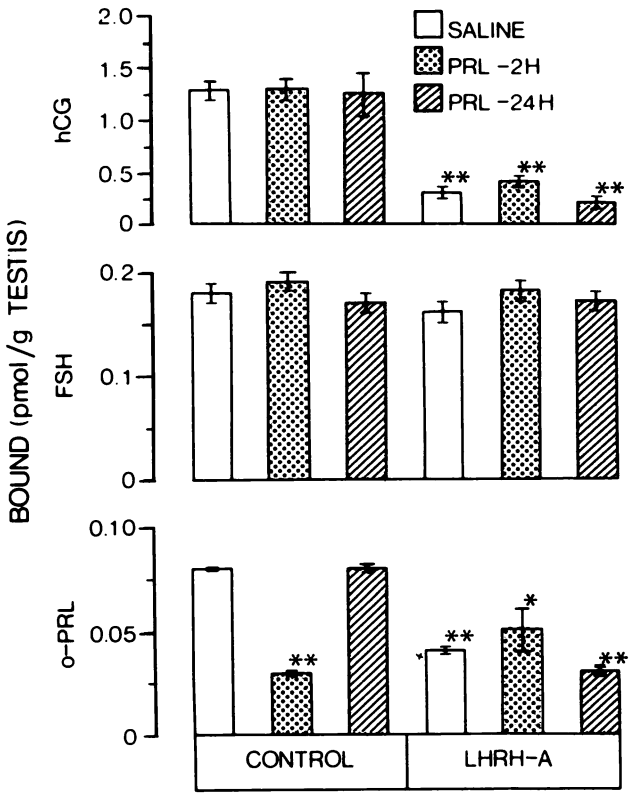

Fig. 2. Effect of single injection of 2 mg of ovine prolactin (NIH-P-S12) on LH/hCG, FSH, and prolactin receptor levels in normal rats or animals previously treated for 12 days with [D-Ala^6, des-Gly-NH$_2$^{10}]LHRH ethylamide (1 μg every third day). Animals were sacrificed 2 hours or 24 hours after injection of ovine prolactin. The values are presented as means ± SEM of values obtained in eight rats. ** = $P < 0.01$; * = $P < 0.05$ as compared with saline-treated control.

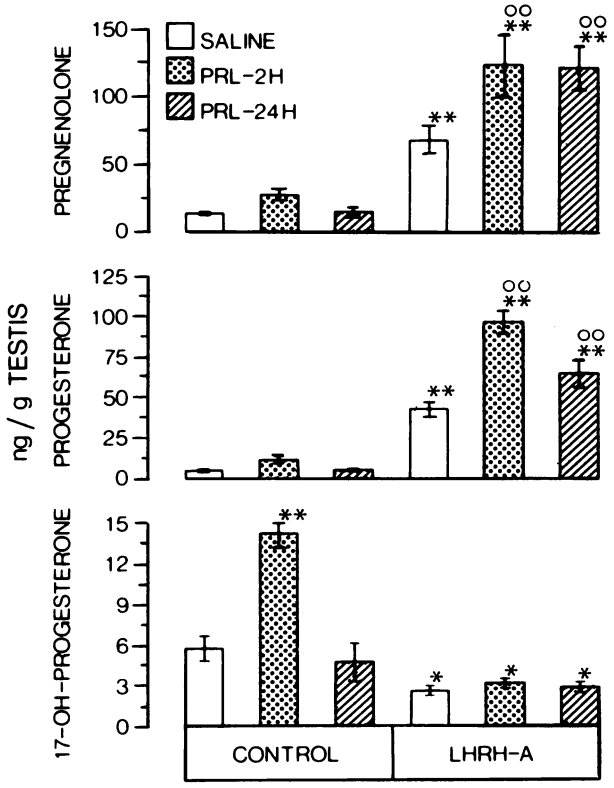

Fig. 3. Effect of single injection of 2 mg of ovine prolactin on testicular pregnenolone, progesterone, and 17-OH-progesterone levels in normal rats or animals previously treated for 12 days with [D-Ala^6, des-Gly-NH$_2$^{10}]LHRH ethylamide (1 μg every third day). Animals were sacrificed 2 hours or 24 hours after single injection of ovine prolactin. The values are presented as means ± SEM of values obtained in eight rats. ** = $P < 0.01$, * = $P < 0.05$ as compared with saline-treated controls. oo = $P < 0.01$ as compared with LHRH-agonist alone.

progesterone clearly indicates a blockage of the steroidogenic pathway at the 17-hydroxylase step, following treatment with LHRH-agonist.

As shown in Fig. 4, the administration of prolactin in control animals causes a two-fold increase in testicular androstenedione, testosterone, and dihydrotestosterone levels after 2 hours, whereas no effect of prolactin can be detected after 24 hours. As observed for 17-OH-progesterone, administration of the LHRH-analogue causes an inhibition of testicular androgen levels, and, furthermore, injection of 2 mg of prolactin has no effect on these low androgen levels. Although the levels of progestins and androgens are affected by treatment with the LHRH-agonist, no effect of LHRH-agonist treatment could be detected on the levels of testicular estradiol and estrone (data not shown).

It can be seen in Fig. 5 that the stimulatory effects of LHRH-agonist treatment on testicular pregnenolone and progesterone levels (Fig. 3) are also reflected in the plasma concentration of these steroids. In fact, a four- to six-fold increase in basal plasma pregnenolone and progesterone is observed 26 hours after the last injection of LHRH-agonist. Moreover, it can be seen in Fig. 5 that a marked stimulation of the plasma levels of these two progestins is found 2 and 24 hours after injection of prolactin in LHRH-agonist-treated animals. Whereas the stimulatory effect of prolactin on plasma pregnenolone levels is observed in the controls, the stimulation of progesterone is not significant.

As with the effects of prolactin on testicular levels of testosterone and dihydrotestosterone, plasma concentrations of testosterone and dihydrotestosterone are significantly increased 2 hours after prolactin injection in controls, whereas the low levels of circulating androgen in animals

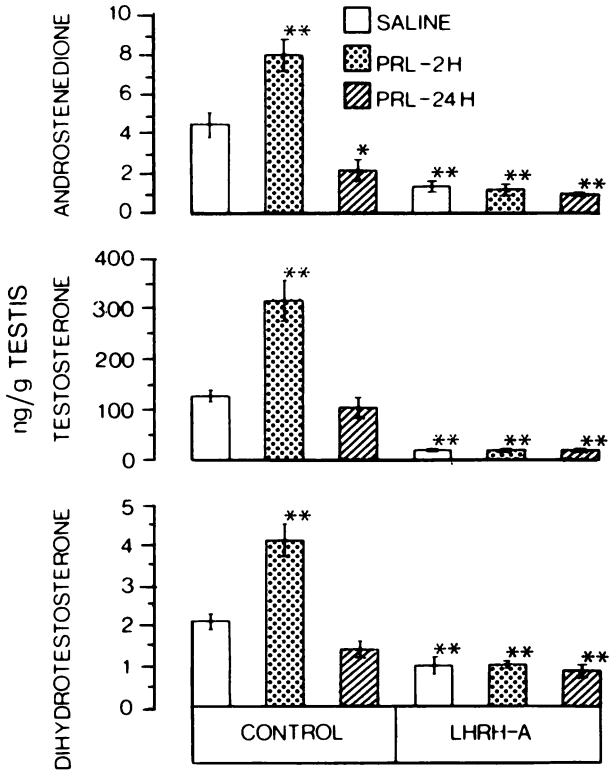

Fig. 4. Effect of single injection of 2 mg of ovine prolactin on testicular androstenedione, testosterone, and dihydrotestosterone levels in normal rats or animals previously treated for 12 days with [D-Ala^6, des-Gly-NH_2^{10}]-LHRH ethylamide (1 μg every third day). Animals were sacrificed 2 hours or 24 hours after single injection of ovine prolactin. The values are presented as means ± SEM of groups of eight rats. ** = $P < 0.01$ or * = $P < 0.05$ as compared with saline-treated controls.

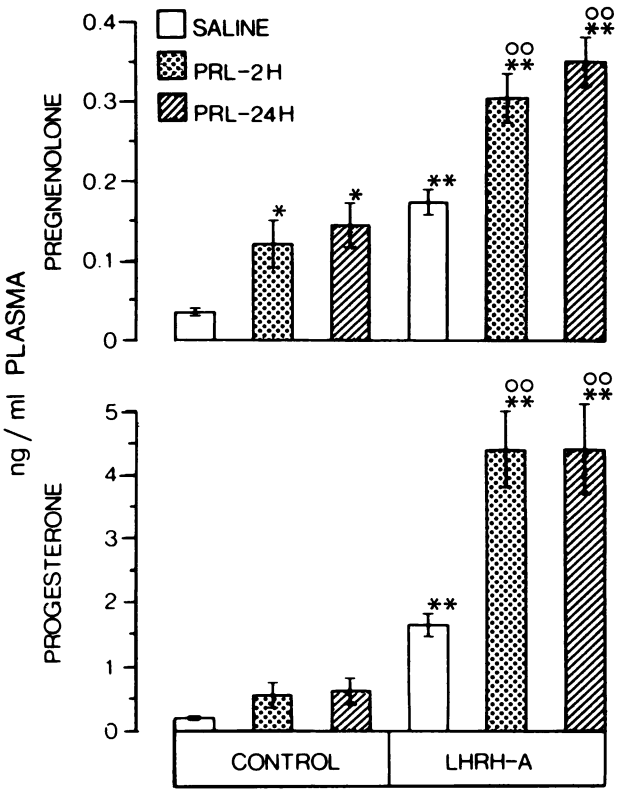

Fig. 5. Effect of single injection of 2 mg of ovine prolactin on plasma pregnenolone and progesterone levels in normal rats or animals previously treated for 12 days with [D-Ala^6, des-Gly-NH_2^{10}]LHRH ethylamide (1 μg every third day). Animals were sacrificed 2 hours or 24 hours after single injection of ovine prolactin. The values are presented as means ± SEM of values obtained in eight rats. ** = $P < 0.01$, * = $P < 0.05$ as compared with saline-treated controls. oo = $P < 0.01$ as compared with LHRH-agonist alone.

treated with the LHRH-agonist are not affected (Fig. 6).

Discussion

The present data clearly show that prolactin has an acute stimulatory effect on testicular steroidogenesis. This stimulatory effect of prolactin is reflected by increased testicular and plasma levels of 17-OH-progesterone and androgen in intact adult male rats. In LHRH-agonist-treated animals, the concentrations of testicular progesterone and pregnenolone are markedly increased in the presence of low 17-OH-progesterone and androgen levels, thus indicating a blockage at the 17-hydroxylase enzymatic step. A similar effect on progesterone levels has already been observed in animals having high circulating prolactin concentrations induced by pituitary implants and treated with an LHRH-agonist (Bélanger et al, 1979). It is well known that treatment of hypophysectomized rats with a combination of prolactin and LH leads to higher plasma testosterone levels than treatment with LH alone (Purvis and Hansson, 1978).

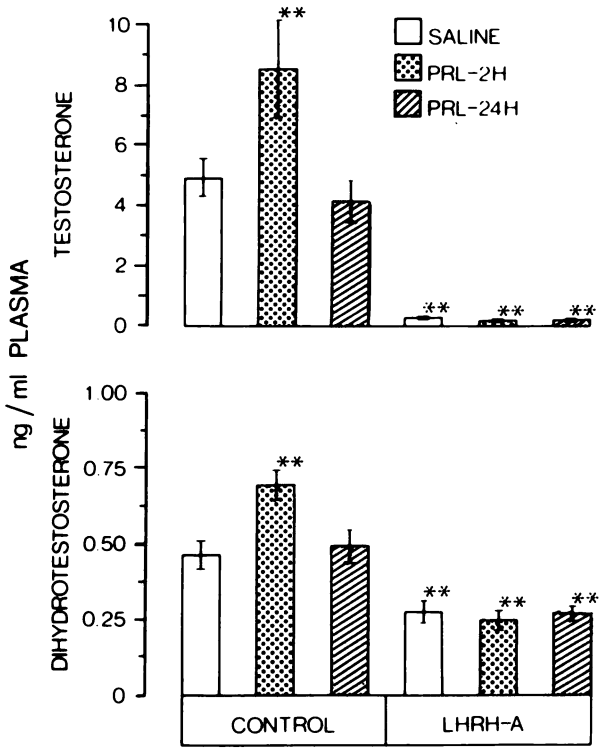

Fig. 6. Effect of single injection of 2 mg of ovine prolactin on plasma testosterone and dihydrotestosterone levels in normal rats or animals previously treated for 12 days with [D-Ala^6, des-Gly-NH$_2^{10}$]LHRH ethylamide (1 μg every third day). Animals were sacrificed 2 hours or 24 hours after single injection of ovine prolactin. The values are presented as means ± SEM of values obtained in eight rats. ** = $P < 0.01$ as compared with saline-treated control.

The present report is the first to describe a stimulatory effect of prolactin on testicular steroidogenesis. Although these results could be explained by contamination by LH of the prolactin preparation used, the LH activity determined by LH radioreceptor assay is below the level expected to exert any significant effect on steroidogenesis. Data reflected in Fig. 1 indicate the presence of 0.2 μg of oLH in 2 mg of the injected prolactin, although 2 μg of oLH are required to induce a significant increase of plasma steroid levels (Bélanger, Séguin, and Ferland, unpublished observation). A previous report (Smith and Hafs, 1973) did not show a stimulatory effect of prolactin injected in rabbits at a much lower dose (100 μg).

In the present study, no change in testicular LH receptors occurred 2 or 24 hours after the injection of prolactin, even though it is well known that prolactin increases testicular LH receptor levels (Bohnet and Friesen, 1976; Bex and Bartke, 1977; Bex et al, 1978; Zipf et al, 1978; Purvis and Hansson, 1978) and can prevent the loss of homologous receptors after injection of LH (Purvis and Hansson, 1978). The discrepancy between changes in steroid production and in LH binding suggests that the stimulatory effect of prolactin on steroidogenesis can be independent of changes in LH receptor levels. Although previous observations have shown that chronic treatment with LHRH-agonists decreases plasma prolactin levels (Sandow et al, 1978; Belanger et al, 1979; Rivier et al, 1979), the present data show that prolactin cannot reverse the inhibitory effects of LHRH-agonist treatment on testicular steroidogenesis. Such findings indicate that decreased plasma prolactin levels are unlikely to play a major role in the inhibition of testicular steroid biosynthesis induced by LHRH-agonist treatment in male rats.

The previous observations of the ability of prolactin to stimulate testicular 3β- and 17β-hydroxy steroid dehydrogenase (Hafiez et al, 1971; Musto et al, 1972) and to increase accumulation of esterified cholesterol in the testis (Bartke, 1971b) may offer an explanation for this acute stimulation of testicular steroidogenesis. In the evaluation of the effects of prolactin on testicular steroidogenesis, it should be mentioned that high levels of circulating prolactin in the human inhibit

androgen biosynthesis (Boyar et al, 1974), whereas, in the rat, transplantation of prolactin-secreting tumors can induce testicular atrophy (Fang et al, 1974). It is thus apparent that the effect of prolactin on testicular steroidogenesis is markedly dependent upon time and concentration factors. Moreover, the physiologic importance of the present findings obtained with a high dose of prolactin remains to be assessed.

The present study confirms and extends our previous observation of an increase in testicular pregnenolone and progesterone levels in animals treated with LHRH-agonist (Bélanger et al, 1980b). It is, in fact, apparent from these data that LHRH-agonist treatment leads to a preferential blockage of the testicular steroidogenic pathway between progesterone and 17-OH-progesterone. Depending upon the dose of the LHRH-agonist and the schedule of administration, we have observed that both 17-hydroxylase and 17,20-desmolase activities can be inhibited. This LHRH-agonist-induced inhibition of the steroidogenic pathway is in agreement with the data of Chasalow (1979) and Chasalow et al (1979) obtained after treatment with hCG. Although changes in testicular androgen levels could also be involved, investigations are in progress to assess the possible role of high levels of progesterone in the marked degenerative changes of the seminiferous tubules and cessation of spermatogenesis observed in animals chronically treated with LHRH-agonists (Pelletier et al, 1978; Labrie et al, 1978).

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