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# Temporal Augmentation of LH by Prolactin in Stimulation of Androgen Production by the Testes of Hypophysectomized Male Rats[1] (37860)

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Diurnal variations in the serum levels of all of the hypophysial gonadotropins have been demonstrated for the rat (1, 2). In the male, elevations in the serum concentrations of prolactin and luteinizing hormone (LH, ICSH) appear to occur in the first half of each dark period when the animals are exposed to 14 hr light/day (3). The highest concentrations of serum follicle-stimulating hormone (FSH) were found 1 hr before the dark period began (2). There are no reports concerning the effects of light periods on the diurnal rhythms. The changes in circadian rhythms induced by light for other pituitary hormones, such as ACTH, suggest that it probably also affects periods of gonadotropin output.

Rhythmic fluctuations in physiologic functions and their sensitivity to a variety of agents are well-known (4, 5). However, little concern has been given to periodic changes in hormonal effects. The importance of changes in response was emphasized by the experiments of Meier and his associates (references in 6). They showed that the time relationship between the administration of corticosterone and prolactin had profound effects upon such diverse functions as testicular growth in frogs and lipogenesis in pigeons. Furthermore, the changes were not purely quantitative because some combinations stimulated lipogenesis while others appeared to produce lipolysis in the birds. If this kind of response is a general biological phenomenon applicable to many systems, it certainly has significant implications for the understanding and the control of cyclic changes in reproductive functions.

The production of androgen by the testes of hypophysectomized male rats is stimulated by LH. Prolactin does not have this effect, but it does enhance very significantly the action of LH (7). This combination of hormones appeared to be a suitable model for investigating the possible effects of altering the time of tropic stimulation upon the androgen output of the testes.

*Materials and Methods.* Male rats of the Holtzman strain were used. They were kept in air-conditioned quarters with free access to Purina laboratory chow and tap water. The lights were on from 0600 until 2000 daily. After 7 days in this light schedule (animals 30 days old), they were hypophysectomized by the parapharyngeal route using ether anesthesia. This operation was done on groups of about 40 rats between 0900 and 1000. A solution of 5% glucose was substituted for the drinking water after hypophysectomy. The next day the animals were randomly placed into treatment groups and injected subcutaneously daily for 5 days with 25 μg of ovine LH (NIH-LH-S-15) and/or 500 μg of ovine prolactin (NIH-P-9). These materials were injected at different sites. The LH was dissolved in normal saline, but the prolactin was a suspension in saline. At autopsy on the 6th day, the body weight was recorded and the sella of each animal examined with the aid of an eye loop; all animals with pituitary frag-

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ments were discarded without further dissection. The sex accessory organs (prostates and seminal vesicles) were dissected *in toto* and weighed, as were the testes. Data were statistically analyzed using Student's $t$ test or Duncan's new multiple range test (8).

*Results and Discussion.* The dose of LH used stimulated the production of sufficient androgen by the testes to maintain the sex accessory organ weights about 25% above those of controls injected with saline only (Table I). Prolactin alone was without effect on accessory weights, but when combined with LH, the average weight of the accessories was about double that of saline controls and significantly ($P < 0.01$) above that found in animals treated only with LH. In addition, the accessory weight obtained with the combination of hormones was related to the times they were administered. In series A (Table I), the least effective schedule for the combination was at 0900. Injection of prolactin at 2100 and LH at 0900 or 1500 was slightly, but not significantly, more effective. The largest accessories were found in the group treated with both hormones at 2100; in this group, they were 44% ($P < 0.01$) above those of the 0900 treatment group. In series B, the combination of hormones was given either at 1000, 1500, or 2200. As with series A groups, treatment in the dark period was more effective than when it was given in the light period (Table I). Sex accessory organ weights for animals treated at 2200 were about 18% ($P < 0.05$) above those of animals treated at 1000.

Testicular weights for the males of the two series were significantly different from each other and therefore cannot be compared. In series A, testes of males treated with LH plus prolactin at 2100 were not different in weight from those of males injected with LH alone at 0900 or LH at 0900 and prolactin at 1500. However, when prolactin plus LH was given at 0900, or LH at 1500 and prolactin at 2100, testes weights were significantly lower ($P < 0.05$) than those of animals treated with LH alone at 0900 or the combination of hormones at 2100; the difference in weight has the same statistical significance whether calculated as relative or absolute weight. In series B, the males injected with both LH and prolactin at 1000 or 1500 had significantly ($P < .05$) smaller testes than those of animals treated with LH alone at 2200; the combination of hormones given at 2200 did not produce smaller testes, however.

The greater effect of injections in the dark

TABLE I. The Effect of LH and Prolactin on Testicular and Sex Accessory Organ Weight in Hypophysectomized Male Rats.[a]

| Series | Time of injection | | No. of rats | Body weight (g) | Testes (mg/100 g body wt) | Sex accessories (mg/100 g body wt) |
|---|---|---|---|---|---|---|
| | LH | PROL | | | | |
| A | — | — | 4 | 101 ± 3[b] | 632 ± 53 | 66.5 ± 3.7 |
| A | 0900 | — | 4 | 102 ± 3 | 732 ± 45[2,3] | 85.6 ± 5.6 |
| B | 2200 | — | 5 | 110 ± 5 | 923 ± 19[d] | 92.3 ± 2.8 |
| A | — | 0900 | 4 | 103 ± 3 | 475 ± 12 | 66.1 ± 1.1 |
| B | — | 2200 | 7 | 112 ± 4 | 592 ± 48 | 63.9 ± 3.2 |
| A | 0900 | 0900 | 6 | 100 ± 5 | 604 ± 27[1] | 116.3 ± 6.3[1] |
| B | 1000 | 1000 | 8 | 112 ± 5 | 802 ± 13[c] | 122.8 ± 7.0[c] |
| A | 0900 | 2100 | 5 | 103 ± 4 | 807 ± 40[3] | 130.6 ± 8.8[1] |
| A | 1500 | 2100 | 6 | 100 ± 2 | 693 ± 39[1,2] | 136.9 ± 8.9[1] |
| B | 1500 | 1500 | 8 | 109 ± 3 | 807 ± 33[c] | 135.7 ± 8.6[c] |
| A | 2100 | 2100 | 9 | 101 ± 4 | 783 ± 38[3] | 167.7 ± 6.8 |
| B | 2200 | 2200 | 8 | 108 ± 3 | 849 ± 56[c,d] | 144.9 ± 6.0 |

[a] 25 μg of LH-S-15 and 500 μg of prolactin (S-9) were dissolved in saline and given at the times indicated each day for 5 days. Sex accessory weights include prostatic tissue, seminal vesicles, and the prostatic urethra. Numbers with the same superscript are not significantly different from each other ($P < 0.05$) by Duncan's new multiple range test.

[b] ± SE.

TABLE II. The Effect of LH and Prolactin on Testicular and Sex Accessory Organ Weights in Hypophysectomized Male Rats After Altering the Lighting Schedule.[a]

| Time of injection | | No. of rats | Body weight (g) | Testes (mg/100 g body wt) | Sex accessories (mg/100 g body wt) |
|---|---|---|---|---|---|
| LH | Prolactin | | | | |
| 1300 | 1300 | 7 | 97.8 ± 2 | 768 ± 35 | 151.7 ± 8.5 |
| 1700 | 1300 | 6 | 88.7 ± 3 | 701 ± 51 | 136.8 ± 6.3[2] |
| 2100 | 1300 | 7 | 88.7 ± 2 | 695 ± 66 | 123.0 ± 6.1[1,2] |
| 2100 | 2100 | 5 | 84.4 ± 4 | 638 ± 105 | 128.9 ± 9.8[1,2] |
| 0900 | 1300 | 4 | 95.5 ± 3 | 662 ± 85 | 119.0 ± 5.1[1] |

[a] Animals were placed in 14 hr of light/day (2200–1200) 8 days prior to hypophysectomy. LH (25 μg) and prolactin (500 μg) were given once daily for 5 days at times indicated. Numbers with the same superscript are not statistically different ($P < 0.05$) from each other using Duncan's new multiple range test.

period suggested the possibility of a light-controlled rhythm. In order to check this further, five groups of hypophysectomized males were treated with LH plus prolactin at various times of the day after they had been acclimated to a different lighting schedule; 8 days before hypophysectomy and during treatment, the room lights were on between 2200 and 1200. The results are shown in Table II. Testes weights were not different between groups, but the accessories in males injected with both hormones at 1300, i.e., 1 hr into the dark period, were significantly heavier than those of males injected at other times.

The larger accessories in animals treated early in the dark period could be the result of an increased sensitivity of these tissues to androgen or a combination of androgen and prolactin. Prolactin augmentation of androgenic effects upon accessories is well-known and has recently been discussed by Moger and Geschwind (9). Four groups of hypophysectomized males were given either testosterone (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 ml sesame oil or the androgen plus 500 μg of prolactin at two different times. The results are shown in Table III. The dose of testosterone (50 μg) produced sex accessories of about the same weight as did the LH plus prolactin combination in the previous groups (Tables I and II). Prolactin did not, however, augment the effect of testosterone, but caused a slight reduction in accessory weights. When both testosterone and prolactin were given at 0900, relative, but not absolute, testes weights were reduced compared with those of males treated with testosterone alone or even oil-treated controls.

The results do not indicate a rhythm in the response of the sex accessories to androgenic stimulation but rather a rhythm in the sensitivity of the steroidigenic mechanisms

TABLE III. The Effect of Prolactin and Testosterone of the Weights of the Testes and Sex Accessory Organs of Hypophysectomized Male Rats.[a]

| Time of injection | | No. of rats | Body weight (g) | Testes (mg/100 g body wt) | Sex accessories (mg/100 g body wt) |
|---|---|---|---|---|---|
| Testosterone | Prolactin | | | | |
| — | — | 5 | 88.8 ± 2[b] | 640 ± 26[1,3] | 67.8 ± 3.3 |
| 0900 | — | 5 | 84.6 ± 2 | 695 ± 35[1] | 133.4 ± 17.1[1] |
| 2100 | — | 4 | 89.0 ± 2 | 639 ± 40[1,3] | 116.5 ± 4.1[1] |
| 0900 | 0900 | 6 | 101.7 ± 2 | 555 ± 24[2] | 104.4 ± 3.1[2] |
| 2100 | 2100 | 5 | 94.8 ± 4 | 598 ± 27[2,3] | 105.8 ± 4.5[2] |

[a] 50 μg testosterone dissolved in 0.1 ml sesame oil and 500 μg prolactin in saline were given once daily at the times indicated for 5 days. Numbers with the same superscript are not statistically different from each other ($P < 0.05$) using Duncan's new multiple range test.

[b] ± SE.

in the testes to gonadotropic stimulation. What the latter involves is obscure but appears to involve more than simply stimulating testosterone and androstenedione production. Temporal variations in androgen production in response to LH, for example, were not apparent in these studies. This was examined further in two groups of males hypophysectomized at the same time and given LH at 0900 or 2100. No differences in sex accessory weights were found; they weighed 113.6 ± 3.5 mg ($n = 7$) when injected at 0900 and 119.7 ± 6.7 mg ($n = 8$) when injected at 2100. There is the possibility, of course, that qualitative, rather than quantitative, changes are involved in the responses to two gonadotropins. The unstimulated testes of hypophysectomized male rats secrete considerable quantities of androstenedione, but very little testosterone. Prolactin reduces the amount of androstenedione produced but does not affect testosterone (7). The possibility of changing the ratio of these androgens, and probably others that have not been measured, invites investigations into the consequences of altering the periodicity of gonadotropic stimulation.

The testes apparently have an optimal time for LH and prolactin stimulation, but precisely what this time is has not been determined. The present results can only suggest that it is more likely to be early in the dark period rather than early in the light period. Furthermore, the period of sensitivity may be influenced by light since in those animals exposed to an altered light regimen, the largest accessory organs were found in animals given the gonadotropins 1 hr into the dark period as was the case in the animals on the regular light schedule. The sensitivity may of course depend upon many factors including some under pituitary hormone control. The important question raised is whether the period of greatest sensitivity in responsive tissues coincides with the time of maximal output of the tropic hormones which stimulate them. If either the response and/or the output are subject to predictable variations, then quantitative changes in functions are quite understandable and very possibly controllable. The finding of increased sensitivity to LH and prolactin at the time when the serum level of these hormones has been reported to be maximal encourages further study into this area.

*Summary.* Sex accessory organ weight was used as an index of androgen production by the testes of hypophysectomized male rats treated with ovine LH for 5 days. Prolactin augmented the testicular response to LH to a greater extent when both hormones were given simultaneously, but at different sites, early in the dark period than early in the light period. The effect of testosterone upon the accessories was not augmented by prolactin. The results suggest that the sensitivity to tropic hormone stimulation is rhythmic and that gradients in function may have a temporal relationship to the periodicity of tropic hormone release by the pituitary.

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