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Effects of Serotonin on Reproductive Hormone Levels and Testis Morphology in Adult Male Rats (41460)

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Abstract. The effects of subcutaneous implantation of 5-hydroxytryptamine (5-HT, serotonin) on serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), testosterone, 5α-dihydrotestosterone (DHT), and on testicular morphology were investigated in adult male rats. Four weeks after a single implantation of 5-HT (10 mg/kg body weight) a significant increase in serum FSH and a decrease in testes weights were observed. Histological examination of the testes indicated degenerative changes in a few seminiferous tubules. Implantation of 5-HT at two successive 4-week intervals or implantation of 5-HT once followed by methysergide maleate 4 weeks later resulted in reduction of testes size, increase in serum FSH and LH, and extensive degenerative changes in the seminiferous tubules. Implantation of methysergide maleate alone did not have any effect on hormonal profiles, body weight, and testes weight. It is suggested that the serum gonadotropin increase is secondary to the 5-HT effect on testes and is probably attributable to the interference in the feedback mechanism(s) from the testis to the anterior pituitary gland.

The reported effects of excess serotonin (5-HT) on reproductive organs and their function consist of gonadal atrophy (1), ovulation block (2), inhibition of sexual behavior (3, 4) in rats, and inhibition of sexual maturity in mice (5). Serotonin prevents the compensatory increase of testosterone following hemicastration (6) and also inhibits the compensatory ovarian hypertrophy following unilateral ovariectomy (7). Chronic treatment of men of normal sexual potency with tryptophan, a precursor of 5-HT, results in inhibition of libido (8). Excessive daily urinary secretion of 5-HT and 5-hydroxy indoleacetic acid (5-HIAA) were observed in some infertile men with oligospermia, azoospermia, or impaired sperm motility (9). The effect of 5-HT or its precursors on elevating serum prolactin (PRL) level is well documented (10–13). Although the role of 5-HT in the secretion of gonadotropins is not clearly established, stimulation of luteinizing hormone (LH) release in adult male rats (14) as well as inhibition of LH secretion in acutely castrated, but not in chronically castrated, male rats (15) have been reported. The present study was designed to investigate the effect of sc implantation of 5-HT on serum levels of FSH, LH, PRL, testosterone, 5α-DHT, and to determine whether changes in hormone levels correlated with histologic appearance of the testes of adult male rats.

Materials and Methods. *Animals.* Adult male Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) were used in all the experiments. The animals were housed singly, given food and water *ad libitum,* and maintained under controlled lighting (14 hr light, 10 hr darkness, lights on 0600 hr) with temperature at 23°. The subcutaneous implantation of pellets in the neck and orchiectomies were performed under ether anesthesia when body weight reached approximately 300 g.

Preparation of placebo and medicated agarose pellets. Three milliliters of distilled water was added to 300 mg agarose (Marine Colloids, Inc.) heated at 55° over a heater block for 10 min, vortexed, poured into a polyethylene weigh-boat, and allowed to solidify at room temperature. After reaching a firm consistency, the agarose was cut

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into cubes (5 mm). For pellets containing 5-HT, serotonin creatinine sulfate (Sigma Chemical Co.) was dissolved in phosphate-buffered saline (PBS, 0.01 *M* PO_4, 0.14 *M* NaCl, pH 7.5) and added to the agarose slurry which was then allowed to reach a firm consistency. The doses of 5-HT used in this study were based upon the report showing the effect of 5-HT on testes (17). Pellets containing methysergide maleate (10 mg/kg body weight) were prepared following the procedure used for 5-HT.

Experimental procedures. Experiment 1. Two groups of six rats were implanted with either agarose pellets or pellets containing 10 mg 5-HT/kg body weight. Four weeks later the animals were decapitated, trunk blood collected, and testes removed and weighed. Testes were fixed in Bouin's fluid, sectioned, and stained with hematoxylin and eosin. Blood was allowed to clot, serum was separated and stored frozen at −20° until assayed for hormone activity.

Experiment 2. A control group of rats (n = 8) was implanted with agarose pellets and two experimental groups (eight/group) were implanted with pellets containing 5-HT at a dosage of 10 mg/kg body weight. Four weeks later the control group were again implanted with agarose pellets, one of the groups of experimental rats received implants containing 5-HT (20 mg/kg body wt) and the second group was implanted with pellets containing methysergide maleate (10 mg/kg body wt). Following an additional period of 4 weeks, the rats were decapitated and blood and testes processed as described above. A fourth group of rats (n = 8) implanted two times at 4-week intervals with methysergide maleate (10 mg/kg body weight) served as an additional control group.

Experiment 3. At the time of implantation with either placebo or agarose pellets containing 10 mg 5-HT/kg body weight, both control and experimental animals (six/group) were orchiectomized. Four weeks after implantation, rats were decapitated, trunk blood collected, and the serum stored at −20° until assayed for FSH, LH, and PRL.

Assays. The serum samples were assayed for FSH, LH, and PRL according to the procedure outlined by the Rat Pituitary Program of the NIH, Bethesda, Maryland. The values are expressed in terms of NIAMDD-Rat-FSH-RP-1, NIAMDD-Rat-LH-RP-1, NIAMDD-Rat-PRL-RP-1 reference preparations, respectively. Testosterone and DHT were assayed by RIA following celite microcolumn chromatographic purification (16). Briefly, the steroid assay procedure involved ether extraction of the sample followed by partition chromatography of the steroids using a celite microcolumn with ethylene glycol−propylene glycol (50:50) as the stationary phase and a combination of isooctane and ethylacetate as the mobile phase. The fractions were then dried, dissolved in buffer, and assayed for hormone activity by RIA. Small amounts of ^{3}H-labeled steroids (2000 dpm) were added to serum samples prior to ether extraction to calculate recovery values. Reagents used in the steroid assay were: [1,2,6,7-^{3}H]testosterone and [1,2,4,5,6,7-^{3}H]dihydrotestosterone (New England Nuclear); testosterone and 5α-DHT (Steroloids, Inc.); 5α-DHT-1 BSA antiserum (Miles Laboratory, Inc.); and testosterone antiserum (Dr. G. E. Abraham, Harbor General Hospital, Calif.). The intra- and interassay variances, respectively, for various assays expressed as the coefficient of variation are as follows: FSH (4.1, 8.7), LH (6.4, 7.8), PRL (2.4, 2.7), 5α-DHT (4.1, 15.1), and testosterone (4.0, 10.6). The sensitivity estimates (mean ± SE) derived from 100% (buffer control) ±2 SD for the different hormones are: FSH 15.6 ± 2.4 (ng), LH 0.53 ± 0.08 (ng), PRL 0.16 ± 0.01 (ng), 5α-DHT 4.29 ± 1.0 (pg), and testosterone 5.10 ± 1.0 (pg).

Data were analyzed using Student's t test for comparisons between two groups, or by analysis of variance for significance of difference and Newman−Keul's procedure for comparisons involving four groups.

Results. *Experiment 1.* For male rats implanted with 10 mg 5-HT/kg body weight and killed after 4 weeks, the serum FSH level increased by 100% over that of the placebo agarose pellet control group (P <

TABLE I. SERUM LEVELS OF FSH, LH, PRL, T, 5α-DHT, AND TESTES WEIGHT IN ADULT MALE RATS IMPLANTED WITH AGAROSE PELLETS (CONTROL) OR AGAROSE PELLETS CONTAINING 5-HT, 10 mg/kg BODY WEIGHT (EXPERIMENTAL) FOR 4 WEEKS

	Control (mean ± SE)	Experimental (mean ± SE)
Body weight (g)	421 ± 7.0	423 ± 8.6
Testes wt/100 g body wt (g)	0.96 ± 0.05	0.66 ± 0.07**
FSH (ng/ml)	152 ± 24.8	309 ± 30.0**
LH (ng/ml)	13 ± 3.6	29 ± 7.3
PRL (ng/ml)	25 ± 3.9	25 ± 2.5
T (ng/ml)	2.25 ± 0.88	2.84 ± 1.09
5α-DHT (ng/ml)	0.16 ± 0.02	0.11 ± 0.02

Note. Values represent mean ± SE of six observations.
** Significantly different from control ($P < 0.01$).

0.01). LH, PRL, testosterone, and DHT levels were not significantly different. Testes weights were significantly reduced ($P < 0.01$) in the 5-HT-implanted group as compared to controls (Table I). However, damage to the seminiferous tubules was not extensive and only a few tubules demonstrated some degenerative changes (Fig. 2) as compared to controls (Fig. 1). Body weight in the two groups did not differ.

Experiment 2. Implantation of 5-HT, 10 mg/kg body weight for 4 weeks and 20 mg/kg body weight for an additional 4 weeks, resulted in significant increases in the levels of FSH and LH and a decrease in testes size ($P < 0.05$) as compared to the placebo or methysergide maleate-implanted control groups (Table II). The histologic preparations showed extensive degenerative changes. Normal spermatogenesis was found to proceed within a few remaining normal-appearing tubules (Fig. 3). Implan-

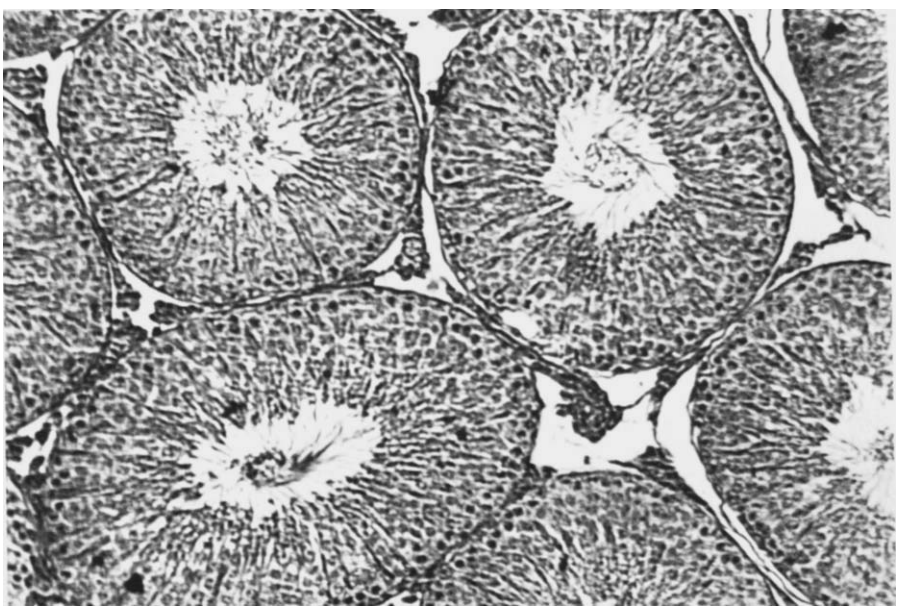

FIG. 1. Section of testis from adult male rat implanted subcutaneously with placebo agarose pellets for 4 weeks (control) (×350).

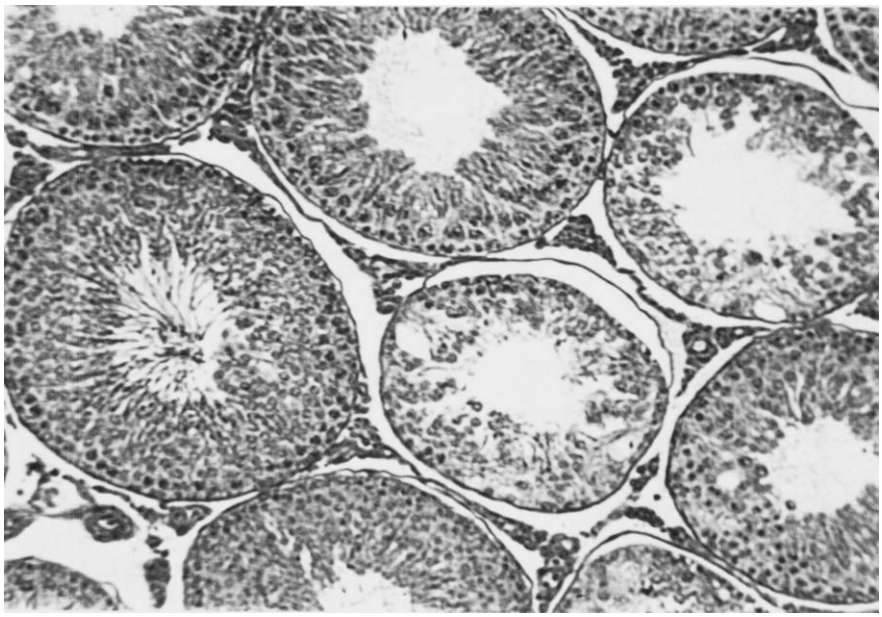

FIG. 2. Section of testis from adult male rat implanted subcutaneously with 5-HT, 10 mg/kg body weight, for 4 weeks. Seminiferous epithelia in some areas are comparable to the control (Fig. 1) and other tubules illustrate the onset of degenerative changes (×350).

tation of 10 mg/kg body weight of 5-HT followed by that of methysergide maleate for an additional 4 weeks also resulted in significant increases in serum FSH and LH levels and a decrease in testes weight ($P < 0.05$) (Table II). There were no significant differences in body weight, PRL, or androgens between the control and experimental groups (Table II).

Experiment 3. Serum levels of pituitary hormones in orchiectomized rats following implantation of agarose pellets (control)

TABLE II. SERUM LEVELS OF FSH, LH, PRL, T, 5α-DHT, AND TESTES WEIGHT IN ADULT MALE RATS IMPLANTED WITH AGAROSE PELLETS, AGAROSE PELLETS CONTAINING 5-HT OR METHYSERGIDE MALEATE

	Group I[a] (control)	Group II[b] (methysergide)	Group III[c] (5-HT × 2)	Group IV[d] (5-HT + MS)
Body wt (g)	459 ± 14	474 ± 7	458 ± 10	464 ± 7
Testes wt (g/100 g body wt)	0.90 ± 0.04	0.84 ± 0.02	0.57 ± 0.05*	0.55 ± 0.05*
FSH (ng/ml)	181 ± 33	209 ± 19	340 ± 56*	316 ± 20*
LH (ng/ml)	27 ± 4.5	16 ± 2.8	86 ± 19.4*	67 ± 6.3*
PRL (ng/ml)	40 ± 7.8	53 ± 7.3	31 ± 9.5	40 ± 9.3
T (ng/ml)	2.65 ± 0.5	1.88 ± 0.3	2.49 ± 0.3	2.61 ± 0.6
5α-DHT (ng/ml)	0.31 ± 0.1	0.20 ± 0.4	0.20 ± 0.01	0.16 ± 0.01

Note. Values represent the mean ± SE of five or more observations. Statistical analyses were done by analysis of variance and differences among groups were estimated by Newman–Keuls procedure. Differences at 0.05 level were considered significantly different. Asterisks indicate testes weight, FSH, and LH levels in Groups III and IV are significantly different from those of Groups I and II.

[a] Agarose pellets (control).
[b] Methysergide maleate × 2 (10 mg/kg).
[c] 5-HT × 2 (10 mg/kg + 20 mg/kg).
[d] 5-HT + methysergide maleate (10 mg/kg).

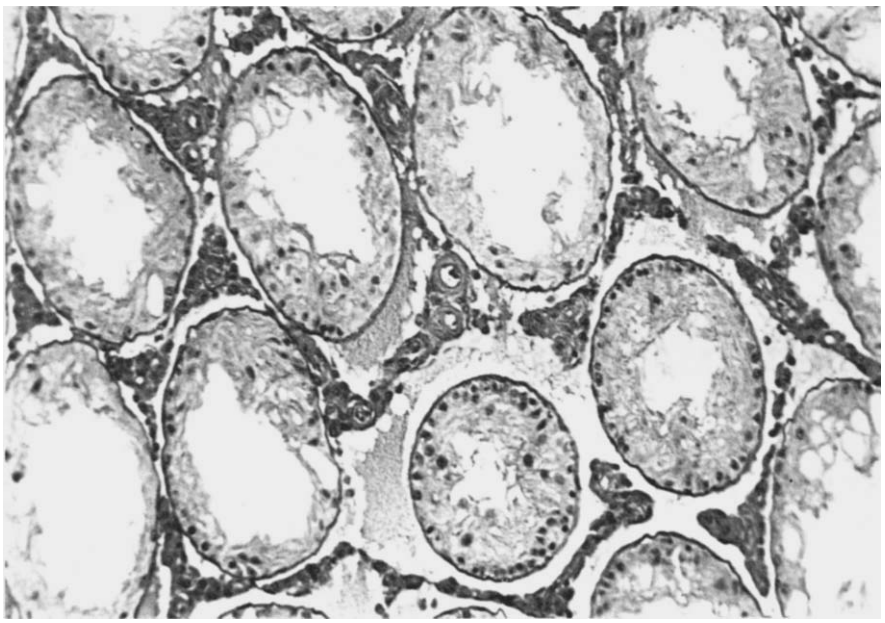

FIG. 3. Section of testis from adult male rat implanted subcutaneously with 5-HT, 10 mg/kg body weight for 4 weeks and 20 mg/kg body weight for an additional 4 weeks. Degeneration of the seminiferous tubules is extensive and very few tubules show the presence of multiple layers of seminiferous epithelia (×350).

compared to those implanted with agarose pellets containing 5-HT, 10 mg/kg body weight for 4 weeks, were not significantly different (Table III).

Discussion. The adverse effect of 5-HT on reproductive organs and sexual maturity in rats has been reported (1, 5, 17). Serotonin has been shown to inhibit the testosterone activated heterosexual copulatory behavior in castrated male rats (3) and chronic treatment of normal men with tryptophan, a precursor of 5-HT, decreased sexual activity (8). Experiments to understand the role of 5-HT as a neurotransmitter in pituitary function are usually performed by the administration of serotonergic agonists and antagonists and measuring acute responses in serum hormone levels (14, 15, 18, 19).

The present study describes the long-term effect of 5-HT exposure on serum hormone levels and concomitant changes in testicular morphology. Four weeks following implantation of 5-HT pellets, a significant reduction in testes weight, minimal degenerative changes in seminiferous tubules, and an increase in serum FSH were detected (Table I). Although the rate of release of 5-HT from the implant is not available from this study the above observations are suggestive of a direct effect of 5-HT on the testes and may possibly indicate the disruption of a negative feedback mechanism (inhibin like?) from testis to adenohypophysis. Also, the absence of any changes in serum levels of testosterone, DHT, and LH may indicate that the pituitary–testis axis for androgen secretion is not affected (Table I). The administration of 5-HT to rats (10, 12) or its precursor 5-hydroxytryptophan to humans (11) or rats (13) has previously been demonstrated to result in augmented PRL release. The absence of differences of serum PRL levels between control and 5-HT-implanted rats at 4 weeks postimplantation in our studies is probably due to 5-HT not having been released in significant amounts by the time the animals were sacrificed.

The results of the second experiment, involving twice-implanted 5-HT, indicate

TABLE III. SERUM LEVELS OF FSH, LH, AND PRL IN ORCHIECTOMIZED ADULT MALE RATS IMPLANTED WITH AGAROSE (CONTROL) OR AGAROSE PELLETS CONTAINING 5-HT, 10 mg/kg BODY WEIGHT (EXPERIMENTAL) FOR 4 WEEKS

	Control (mean ± SE)	Experimental (mean ± SE)
Body weight (g)	419 ± 8.8	403 ± 15.0
FSH (ng/ml)	1130 ± 101	947 ± 70
LH (ng/ml)	403 ± 61	450 ± 40
PRL (ng/ml)	29 ± 12.9	18 ± 4.1

Note. Values represent mean ± SE of six or more observations. For all experimental values, $P > 0.1$ when compared with the corresponding control value.

there was a significant decrease in testis size, increase in serum FSH and LH (Table II), and extensive degenerative changes of the seminiferous tubules (Fig. 3). The maintenance of normal serum androgens coupled with increase in serum LH could be due to subtle degenerative changes in the Leydig cells and/or inhibition of their functional capacity leading, in turn, to compensatory increase in serum LH level to provide adequate stimulus to androgen-secreting cells. Such a direct inhibitory effect of 5-HT on Leydig cells has been reported previously (20). A similar increase in serum LH level was also observed in the group of rats implanted with 5-HT for 4 weeks followed by methysergide maleate for an additional 4 weeks (Table II), but not in rats implanted with 5-HT alone for 4 weeks (Table I). This is probably indicative of the effects of 5-HT on seminiferous tubules which are manifested (rise in FSH alone) before the effects on Leydig cells are observed (rise in LH). The purpose of methysergide maleate implantation to rats previously implanted with 5-HT was to establish whether the recovery process from the 5-HT-induced damages could be hastened by providing the alleged antagonist for 5-HT. Since the effect seen in this group and in the rats implanted twice with 5-HT were similar with respect to testes weight, serum FSH and LH levels, it was concluded that: (1) the effect of 5-HT is more a time-dependent than dose-dependent phenomenon since one time implantation followed by the antagonist had the same effect as seen in rats implanted twice, and (2) there appeared to be no recovery from the effects of the drug for the duration of the experiment. Methysergide maleate-implanted group did not differ significantly from the control group for the variables tested (i.e., body weight, testis weight, and serum hormone levels). Furthermore, no differences were noted in serum prolactin levels among the four groups (Table II).

To determine whether 5-HT would have any direct effect upon the pituitary, pellets containing 5-HT were also implanted in orchiectomized rats. Serum levels of FSH, LH, and PRL were not significantly different between the control and the experimental groups (Table III). It has been reported that the administration of 5-HT inhibited LH release in acutely castrated male rats but not in chronically castrated and intact male rats (15). The magnitude of gonadotropin increase in serum 4 weeks postorchiectomy in our study probably masked any drug-induced effect.

Our data indicate that there is a correlation between the 5-HT-induced degenerative changes in the testes and secondary increases in serum FSH and LH in intact rats. The decrease in testis weight observed in this study, the reported effects of 5-HT on various aspects of reproduction (1–7), and the observation of poor semen quality in infertile men with concomitant excessive daily urinary secretion of 5-HT and 5-HIAA (9) are all indicative of possible action of 5-HT directly on the testis. The rise in serum FSH alone in 4 weeks (Table I) and both FSH and LH in 8 weeks (Table II) also suggests that the interference in the feedback mechanism from testis to pituitary gland probably occurs at different time sequence with inhibitory effect on FSH being affected earlier than that of LH.

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1. Kormano M, Penttila A. Distribution of endogenous and administered 5-hydroxytryptamine in the rat testis and epididymis. Ann Med Exp Biol Fenn 46:468–473, 1968.
2. Meyerson BJ, Sawyer ChH. Monoamines and

ovulation in the rat. Endocrinology **83**:170–176, 1968.
3. Malmnas CO. Monamine precursors and copulatory behavior in the male rat. Acta Physiol Scand Suppl **395**:47–68, 1973.
4. Meyerson BJ. Drug and sexual motivation in the male rat. In: Sandler M, Gessa GL eds. Sexual Behavior—Pharmacology and Biochemistry. New York, Raven Press, p2, 1975.
5. Robson JM, Botros M. The effect of 5-hydroxytryptamine and monamine oxidase inhibitors on sexual maturity. J Endocrinol **22**:165–176, 1961.
6. Naumenko EV, Shishkina GT. Role of serotonin in feedback control of hypothalamic–pituitary–testicular complex in male rats. Neuroendocrinology **26**:359–366, 1978.
7. Vaughan MK, Benson B, Norris JT. Inhibition of compensatory ovarian hypertrophy in mice by 5-hydroxytryptamine and melatonin. J Endocrinol **47**:397–398, 1970.
8. Sicuteri F. Serotonin and sex in man. Pharmacol Res Commun **6**:403–411, 1974.
9. Segal S, Ron M, Yaffe H, Laufer N, Palti Z. Anti-serotonin Therapy for Male Infertility. Fourth Annual Meeting of the American Society of Andrology (abstract), p62, 1979.
10. Kamberi IA, Mical RS, Porter JC. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology **88**:1288–1293, 1971.
11. MacIndoe JH, Turkington RW. Stimulation of human prolactin secretion by intravenous infusion of L-tryptophan. J Clin Invest **52**:1972–1978, 1973.
12. Lawson DM, Gala RR. The influence of pharmacological manipulations of serotonergic and dopaminergic mechanisms on plasma prolactin in ovariectomized estrogen treated rats. Endocrinology **102**:973–981, 1978.
13. Lamberts SWJ, MacLeod RM. The interaction of serotonergic and dopaminergic systems on prolactin secretion in the rat. Endocrinology **103**:287–295, 1978.
14. Baumgarten HG, Bjorklund A, Wuttke W. Neural control of pituitary LH, FSH, and prolactin secretion. In: Scott DE, Kozlowski GP, Weindle A. eds. Neural Hormones and Reproduction: Proceedings of the Third International Symposium on Brain–Endocrine Interaction. Basel, Karger, p327, 1978.
15. Pilotte NS, Porter JC. Circulating luteinizing hormone and prolactin concentrations in intact or castrated male rats treated with 5-hydroxytryptamine. Endocrinology **105**:875–878, 1979.
16. Coyotupa J, Parlow AF, Abraham GE. Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. Anal Lett **5**:329–340, 1972.
17. Segal S. Serotonin and male reproduction: Age difference on the effect of serotonin on testicular morphology in prepubertal and adult rats. Fertil Steril **30**:739, 1978 (abstract).
18. Subramanian MG, Gala RR. The influence of cholinergic, adrenergic and serotonergic drugs on the afternoon surge of plasma prolactin in ovariectomized estrogen treated rats. Endocrinology **98**:842–848, 1976.
19. Subramanian MG, Gala RR. Further studies on the effects of adrenergic, serotonergic and cholinergic drugs on the afternoon surge of plasma prolactin in ovariectomized estrogen treated rats. Neuroendocrinology **22**:240–249, 1976.
20. Kalla NR. Demonstration of direct effect of serotonin on rat Leydig cells. Andrologia **11**:259–262, 1979.

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