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Serum Concentrations of Reproductive Hormones after Administration of Various Anesthetics to Immature and Young Adult Male Rats (42692)

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Abstract. Immature and young adult male rats were either castrated or unoperated. One of seven anesthetic agents (Rompun, Bio-Tal, Thiopental, pentobarbital, ketamine, halothane, or ether) was administered. When the animals were clearly anesthetized, they were decapitated. Control rats were decapitated without anesthesia. Serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, testosterone, and androstenedione were determined by radioimmunoassay. None of the anesthetics was clearly suitable for study of all these hormones. Most would be suitable for acute LH studies. Ketamine and halothane appeared inappropriate for FSH studies in immature rats. Pentobarbital, Rompun, and ether caused increases in serum prolactin. Most of the agents appeared to cause a reduction in serum testosterone in intact rats but an increase in castrated animals, suggesting an inhibition of testicular androgen secretion and a stimulation of adrenal androgen secretion.

The recent report of the American Veterinary Medical Association's Panel on Euthanasia (1) recommended that decapitation of rodents "should be used only after that animal has been sedated or lightly anesthetized, unless the head will be immediately frozen in liquid nitrogen . . ." Traditionally it was felt that decapitation would eliminate the stress associated with the induction of anesthesia as well as eliminating any effects of the anesthetic agent itself. For this reason decapitation of rats has been used extensively and for many years to study reproductive hormones, especially those of pituitary origin. This method has also been used as the control technique in studies of the effects of stress on reproductive hormones (2–6). An occasional older study directly addressed the question of the effect of anesthetic agent and/or blood sampling method (6–9) on serum hormone concentrations. More commonly, however, such information is described as the result of preliminary experiments, unpublished observations, or personal communications with little or no hard data provided. Since we frequently need to examine the serum concentrations of luteinizing hormone (LH), follicle stimulating hormine (FSH), prolactin, testosterone, and/or androstenedione, we decided to examine the serum concentrations of these hormones after the administration of a number of common anesthetic agents.

Materials and Methods. *Animals.* Male rats of a Sprague–Dawley-derived strain were obtained from Holtzman Co. (Madison, WI). They were housed four per cage in hanging wire-mesh cages with food and water available *ad libitum* and a controlled light cycle of 14 hr of light and 10 hr of darkness (lights on at 0600). All animals were permitted a minimum of 1 week acclimation to local conditions prior to sacrifice. During this time they were handled daily, Monday through Friday. This consisted of removing the animals from their home cage, placing them in a clear plastic cage, transporting them a short distance on a cart, and returning them to their home cage. Young adult animals weighed 275–300 g when received and 320–360 g when sacrificed. Immature rats were 22 days old when received and 28–30 days old when sacrificed.

Four separate animal models were used: intact adult, castrated adult, intact immature, and castrated immature. Intact adult rats were sacrificed 8 days after receipt. Approximately 1 week after receipt the castrated adult group had their testes removed via a scrotal incision while under ether anesthesia. They were sacrificed 8 days after surgery. Intact immature animals were sacrificed at 28 days of age. The castrated immature group was castrated the day after receipt (23 days of age) and sacrificed at 30 days of

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age. Weight of the immature rats at sacrifice ranged from 100 to 135 g.

On the morning of the day the animals were to be sacrificed, they were placed in clear plastic cages (six per cage) and transported from the animal quarters to the laboratory where they were isolated for 2 hr. Although the laboratory itself was vacant during this period, animals were potentially exposed to noise from the hallway. Water was freely available during this time. Rats were then anesthetized with one of the following agents: Rompun (xylazine), 10 mg/kg ip; Bio-Tal (thiamylal), 40 mg/kg ip; Thiopental (pentothal) 40 mg/kg ip; pentobarbital, 50 mg/kg ip; ketamine (Vetalar), 200 mg/kg im; halothane, vapor to effect or ether, vapor to effect. When the animals were clearly anesthetized (usually 10–15 min for the injectable anesthetics, approximately 30 sec for the ether), they were decapitated and trunk blood was collected. Control rats were decapitated without anesthesia. Serum was obtained and frozen until assayed for LH, FSH, prolactin, testosterone, and androstenedione by radioimmunoassay as described below. These experiments were performed with the prior approval of the University of South Florida College of Medicine Laboratory Animal Medicine Ethics Committee (the local Institutional Animal Care and Use Committee).

Assays and statistics. Serum concentrations of LH, FSH, and prolactin were determined using kits provided by the National Hormone and Pituitary Agency (10–12). First antibodies used were anti-rat LH S5, anti-rat FSH S11, and anti-rat prolactin S8. Results were expressed in terms of the appropriate rat standard (LH RP-2, FSH RP-1, prolactin RP-3). All samples from a given animal model were run in the same assay.

Concentrations of total testosterone were determined by radioimmunoassay after extraction of 100–120 μl of serum with diethyl ether (10) using a double antibody procedure similar to that described by Fuderburgh *et al.* (13). First antibody (R-181-I) and radioiodinated testosterone (19 position) were obtained from Radioassay Systems Laboratories (Carson, CA). The cross-reactivity of this antiserum is 3.4% for dihydrotestosterone and less than 2.5% for a number of other steroids. All samples from a given animal model were run in the same assay. Extracts of buffer or serum from castrated/adrenalectomized animals were undetectable. The between assay coefficient of variation was determined from a pool of adult male rat serum run in each assay and was 15.1% for these assays. Within assay coefficient of variation was 10.8% for samples containing 3 pg or more testosterone. The minimum detectable dose as determined from the variability of the 100% bound tubes averaged 0.5 pg. The working detection limit was defined as the displacement at 95% bound and averaged 0.7 pg. When corrected for recovery and volume extracted, sensitivity in these assays was approximately 20 pg/ml.

Androstenedione concentrations were also measured by radioimmunoassay as described previously (10).

Data were analyzed and signficance determined using Duncan's New Multiple Range test to compare controls to each of the anesthetic groups. Six animals were decapitated per group. Several samples were lost during the processing of the FSH assay. Two groups (intact adult, Bio-Tal; and intact adult, ketamine) contained only three observations for FSH. For some animals insufficient plasma remained to perform the androstenedione assay. In no group did the n for androstenedione drop below 4. For statistical purposes any sample that was undetectable (>95% bound) in the radioimmunoassay was set equal to the assay sensitivity.

Results. *LH (Fig. 1).* Acute exposure to these anesthetics had little or no effect on LH concentrations in intact adult male rats. In castrated, adult animals there were no statistically significant changes in LH. In intact immature male rats anesthesia had no statistically significant effect on serum LH, and ether treatment of castrated immature rats caused a statistically significant ($P < 0.05$) increase in LH secretion.

FSH (Fig. 2). None of the anesthetics used had a significant effect on FSH secretion in adult animals whether they were castrated or intact. In immature animals, however, ketamine ($P < 0.01$) and halothane ($P < 0.05$) significantly reduced serum FSH concentrations in intact rats. No significant effects of any of these anesthetics was seen in castrated immature rats.

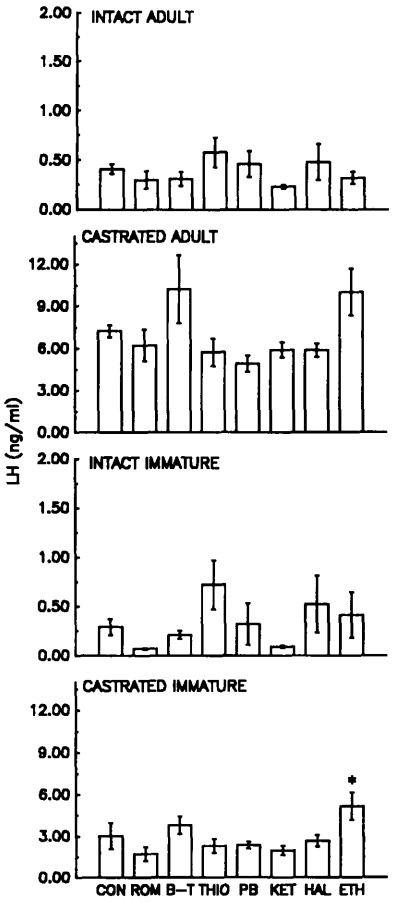

FIG. 1. Serum concentrations of LH (mean ± SEM) in intact or castrated young adult male rats and intact or castrated immature male rats decapitated after the administration of various anesthetics. CON, control—no anesthesia; ROM, Rompun; B-T, Bio-Tal; THIO, Thiopental; PB, pentobarbital; KET, ketamine; HAL, halothane; ETH, ether. *$P < 0.05$ vs control.

Prolactin (Fig. 3). Pentobarbital consistently raised prolactin concentrations regardless of age or testicular status ($P < 0.01$). Rompun treatment of adult male rats resulted in increased ($P < 0.01$) concentrations of serum prolactin whether the animals were castrated or intact. Ether resulted in increased prolactin concentrations in the castrated immature rats only ($P < 0.05$).

Testosterone (Fig. 4). There was a consistent, although not statistically significant decline, in serum testosterone in intact adult male rats in response to anesthesia. Administration of Bio-Tal to castrated adult rats resulted in an increase in serum testosterone concentrations ($P < 0.01$). Serum testosterone levels declined in intact immature male rats in response to Rompun, pentobarbital, ketamine, halothane, and ether ($P < 0.05$). Castrated immature male rats responded to Rompun, Bio-Tal, Thiopental, and ketamine with significant ($P < 0.01$) increases in serum testosterone.

Androstenedione (Fig. 5). No anesthetic had a statistically significant effect on androstenedione concentrations in intact rats regardless of age. Ketamine administration resulted in an increase in serum androstenedione in castrated rats whether adult ($P < 0.01$) or immature ($P < 0.05$). No other agent af-

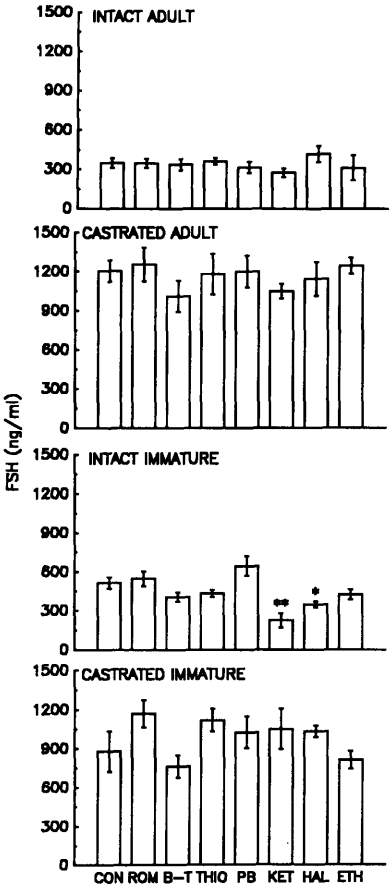

FIG. 2. Serum concentrations of FSH (mean ± SEM) in intact or castrated young adult male rats and intact or castrated immature male rats decapitated after the administration of various anesthetics. CON, control—no anesthesia; ROM, Rompun; B-T, Bio-Tal; THIO, Thiopental; PB, pentobarbital; KET, ketamine; HAL, halothane; ETH, ether. *$P < 0.05$, **$P < 0.01$ vs control.

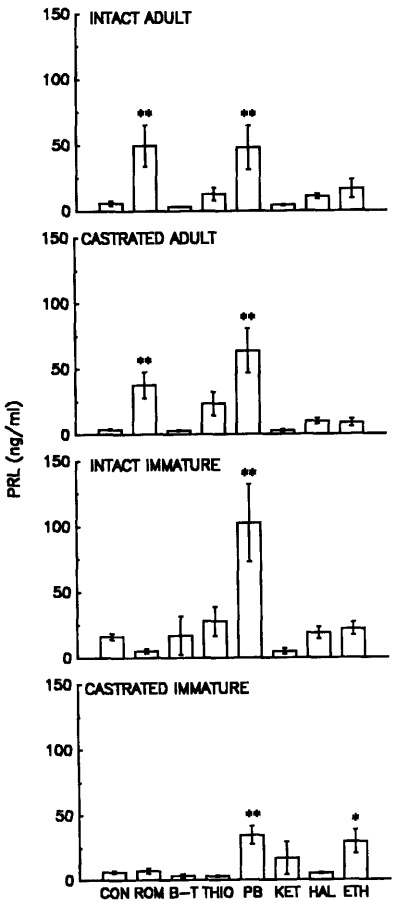

FIG. 3. Serum concentrations of prolactin (mean ± SEM) in intact or castrated young adult male rats and intact or castrated immature male rats decapitated after the administration of various anesthetics. CON, control —no anesthesia; ROM, Rompun; B-T, Bio-Tal; THIO, Thiopental; PB, pentobarbital; KET, ketamine; HAL, halothane; ETH, ether. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control.

fected androstenedione concentrations in castrated animals.

Discussion. These experiments evaluated the response of LH, FSH, prolactin, testosterone, and androstenedione to acute exposure to various anesthetics in the male rat. Given the recent report of the American Veterinary Medical Association (1), such information is important for proper experimental design. With the possible exception of ether, any of these anesthetics appear to be appropriate for use in studies interested in basal LH secretion.

Rather surprisingly two anesthetics generally considered to be rather innocuous, ketamine and halothane appeared to suppress FSH, at least in intact immature animals. Whether this is due to a direct effect on the pituitary or an effect that suppresses hypothalamic secretion of luteinizing hormone-releasing hormone (LHRH) cannot be determined from this data. Little is known about hypothalamic control of FSH secretion at this age (14). The fact that, in the same rats, there were no differences in LH secretion argues against an effect on LHRH. The involvement of inhibin (15) or the putative FSH releasing factor (16) in this effect cannot be determined from these experiments.

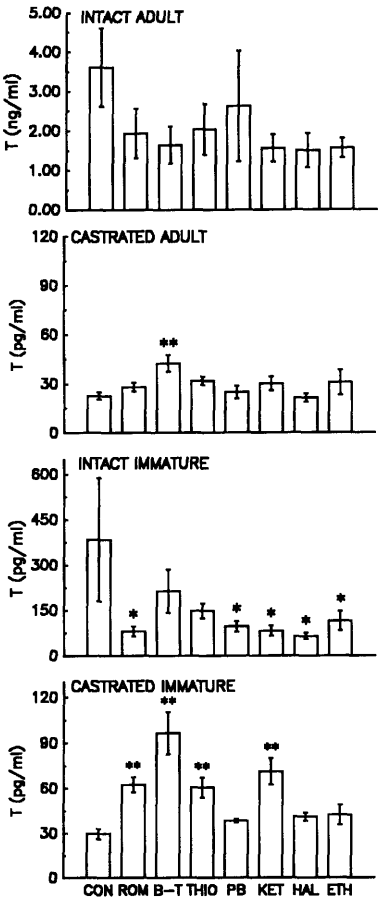

FIG. 4. Serum concentrations of testosterone (mean ± SEM, note scale differences) in intact or castrated young adult male rats and intact or castrated immature male rats decapitated after the administration of various anesthetics. CON, control—no anesthesia; ROM, Rompun; B-T, Bio-Tal; THIO, Thiopental; PB, pentobarbital; KET, ketamine; HAL, halothane; ETH, ether. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control.

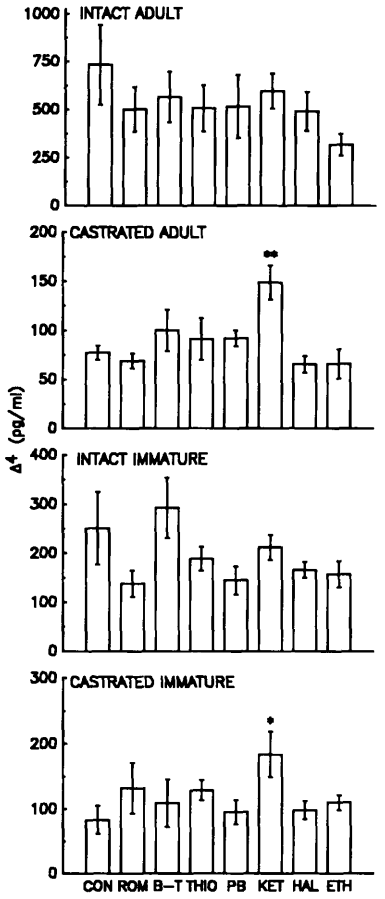

FIG. 5. Serum concentrations of androstenedione (mean ± SEM, note scale differences) in intact or castrated young adult male rats and intact or castrated immature male rats decapitated after the administration of various anesthetics. CON, control—no anesthesia; ROM, Rompun; B-T, Bio-Tal; THIO, Thiopental; PB, pentobarbital; KET, ketamine; HAL, halothane; ETH, ether. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control.

Prolactin is known to be highly sensitive to "stress" (2–5). These rats were handled daily primarily to accustom them to such procedures and prevent any release of prolactin during the subsequent experimental period. Pentobarbital clearly increases prolactin and thus is unsuitable for acute studies of prolactin secretion. Rompun was also capable of increasing prolactin in adult animals and thus would not be an appropriate anesthetic for acute studies. Exposure to ether vapor for 1 min or longer is well known to be a potent inducer of prolactin release (2). The technique used here (exposure to an ether saturated atmosphere for 30 sec) was effective at avoiding this increase except in the castrated immature rats. The problems imposed by the short time intervals and the need to be certain that the animals are completely anesthetized suggests that for larger numbers of animals, ether would not be an appropriate anesthetic for studies of unstressed prolactin levels. Based on the data reported here, ketamine or halothane would appear to be acceptable anesthetics for prolactin studies.

Although not statistically significant, in intact adult animals the overall trend of serum testosterone concentrations after any of these anesthetics is a reduction. Part of the reason for the lack of statistical significance in this animal model is the presence of one very high (>9 ng/ml) observation in the pentobarbital group. If this observation is excluded from the analysis, the Bio-Tal, pentobarbital, ketamine, halothane, and ether treatment groups become significantly ($P < 0.05$) lower than the control group. This decline is confirmed in the intact immature rats where five of the seven anesthetics resulted in significant reductions in serum testosterone. This is probably a direct effect on the testes. Serum LH concentrations were not altered and adrenal output of testosterone (as indicated by the results in castrated rats) was actually increased by many of these anesthetics, especially in the immature rats. Ketamine appears to be especially effective at increasing adrenal androgen production since it also increased serum concentrations of androstenedione in animals lacking testes. Presumably this is due to a stress-induced release of adrenocorticotropic hormone. Clearly, none of these anesthetics can be recommended for studies of serum androgens, especially studies that could anticipate subtle but potentially important changes in testicular function.

It should be emphasized that these studies are concerned only with the acute effects of these anesthetics in their use as a means of reducing pain and stress immediately prior to decapitation. It is well known that long-term (hours) administration of many of these agents (for example pentobarbital (17) or ketamine (18)) can result in reductions in the serum concentrations of these hormones in the male rat. On the other hand, experiments in which an exogenous stimulus is adminis-

tered (such as would occur for example in studies of pituitary sensitivity to LHRH) probably would not be seriously affected by the choice of the anesthetic.

The anesthetic agents used in these studies are all readily available to scientists. Although some present special problems due to licensing of controlled substances or mode of administration, they are all relatively simple to use. Unfortunately the use of none of these has been demonstrated to be appropriate for acute studies dealing with all of these reproductive hormones. It is incumbent upon the individual investigator to demonstrate that any anesthetic utilized does not introduce bias into the results.

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1. Smith AW, Houpt KA, Kitchell RL, Kohn DF, McDonald LE, Passagalia M, Thurmon JC, Ames ER. Report of the AVMA panel on euthanasia. J Amer Vet Med Soc **188**:252–268, 1986.
2. Neill JD. Effect of "stress" on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. Endocrinology **87**:1192–1197, 1970.
3. Fenske M, Wuttke W. Development of stress-induced pituitary prolactin and TSH release in male rats. Acta Endocrinol **85**:729–735, 1977.
4. Dunn JD, Arimura A, Scheving LE. Effect of stress on circadian periodicity in serum LH and prolactin concentration. Endocrinology **90**:29–33, 1972.
5. DuRusseau P, Tache Y, Brazeau P, Collu R. Pattern of adenohypophyseal hormone changes induced by various stressors in female and male rats. Neuroendocrinology **27**: 257–271, 1978.
6. Dohler K-D, von zur Muhnlen A, Gartner K, Dahler V. Effect of various blood sampling techniques on serum levels of pituitary and thyroid hormones in the rat. J Endocr **74**:341–342, 1977.
7. Neill JD. Comparison of plasma prolactin levels in cannulated and decapitated rats. Endocrinology **90**:568–572, 1972.
8. Mock EJ, Frankel AI. Blood-collecting methodology does not affect the maturational pattern of serum hormone concentrations (testosterone, LH, FSH and prolactin) in the male rat. Neuroendocrinology **26**:202–207, 1978.
9. Wuttke W, Meites J. Effects of ether and pentobarbital on serum prolactin and LH levels in proestrous rats. Proc Soc Exp Biol Med **135**:648–652, 1970.
10. Nazian SJ. Temporal relationship between the development of the self-priming effect of LHRH and changes in serum concentrations of testosterone and androstenedione during the sexual maturation of the male rat. Biol Reprod **25**:977–982, 1981.
11. Nazian SJ. Role of adrenal in control of gonadotropin secretion in the immature male rat. Arch Androl **8**:37–42, 1982.
12. Nazian SJ. Pituitary function during the sexual maturation of the male rat: Inhibition of prolactin secretion by exogenous dopamine. Biol Reprod **28**:645–651, 1983.
13. Funderburgh LJ, Zipf WB, Sotos JF. Direct measurement of testosterone in a pediatric center, with use of a radioimmunoassay kit and unextracted serum. Clin Chem **29**:1796–1798, 1983.
14. Nazian SJ. Hypothalamic pituitary, testicular, and secondary organ functions and interactions during the sexual maturation of the male rat. Arch Androl **4**:283–303, 1980.
15. deJong FH, Robertson DM. Inhibin: 1985 update on action and purification. Mol Cell Endocrinol **42**:95–103, 1985.
16. Mizunuma H, Sampson WK, Lumpkin MD, Moltz JH, Fawcett CP, McCann SM. Purification of a bioactive FSH-releasing factor (FSHRF). Brain Res Bull **10**:623–629, 1983.
17. Nansel DD, Aiyer MS, Meinzer WH, Bogdanove EM. Rapid direct effects of castration and androgen treatment on luteinizing hormone-releasing hormone-induced luteinizing hormone release in the phenobarbital-treated male rat: Examination of the roles direct and indirect androgen feedback mechanisms might play in the physiologic control of luteinizing hormone release. Endocrinology **104**:524–531, 1979.
18. Nazian SJ. Serum and pituitary luteinizing hormone and serum androgens during luteinizing hormone releasing hormone self-priming in immature and pubertal male rats. Biol Reprod **29**:912–918, 1983.

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