PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 613–619 (1983)

Effect of Vagotomy on Postcastration Gonadotropin Secretion in Male Rats[1,2] (41695)

L. GREGORY ALLEN,*[,3] CHARLES A. HODSON,† HUBERT W. BURDEN,* AND IRVIN E. LAWRENCE, JR.*

*Departments of *Anatomy and †Obstetrics and Gynecology, School of Medicine, East Carolina University, Greenville, North Carolina 27834*

Abstract. The postcastration increase in gonadotropins was studied in intact and vagotomized male rats. Rats underwent vagotomy or sham surgery immediately prior to castration. In the first experiment, rats were bled before castration and at 1, 2, 4, and 7 days after castration. Serum LH and FSH were significantly lower in vagotomized rats 1 day after castration. On Days 2, 4, and 7 postcastration, serum gonadotropin levels were generally not different among experimental groups. In a second experiment, rats were decapitated at 12 or 24 hr after surgery and castration. Trunk blood was collected for assay of LH. Vagotomy had no effect on LH levels at 12 hr postcastration, but, at 24 hr postcastration, vagotomized rats had significantly lower serum LH than did sham-operated rats. These experiments indicate that vagotomy has a transient suppressive effect on gonadotropin release following castration. Such observations support the hypothesis that the vagus nerve may play a modulatory role in gonadotropin secretion.

The rat ovary receives innervation via the vagus nerve (1–3). It is likely that these vagal components are predominantly sensory since most of the subdiaphragmatic vagal fibers are sensory and mediate visceral reflexes (4, 5). Sensory fibers for the general visceral afferent component of the vagus nerve have their cell bodies in the nodose ganglion and terminate in the nucleus of the solitary tract (6). It has been shown that there are connections between the nucleus of the solitary tract and hypothalamic centers which regulate gonadotropin release (7, 8). Two studies have suggested that gonadotropin release is suppressed by vagotomy (9, 10).

In the present study, the effects of vagotomy on gonadotropin release following castration were studied in the male rat. The male rat was chosen as the experimental model because the postcastration increase of gonadotropins in the male rat is more rapid than in the female rat (11, 12).

Materials and Methods. *Experiment I.* Thirty-six male rats (Harlan Sprague–Dawley, Madison, Wisc.) weighing 300 to 400 g were used. Animals were maintained on a 14:10 light:dark cycle and were housed three to a cage.

The animals were divided into four experimental groups of nine rats each (Group 1: castrate, sham vagotomy, solid diet; Group 2: castrate, vagotomy, solid diet; Group 3: castrate, sham vagotomy, liquid diet; Group 4: castrate, vagotomy, liquid diet). Before surgery, precastration blood samples (2 ml) were obtained from all animals by orbital sinus puncture under light ether anesthesia.

Surgery. Surgery was performed under ether anesthesia. Rats were castrated through a midventral incision in the lower abdominal wall. Immediately prior to castration, half of the rats (Groups 2 and 4) were vagotomized by the following procedure: A midventral incision was made through the upper abdominal wall along the linea alba. The stomach was gently pulled caudally and held in place. The anterior trunk of the vagus nerve was located on the anterior surface of the esophagus and was severed rostral to the level of branching. The posterior trunk was also located, isolated, and avulsed. In sham-vagotomized rats (Groups 1 and 3), the vagus was viewed but not touched. Following surgery, the abdominal wall was sutured together and the skin closed with wound clips. A prophylactic dose of 20,000 IU of penicillin was injected im.

[1] This work was presented in part at the 22nd Annual Meeting of the Southern Society of Anatomists, October 6–9, 1982, Johnson City, Tennessee.

[2] Supported by NICHHD Grants 06899 (to H.W.B.) and 16364 (to C.A.H.).

[3] To whom all correspondence should be addressed.

0037-9727/83 $1.50

Feeding. Since vagotomy has been shown to delay stomach emptying of solid food (13), half of the animals were placed on a liquid diet (Sustacal, Mead Johnson, Evansville, Ind.) following surgery. The other groups were fed Wayne lab chow (Chicago, Ill). Animals were weighed daily and food consumption per cage (three rats) was determined. Both food and water were available *ad libitum.*

Blood collection. All animals were bled under light ether anesthesia by orbital sinus puncture before castration and at 1, 2, and 4 days after castration. On the seventh day after castration, all rats were decapitated and trunk blood was collected. Blood samples were allowed to clot overnight at 4°C. Serum was separated and stored (−20°C) until assayed.

Experiment II. To further evaluate the immediate response of LH to vagotomy, 59 male Sprague–Dawley rats were either vagotomized or sham vagotomized immediately prior to bilateral castration. Following surgery, water and lab chow were available *ad libitum.* Rats were decapitated 12 or 24 hr after castration and trunk blood was collected. Serum LH was determined by radioimmunoassay (RIA).

Analytical methods. Serum LH and FSH were measured using radioimmunoassay reagents supplied by the Hormone Distribution Program of the National Institutes of Health. Assays were performed by the double-antibody method using the instructions enclosed with the kits. All samples for a particular study were run in the same RIA. Intraassay coefficients of variation were 9.03% for the FSH assay and 6.6% for the LH assay.

Statistical analysis of serum LH and FSH levels was accomplished by a one way analysis of variance. Values for each day were analyzed separately and if a significant F value was found ($P < 0.05$), multiple comparisons tests for differences between means were applied

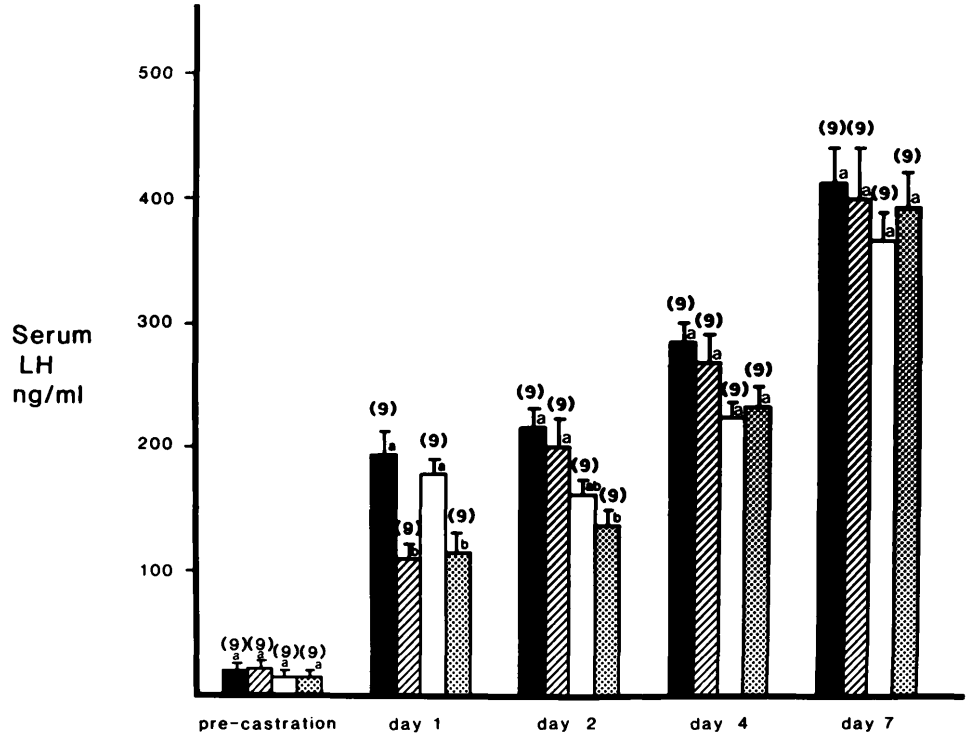

FIG. 1. Mean (±SEM) serum LH concentrations in male rats before and after castration. Experimental groups were (1) sham-vagotomized rats on solid diet (solid bars), (2) vagotomized rats on solid diet (hatched bars), (3) sham-vagotomized rats on liquid diet (open bars), and (4) vagotomized rats on liquid diet (stippled bars). Numbers in parentheses represent the number of animals in each group. Statistical analyses were performed for each separate day, and groups with identical subscripts are not significantly different ($P > 0.05$).

using the Student–Newman–Keuls test. Feeding data were analyzed by the two-way analysis of variance with subsequent comparisons by Student–Newman–Keuls tests. Data for solid and liquid diets were analyzed separately.

Results. *Experiment I. Effects of vagotomy on serum LH levels.* Serum LH levels are shown in Fig. 1. Before castration, serum LH levels were similar in all groups. By Day 1, the characteristic postcastration increase in LH was apparent. At this time, mean serum LH levels in vagotomized rats were significantly lower than levels in sham-vagotomized rats, regardless of diet. On Day 2, serum LH levels in vagotomized rats on liquid diet were lower than levels in both vagotomized and sham-vagotomized rats on solid diet. However, LH levels of vagotomized rats on liquid diet were not different from LH levels of sham-vagotomized rats on liquid diet. At 4 and 7 days

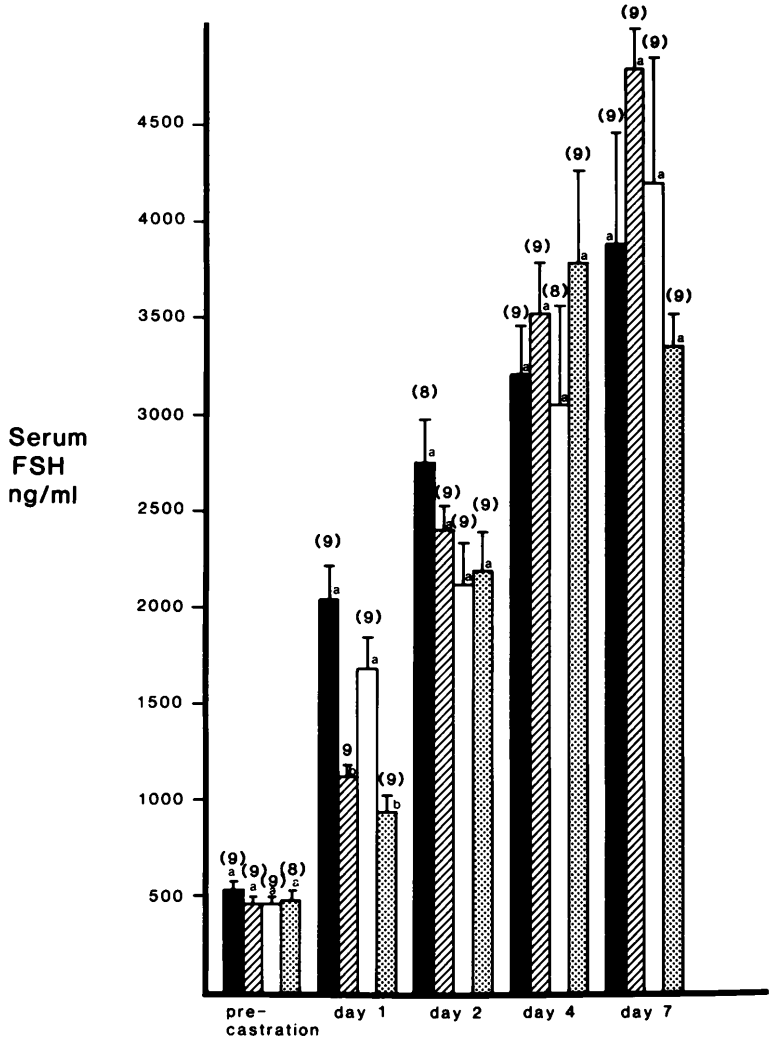

FIG. 2. Mean (±SEM) serum FSH concentrations in male rats before and after castration. Experimental groups were (1) sham-vagotomized rats on solid diet (solid bars), (2) vagotomized rats on solid diet (hatched bars), (3) sham-vagotomized rats on liquid diet (open bars), and (4) vagotomized rats on liquid diet (stippled bars). Numbers in parentheses represent the number of animals in each group. Statistical analyses were performed for each separate day, and groups with identical subscripts are not significantly different ($P > 0.05$).

after castration, there were no significant differences in serum LH among the experimental groups.

Effects of vagotomy on serum FSH levels. Serum FSH levels are shown in Fig. 2. Precastration levels were similar in all groups. On Day 1 postcastration, serum FSH levels were above precastration levels in all groups. At this time, serum FSH levels in vagotomized rats were significantly lower than serum FSH levels in sham-vagotomized rats. On the remaining days of the study (Days 2, 4, and 7 postcastration), serum FSH levels continued to rise, but there were no statistically significant differences between experimental groups on any of these days.

Effects of vagotomy on feeding and body weight in castrated male rats. Feeding data for the experimental groups are shown in Table I. During the experimental period, vagotomized animals ingested less of both solid and liquid diets than did the sham-vagotomized rats. Body weight data (Fig. 3) show that the decreased food intake by vagotomized rats resulted in a decline in body weights throughout the experimental period. This body weight decline was less apparent in vagotomized rats on liquid diets than in vagotomized rats on solid diets.

Experiment II. Data from Experiment II are shown in Table II. Statistical analysis reveals that vagotomy significantly decreased serum LH at 24 hr after castration but had no effect on serum LH at 12 hr after castration.

Discussion. The data presented have shown that subdiaphragmatic vagotomy transiently dampens gonadotropin release after castration. Both FSH and LH were lower in vagotomized male rats than in sham-vagotomized male rats 1 day after castration. With one exception, there were no other differences in gonadotropin concentrations between experimental groups after the first day postcastration.

These results have also confirmed those of other authors who have shown that vagotomy decreases food intake and therefore results in a loss of body weight (13). Although starvation is known to cause decreased gonadotropin secretion (14), it is unlikely that the observed difference in serum gonadotropins between vagotomized and sham-vagotomized rats at 1 day after castration was due to starvation. At 7 days after castration, vagotomized rats consumed less food and weighed less than sham-vagotomized rats. Serum gonadotropin levels were not different between groups at that time.

From the present data, it appears that vagotomy may suppress gonadotropin release following castration. It also appears that this suppression is transient and that, in time, normal gonadotropin secretion is resumed. The mechanism by which vagotomy transiently suppresses gonadotropin secretion following castration is unknown.

One possible interpretation is that testosterone may still be present in the blood in significant levels, differentially affecting LH levels in vagotomized and sham-vagotomized rats. It has been shown that serum testosterone levels decrease rapidly following orchidectomy (15). Less than 4% of control serum levels of testosterone remain 2 hr after castration. At 24 hr after castration, the time at which we observed a difference between serum gonadotropins in vagotomized and sham-vagotomized rats, others have found serum testosterone levels to be less than 1% of control values (15). Unless vagotomy markedly decreases clearance of testosterone from the blood, it would be considered unlikely that

TABLE I. FOOD CONSUMPTION OF THE EXPERIMENTAL ANIMALS

			Food consumption (g)					
Group	Surgery	Diet	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Sham	Solid	49.3 ± 1.2	44.6 ± 6.7	58.6 ± 3.5	50.0 ± 10.4	59.3 ± 7.7	68.0 ± 5.2
2	Vagotomy	Solid	11.3 ± 3.2*	11.3 ± 0.6*	20.0 ± 5.3*	18.0 ± 7.9*	22.7 ± 7.6*	26.7 ± 8.5*
3	Sham	Liquid	127 ± 6.6	204 ± 24.8	235 ± 5.1	218 ± 4.6	225 ± 11.6	235 ± 1.1
4	Vagotomy	Liquid	118	81 ± 12.3*	78 ± 37.9*	115 ± 17.7*	106 ± 67.4*	131 ± 70.8*

Note. Each value is a mean (±SD) for consumption in each cage (three rats per cage). Diet and water were available *ad libitum*.
* Statistically different from corresponding sham group ($P < 0.05$).

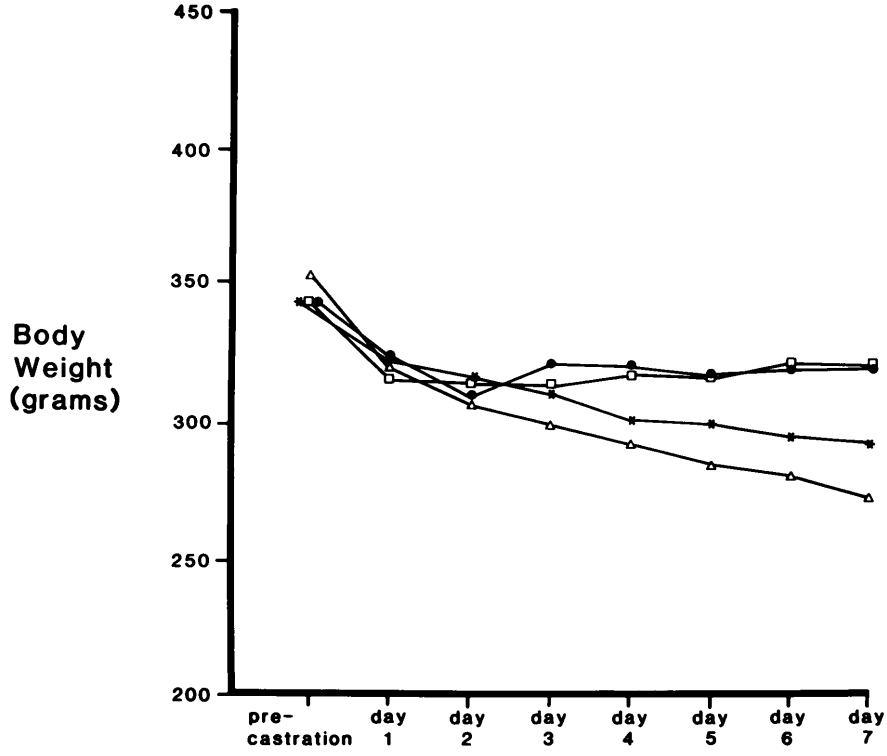

FIG. 3. Mean body weights for male rats before and after castration. Experimental groups were (1) sham-vagotomized rats on solid diet (solid dots), (2) vagotomized rats on solid diet (triangles), (3) sham-vagotomized rats on liquid diet (squares), and (4) vagotomized rats on liquid diets (asterisks).

testosterone remaining after castration would significantly alter serum gonadotropins in the present study.

On the other hand, the following may be suggested as a mechanism for the effect of vagotomy on gonadotropin release: Vagotomy may remove afferent information from the periphery which may participate in the modulation of gonadotropin release, or, alternatively, severing the vagus nerve may constitute

TABLE II. EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY ON SERUM LH LEVELS IN MALE RATS AT 12 AND 24 hrs POSTCASTRATION

	Time after castration			
	12 hr		24 hr	
	Sham vagotomy	Vagotomy	Sham vagotomy	Vagotomy
Serum LH (ng/ml) (mean ± SEM)	68.67 ± 9.1	54.8 ± 9.71	132.7 ± 16.01	92.5 ± 16.97
Number of animals	15	15	14	15
Separation by Student-Newman-Keuls test*	*a*	*a*	*b*	*a*

* Data were analyzed by a one-way analysis of variance for four groups and multiple comparisons were made by Student-Newman-Keuls test. Mean LH levels for groups bearing identical subscripts were not significantly different ($P > 0.05$).

a stimulus to the central nervous system, one which may briefly cause altered gonadotropin release due to effects on relevant hypothalamic centers.

It has been shown that brain stem noradrenergic projections to the hypothalamus may have a role in release of LH in the rat (16–20). This noradrenergic input is considered to be modulatory rather than mandatory (17). Neurons in the nucleus of the solitary tract contribute to the ventral noradrenergic pathway (21). It is possible that vagal input into the nucleus of the solitary tract may influence gonadotropin secretion via the ventral noradrenergic pathway.

Our data show that vagotomy transiently suppresses the postcastration increase in serum LH in male rats. In female rats, surgical transection of the ventral noradrenergic pathway transiently suppresses the postcastration increase in serum LH (16). We suggest that the ventral noradrenergic pathway may be involved in the transmission of vagal input to the hypothalamus and that such input may modulate LH release. In contrast to these findings, Kawakami *et al.* have shown that electrolytic lesions of the A2 noradrenergic region (including the nucleus of the solitary tract) had no effect on ovulation or the proestrus LH surge (22). The effect of such lesions on postcastration LH increase was not investigated in that study.

In conclusion, these data support the hypothesis that the vagus nerve may play a modulatory role in the hypothalamo–hypophyseal–gonadal system. We speculate that the gonadotropin control mechanism includes a vagal sensory contribution from the gonad or, in general, abdominal viscera. The physiological significance of this contribution is unknown. It may be that the vagus nerve acts in concert with the endocrine system to synchronize the gonadotropin control mechanism. Studies are underway to further clarify whether the vagus nerve acts as a modulator of gonadotropin secretion.

The authors wish to thank John Dow for his help with the experimentation, and Shirley Nett for word processing of the manuscript.

1. Burden HW, Lawrence IE Jr. Experimental studies on the acetylcholinesterase-positive nerves in the ovary of the rat. Anat Rec **190**:233–241, 1978.
2. Burden HW, Capps ML, Smith CP, Harris P, Lawrence IE Jr. Extrinsic sensory innervation of the rat ovary. Fed Proc **41**:1492, abstr, 1982.
3. Woodruff ML, Baisden RH, Peppler RD. Medullary sites of origin and termination of the ovarian branch of the vagus in the rat. Anat Rec **204**(4):405, 1982.
4. Agostoni E, Chinnock JE, Daly M, Murray JG. Functional and histological studies of the vagus nerve and its branches to heart, lungs and abdominal viscera in the cat. J Physiol (London) **135**:182–205, 1957.
5. Gabella G, Pease HL. Number of axons in the abdominal vagus of the rat. Brain Res **58**:465–469, 1973.
6. Carpenter, MB. Human Neuroanatomy. Baltimore, Williams & Wilkins, p 310, 1976.
7. Palkovits, M, Zaborsky L. Neural connections of the hypothalamus. In: Morgane PJ, Panksepp J, eds. Handbook of the Hypothalamus: Anatomy of the Hypothalamus: New York, Dekker, Vol I: p 397, 1979.
8. Ricardo JA, Koh ET. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in rat. Brain Res **153**:1–26, 1978.
9. Burden HW, Lawrence IE Jr. The effect of denervation on compensatory ovarian hypertrophy. Neuroendocrinology **23**:368–378, 1977.
10. Lawrence IE Jr, Burden HW, Louis TM. Effect of abdominal vagotomy of the pregnant rat on LH and progesterone concentrations and fetal resorption. J Reprod Fert **33**:131–136, 1978.
11. Gay VL, Midgley AR. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology **84**:1359–1365, 1969.
12. Yamamoto M, Diebel ND, Bogdanove EM. Analysis of initial and delayed effects of orchidectomy and ovariectomy on pituitary and serum LH levels in adult and immature rats. Endocrinology **86**:1102–1111, 1970.
13. Mordes JP, Herrera MG, Silen W. Decreased weight gain and food intake in vagotomized rats. Proc Soc Exp Biol Med **156**:257–260, 1977.
14. Campbell GA, Kurcz M, Marshall S, Meites J. Effects of starvation on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin: Response to LH-releasing hormone and thyrotropin-releasing hormone. Endocrinology **100**:580–587, 1977.
15. Coyotupa J, Parlow AF, Kovacic N. Serum testosterone and dihydrotestosterone levels following orchidectomy in the adult rat. Endocrinology **92**:1579–1581, 1973.
16. Clifton DK, Sawyer CH. Positive and negative feedback effects of ovarian steroids on luteinizing hormone release in ovariectomized rats following chronic depletion of hypothalamic norepinephrine. Endocrinology **106**:1099–1102, 1980.
17. Clifton DK, Sawyer CH. LH release and ovulation

in the rat following depletion of hypothalamic norepinephrine: Chronic vs acute effects. Neuroendocrinology **28**:442–449, 1979.

18. Leung PCK, Arendash GW, Whitmoyer DI, Gorski RA, Sawyer CH. Electrical stimulation of mesencephalic noradrenergic pathway: Effects on luteinizing hormone levels in blood of ovariectomized and ovariectomized, steroid-primed rats. Endocrinology **109**:720–728, 1981.
19. Martinovic JV, McCann SM. Effect of lesions in the ventral noradrenergic tract produced by microinjection of 6-hydroxydopamine on gonadotropin release in the rat. Endocrinology **100**:1206–1213, 1977.
20. Hancke JL, Wuttke W. Effects of chemical lesion of the ventral noradrenergic bundle or of the medial preoptic area on preovulatory LH release in rats. Exp Brain Res **35**:127–134, 1979.
21. Dahlstrom A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand **62**(suppl 232):1–55, 1964.
22. Kawakami M, Ando S, Nishihara M, Tadokoro Y. Participation of the lower brain stem in induction of preovulatory gonadotropin surges in female rats. Endocrinol Jpn **28**:809–818, 1981.

Received January 18, 1983. P.S.E.B.M. 1983, Vol. 173.