Parallel Declines in Fos Activation of the Medial Anteroventral Periventricular Nucleus and LHRH Neurons in Middle-Aged Rats

W.-W. LE, P. M. WISE, A. Z. MURPHY, L. M. COOLEN, AND G. E. HOFFMAN

Department of Anatomy and Neurobiology (W.-W.L., A.Z.M., G.E.H.), University of Maryland, Baltimore, Maryland 21201; Department of Physiology (P.M.W.), University of Kentucky, Lexington, Kentucky 40536; and Department of Cell Biology (L.C.), Neurobiology and Anatomy, University of Cincinnati, Cincinnati, Ohio 45267

The middle-age decline in reproductive function is manifested by reduced LHRH release, resulting in a decreased magnitude and delay of onset of the LH surge. Earlier studies suggested that the reductions in LHRH neural activation in middle-aged rats resulted from deficits in the afferent drive to the LHRH neurons. One critical afferent to the LHRH neurons lies in the anteroventral periventricular preoptic area (AVPv) nucleus. The neurons of the medial AVPv are synchronously activated to express Fos with LHRH neurons at the time of an LH surge in young adult animals. The present study examined whether, in middle age, reductions in the activation of AVPv neurons accompany the reduction in Fos activation in LHRH neurons.

Young (3- to 4-month-old) and middle-aged (10- to 12-month-old) spontaneously cycling and ovariectomized steroid-replaced rats were killed during peak and early descending phase of the LH surge, and their brains were examined for Fos in LHRH and AVPv neurons. Young animals had a characteristic increase in Fos expression in both LHRH and AVPv neurons. In middle-aged rats, the proportion of LHRH neurons expressing Fos at the time of an LH surge was reduced by approximately 10%, irrespective of whether surges were spontaneous or induced by exogenous steroids. A similar reduction in the number of Fos+ cells (by approximately 50%) was noted in the medial AVPv. Linear regression analysis of the relationship between the extent of Fos activation in LHRH and AVPv neurons revealed a strong positive correlation ($r^2 = 0.66$; $P < 0.01$), suggesting that changes in the AVPv’s drive to LHRH neurons underlie the decrease in LHRH activity in middle age.

A second series of experiments examined whether decreased input from the AVPv could account for reduced Fos activation in LHRH neurons seen in middle-aged animals. When the medial AVPv was lesioned, LHRH neurons failed to express Fos on the side ipsilateral to the lesion. Animals with lesioned medial AVPv also had significantly lower LH values than animals with an intact medial AVPv. Taken together, these data suggest that a principal deficit in middle-aged rats is the ability of the medial AVPv to stimulate LHRH neurons.

ENDOCRINOLOGY 142: 4976–4982, 2001

Reproductive function declines in female rats as middle age approaches. In the early phases of the decline, preovulatory and steroid-induced LH surges are reduced in magnitude and delayed in time onset (1–4). Reduced LHRH release (5, 6) accompanies the decline in the magnitude of steroid-induced LH surges, and this lowering of LHRH activity is reflected by a decrease in the number of LHRH neurons that express Fos (7–9) and Jun (9) [markers of cell stimulation (10)]. On the other hand, the number of LHRH neurons and the content of LHRH in the medial basal hypothalamus do not change until long after the reproductive senescence is established (3, 11, 12), suggesting that the deficit during the middle-age period lies in the afferent pathway(s) that regulates LHRH synthesis and/or secretion.

Neurons of the medial-most anteroventral periventricular preoptic area (AVPv) play a critical role in the stimulation of LHRH neurons at the time of the LH surge. The AVPv has had a number of names, making the integration of earlier studies difficult. Bleier (13) initially termed the area the periventricular preoptic area; and our initial studies of this area used the variant pePOA to designate the region (14–17). Studies from Terasawa’s group (18–20) had termed this area the medial preoptic nucleus; but currently, a more caudal preoptic nucleus has adopted that name. Irrespective of its name, this region is located along the rostral half of the preoptic area along the ventricular surface in a strip of tissue approximately 70-μm wide and 300- to 400-μm long. The neurons of this medial AVPv innervate the LHRH neurons (21, 22) and are coordinately activated with LHRH neurons at the time of LH surges (14, 15). Lesions of the AVPv with no concomitant loss of LHRH neurons or their axons in the median eminence (23) block both spontaneous LH surges and surges evoked by exogenous E and progesterone (P) in ovariectomized rats (18–20, 24). These features suggest that a decline in afferent drive from AVPv neurons may account for the attenuation of LHRH activation in middle-aged rats. In the present study, we designed experiments to determine whether changes in the degree of Fos activation within the medial AVPv correlated with changes in LHRH neurons in young and middle-aged rats. A second set of experiments assessed whether elimination of the input from the medial AVPv neurons could mimic the reductions in LH Fos activation and LH secretion seen in middle age.

Materials and Methods

Animals

All protocols were approved by the University Committee on Animal Care and Use at the University of Pittsburgh, where the studies began.
and at the Universities of Maryland and Kentucky, where the studies were completed.

Adult female Sprague Dawley rats that were young (3–4 months of age) or middle-aged (10–12 months) were housed under standard laboratory conditions on a 14-h light, 10-h dark schedule. One group of rats (n = 8 young, and n = 8 middle-aged rats) was ovarioverted and, 1 wk later, administered 17β E2 at 5 h after lights on (180 μg/ml in sesame oil placed into SILASTIC brand capsules (Dow Corning, Midland, MI), 150 mm length for young or 20 mm for middle-aged rats; capsules had an inner diameter of 0.062 in and an outer diameter of 0.15 in).

Two days later, P (1.5 mg in 0.2 ml sesame oil) was injected sc to induce an LH surge. Rats were killed on the day of the expected LH surge, either near the peak of the LH surge (11 h after lights on; n = 4) or during the descending limb of the surge (13 h after lights on; n = 4). To determine whether the relationship extended to animals with spontaneous LH surges, we reanalyzed the AVPv and LHRH cells from sections generated in our earlier study (9). This group of rats (n = 5 young; n = 4 middle-aged) was monitored with daily vaginal smear to establish regular estrous cyclicity. Each rat chosen for study demonstrated consecutive 4-d cycles. Rats were killed on proestrus at the same times indicated for the E2 plus P (E + P) groups (11 and 13 h after lights on).

To assess the role of the medial AVPv in LH surge generation and activation of LHRH neurons, 49 ovariectomized young adult rats were anesthetized with chloral hydrate and placed into a stereotaxic apparatus.

Activation of LHRH neurons, 49 ovariectomized young adult rats were indicated for the E2 plus P (E + P) groups (11 and 13 h after lights on).

Preparation of tissue for histology

Ten to 15 min before death, all animals were anesthetized with an overdose of pentobarbital (100 mg/kg), treated with heparin (1000 U), and perfused transcardially with saline containing 2% sodium nitrite and 2% sodium acetate solution, the sections were processed as described previously (25, 27) until immunocytochemistry for Fos and LHRH was initiated.

Immunocytochemistry of Fos and LHRH

Immunocytochemical procedures were conducted as described previously (25, 28–33). Briefly, sections were rinsed free of the cytochrome antifreeze solution (27) until immunocytochemistry for Fos and LHRH was initiated.

Correlation between AVPv and LHRH Fos activation.
The relationship between the number of medial AVPp neurons and the proportion of LHRH neurons that expressed Fos was determined with a Spearman Rho nonparametric correlation analysis; P < 0.05 was considered significant.
lesion in the brain (n = 21) and animals with missed lesions (n = 21); 2) animals with partial medial AVPv lesions (n = 3); and 3) animals with complete unilateral lesions of the medial AVPv (n = 4). Plasma LH values were compared using nonparametric Mann-Whitney U tests.

Results

Consistent with earlier studies, in both cycling rats and steroid-treated ovariectomized young rats, approximately half of the LHRH neurons expressed Fos at the time of the LH, irrespective of whether the surge was spontaneous (mean 49.9% ± 2.2 SEM) or induced by exogenous steroids (mean 46.4% ± 2.4). As shown in Fig. 2A, the increase in Fos activation in LHRH neurons seen in young animals was significantly reduced in the middle-aged animals (23.8% ± 2.7 for the E+P group, and 20.8% ± 9.5 for cycling rats). On average, Fos activation in LHRH neurons accompanying the LH surge in young rats was just over twice that observed in middle-aged animals, irrespective of the manner in which the LH surge was generated. No differences between animals killed at the peak of LH secretion and those killed during the early phase of the descending limb of the LH surge was noted. Within AVPv (Fig. 2B), Fos activation in the middle-aged animals was also greatly reduced at the time of the LH surge (from 296.8 ± 40.4 to 116.3 ± 40.8 for proestrous rats and from 270.8 ± 23.0 to 132.1 ± 15.8 for ovariectomized steroid-replaced rats). Comparisons between the proportion

result in healthy animals (n = 12), lesion in the brain (n = 21) and animals with missed lesions (n = 21); 2) animals with partial medial AVPv lesions (n = 3); and 3) animals with complete unilateral lesions of the medial AVPv (n = 4). Plasma LH values were compared using nonparametric Mann-Whitney U tests.

Results

Consistent with earlier studies, in both cycling rats and steroid-treated ovariectomized young rats, approximately half of the LHRH neurons expressed Fos at the time of the LH, irrespective of whether the surge was spontaneous (mean 49.9% ± 2.2 SEM) or induced by exogenous steroids (mean 46.4% ± 2.4). As shown in Fig. 2A, the increase in Fos activation in LHRH neurons seen in young animals was significantly reduced in the middle-aged animals (23.8% ± 2.7 for the E+P group, and 20.8% ± 9.5 for cycling rats). On average, Fos activation in LHRH neurons accompanying the LH surge in young rats was just over twice that observed in middle-aged animals, irrespective of the manner in which the LH surge was generated. No differences between animals killed at the peak of LH secretion and those killed during the early phase of the descending limb of the LH surge was noted. Within AVPv (Fig. 2B), Fos activation in the middle-aged animals was also greatly reduced at the time of the LH surge (from 296.8 ± 40.4 to 116.3 ± 40.8 for proestrous rats and from 270.8 ± 23.0 to 132.1 ± 15.8 for ovariectomized steroid-replaced rats). Comparisons between the proportion

FIG. 1. Diagrams and micrographs of sections in three rostral-to-caudal levels through the AVPv. In the diagrams, the borders of the AVPv are indicated on the left. The boxes drawn on the right side show the placement of the 70-micron² eyepiece grid boxes used to count the Fos-positive cells. In the micrographs, the left side shows a Nissl section; the right side is an adjacent section stained for Fos. Levels A–C are 150 µm apart. A, Approximately 75 µm from the organum vasculosum of the lamina terminalis; B, approximately 225 µm from OVLT; C, approximately 375 µm from OVLT. Bar, 100 µm; V3, third ventricle; OC, optic chiasm.

FIG. 2. Comparison of the degree of Fos activation in LHRH (A) and med (B). AVPv neurons from young (filled bars) and middle-aged (open bars) rats during the LH surge. Both surge time groups are combined because they did not show significant differences from each other in either LHRH or AVPv activation. Middle-aged animals were all significantly lower than young animals, for all measures (*, P < 0.05; **, P < 0.01).
of LHRH neurons that expressed Fos and the number of Fos+ medial AVPv neurons for both young and middle-aged animals revealed a striking positive correlation (Fig. 3, $r^2 = 0.66; P < 0.01$).

Ibotenic acid or electrolytic lesions, aimed at the AVPv, produced a wide range of lesion sizes and placements. In 4 of the rats, the medial AVPv was eliminated on 1 side of the brain. In 21 rats, no lesion was found; an equal number of animals had lesions that missed the AVPv altogether. In 3 of the animals, only a portion of the medial AVPv was destroyed by the lesion. What was quite revealing was the observation in animals with large POA lesions that either spared or eliminated the medial AVPv. Fig. 4 shows 2 animals with ibotenic acid lesions that eliminated the majority of neurons in the rostral preoptic area on 1 side of the brain. In one (Fig. 4A), the medial AVPv was eliminated; in the other (Fig. 4B), it was spared. In the animal whose medial AVPv was destroyed, AVPv Fos activation was noted only on the intact side (Fig. 4C), and LHRH Fos activation was blocked on the lesioned side (Fig. 4E). In the other animal, Fos activation in the medial AVPv was quite normal (Fig. 4D), and LHRH neurons were strongly activated on both sides of the brain (Fig. 4F). One of the animals that received an electrolytic lesion had a bilateral lesion of the medial AVPv that extended only 100 μm from the ventricular surface (Fig. 5A). In that animal, LHRH neurons failed to activate on either side of the brain (Fig. 5, B and C). Fig. 6 shows a summary of the plasma LH values for the animals in the lesion study. As more and more of the medial AVPv was eliminated, LH levels at the time of the expected LH surge declined; and when 1 side of the medial AVPv was completely destroyed, plasma LH was significantly lower than that of animals with no detected lesion or with lesions that missed the medial AVPv. The animals bearing partial medial AVPv lesions were intermediate. One of the electrolytically lesioned rats had a lesion placement that shifted rostrally and eliminated the majority of LHRH neurons. That animal had a plasma LH value that was lower than that in the animals with unilateral AVPv lesions and was similar to that in animals that had no LH surge.

**Fig. 3.** Correlation between the proportion of Fos+ LHRH neurons and the number of Fos+ medial AVPv neurons in young (open circles) and middle-aged (filled squares) killed at the time of the expected LH surge. Each symbol represents an individual animal. Intact animals whose surges were spontaneous and ovarioectomized rats whose surges were induced by steroid-replacement are both included.

**Discussion**

The concept that the central nervous system and the hypothalamus, in particular, regulates the transition to reproductive acyclicity follows from studies which demonstrated that the pattern of neurochemical and neuroendocrine signals changes during middle age. It seems that these signals, which dictate the patterns of secretion of the gonadotropins, become less precise and synchronized. Newer data, primarily from studies performed in women during the perimenopausal period, reveal that striking parallel changes may occur in humans. Thus, it becomes critically important to decipher the mechanisms that regulate LH release and how they change with age.

Earlier studies from our laboratories, as well as those of others, indicated that the reduction in the number of LHRH neurons expressing Fos in middle-aged rats predicted the decline in peak LH values at the time of an LH surge. The present data extend these findings and demonstrate that parallel reductions in the activation of neurons in the AVPv accompany the decline in LH activation that occurs during middle age. The reduction of Fos activation within both AVPv and LHRH neurons suggests that the two events are linked, either through parallel common afferents that regulate both LHRH and AVPv neuronal activity or through a sequential series of neurochemical signals. Because the AVPv neurons directly innervate LHRH neurons, this suggests that a deficit in AVPv stimulation determines the reduction in LH activation. This conclusion is supported by earlier studies showing that electrolytic lesions of the entire AVPv block the LH surge. Our present data (which indicate that when ibotenic acid or electrolytic lesions destroy the medial AVPv, steroid treatment fails to evoke LHRH activation on the side ipsilateral to the lesion, and LH secretion is reduced) support this hypothesis. Interestingly, lesions placed more laterally or rostrally that exclude the medial AVPv had no effect on LHRF Fos induction, further suggesting that medial AVPv is the central component. The fact that, in middle-aged rats, the LHRH neurons remain responsive to potassium stimulation to the same extent as cells from younger rats, further indicates that it is stimulatory drive to LHRH neurons that is reduced in aging.

What produces the reduced drive to the AVPv neurons during middle age is unknown. One possibility, based on the initial studies of cycling animals, was that E was either not secreted in sufficient amounts to trigger proper up-regulation of PRs, or that the P secretion was deficient. However, the studies of Wise and Lapolt et al. show that E and ER levels are maintained or elevated in middle-aged compared with young rats. We assayed tissue from young and middle-aged rats and found that binding of labeled P and PR protein patterns (E. Hoffmann, unpublished observation) were indistinguishable in the young and middle-aged preoptic areas. The fact that the deficit was detectable in middle-aged rats that were still regularly cycling, as well as in ovarioectomized rats that received controlled concentrations of E and P, further indicates that decline in steroid levels is not the cause of reproductive failure. More likely, the declining responsiveness of AVPv neurons to other inputs is involved. One of the extrinsic factors needed for LH surges is the diurnal elevation of cAMP levels in the AVPv.
Indeed, our preliminary data suggest that this diurnal rhythm in cAMP levels is no longer detectable in middle-aged rats (P. M. Wise, unpublished observation). Such a loss in available cAMP may render the AVPv neurons less responsive to P and prevent their ability to stimulate LHRH neurons.

**FIG. 4.** A, NeuN staining of the AVPv region of the preoptic area in an animal bearing an ibotenic acid lesion that included the medial AVPv. B, NeuN staining in an animal with a lesion of the same general size as that shown in A but which spared the medial AVPv (arrows). Bar, 500 μm. C, Fos staining within the AVPv of the same animal shown in A. Note the absence of activated medial AVPv neurons ipsilateral to the lesion. D, Bilateral activation of the medial AVPv was noted in the animal depicted in B. Bar, 100 μm. E, High magnification of all the LHRH neurons surrounding the OVLT from the animal shown in A. This section was double-labeled for Fos (black) and LHRH (brown). Note that on the side of the brain ipsilateral to the complete medial AVPv lesion, most LHRH neurons failed to express Fos; whereas on the intact side, the LHRH neurons were strongly Fos-positive. F, In the animal whose medial AVPv was spared, LHRH neurons on both sides of the brain were strongly activated. Bar, 10 μm.
integrates a host of hormonal and neuronal inputs that regulate LHRH secretion. Catecholamines, serotonin, and numerous peptide afferents terminate in this region (41). Moreover, AVPv cells contain both ER and PR (22, 42, 43). Use of either hormonal paradigms associated with surge blockade (14) or administration of transmitter receptor blockers that block LH surges (34) (G. E. Hoffman, unpublished data) block or attenuate Fos expression in the medial AVPv in parallel with blockade of LHRH Fos activation. Earlier studies, which used lesions of the entire AVPv, determined that elimination of the AVPv blocked LH surges (18–20, 24). Our data further indicate that it was the medial AVPv that produced that effect. Together, these data suggest that the AVPv functions as an integrative center for the regulation of LHRH neuron firing and that disruption of any of inputs either hormonal or neuronal, alters the drive these neurons impose on the LHRH system.

In summary, our findings demonstrate that changes in Fos activation in neurons in the AVPv occur in middle-aged proestrus and steroid-treated rats in parallel with changes in Fos activation within LHRH neurons. Lesions of the medial AVPv produce reduced LHRH activation and LH secretion. These data strongly suggest that the changes in Fos activation, during middle age, reflect altered stimulatory activity in the AVPv neurons responsible for the attenuated activity in LHRH neurons, leading to a decline in the amplitude and a delay in the onset of the LH surge that we observed previously.

Acknowledgments

Received December 27, 2000. Accepted July 9, 2001.

Address all correspondence and requests for reprints to: Dr. G. E. Hoffman, Department of Anatomy and Neurobiology, HSF 222, University of Maryland, School of Medicine, 685 West Baltimore Street, Baltimore, Maryland 21201. E-mail: gehoffma@umaryland.edu.

This work was supported by NIH Grants NS-28730 (to G.E.H.) and AG-02224 (to P.M.W.).

References

6. Rubin BS, King JC 1995 A relative depletion of luteinizing hormone-releasing hormone was observed in the median eminence of young but not middle-aged rats on the evening of proestrus. Neuroendocrinology 62:259–269
gene products as markers for neuronal activity in neuroendocrine systems. Front Neuroendocrinol 14:173–213


15. Le WW, Berghorn K, Rassnick S, Hoffman G 1999 Periventricular preoptic area neurons coactivated with luteinizing hormone (LH)-releasing hormone (LHRH) neurons at the time of the LH surge are LHRH afferents. Endocrinology 140:510–519


18. Wiegand SJ, Terasawa E, Bridson WE 1978 Persistent estrus and blockade of progesterone-induced LH release follows lesions which do not damage the suprachiasmatic nucleus. Endocrinology 102:1645–1648


22. Le W, Hayashi S, Hoffman G, Periventricular preoptic area neurons activated at the time of an LH surge, express both estrogen and progesterone receptors, under revision


29. Lee WS, Smith MS, Hoffman GE 1990 Progestosterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. Endocrinology 127:2604–2606


38. Lapolt PS, Matt DW, Judd HL, Lu JK 1986 The relation of ovarian steroid levels to estrogen. II. Role of cyclic adenosine 3′,5′-monophosphate. Endocrinology 126:1736–1741


42. Blaustein JD, Turcotte JC 1989 Estradiol-induced progestin receptor immunoreactivity is found only in ER-immunoreactive cells in guinea pig brain. Neuroendocrinology 49:454–461