Endocrine, *vol. 6, no. 3, 265–270, June 1997* 0969-711X/97/6:265–270/$9.50

Dehydroepiandrosterone Administration Reverses the Inhibitory Influence of Aging on Gonadotrophin-Releasing Hormone Gene Expression in the Male and Female Rat Brain

Songyun Li, Laurent Givalois, and Georges Pelletier

MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, 2705 Laurier Boulevard, Québec, Canada

Dehydroepiandrosterone (DHEA) has been shown to exert a beneficial influence on some aging-associated deficits in rodents. It is well documented that in the rat, aging is associated with a decline in reproductive functions. In order to evaluate the effect of DHEA on GnRH gene expression in aged animals, we have studied the effect of 2.5-d administration of DHEA to young (50–54 d of age) and aged (18 mo of age) rats of both sexes. In the young males, DHEA induced an 18% reduction in the hybridization signal. In the aged animals, the mRNA levels were 10% lower than those observed in the young rats. DHEA completely restored the mRNA levels when compared to those detected in young male animals. In the young female, DHEA produced a 11% increase in GnRH mRNA, whereas, in the aged animals, hybridization signal was decreased by 28%. DHEA administration to aged females induced a 33% increase in the amount of mRNA, thus completely reversing the influence of aging. These results indicate that the decrease in GnRH gene expression which is likely involved in the loss of reproductive functions in aged rats can be totally reversed by a short term administration of DHEA which restored the GnRH neuronal activity. They also suggest that DHEA might play a role in the prevention and/or improvement of some deficits associated with aging through stimulation of GnRH biosynthesis.

Key Words: Dehydroepiandrosterone; aging; gonadotrophin-releasing hormone; reproduction; *in situ* hybridization.

Received November 13, 1996; Revised February 18, 1997; Accepted March 4, 1997.

Author to whom all correspondence and reprint requests should be addressed: Dr. Georges Pelletier, MRC Group in Molecular Endocrinology, CHUL Research Center, 2705 Laurier Boulevard, Québec, G1V 4G2, Canada. E-mail: Georges.Pelletier@crchul.ulaval.ca

Introduction

During the aging process, there are several metabolic changes that eventually lead to decreased lean body and bone mass, as well as reduced immune function *(1)*. Aging is also associated with an increase in cancer incidence as well as atherosclerosis. In human of both sexes, these changes are accompanied by a progressive decrease in the adrenal production of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) and, consequently, an age-related decline in the plasma levels of both steroids starting after the third decade of life *(2–5)*. These steroids are converted in peripheral tissues into androgens and/or estrogens depending on the relative activity of 5α-reductase and aromatase in each tissue *(6)*. Experiments performed in laboratory animals have shown that DHEA can prevent or improve aging-associated deficits *(7–9)*. Recently, Morales et al. *(10)* have reported that in men and women between 40 and 70 yr of age, DHEA replacement therapy induced an increase in serum levels of androgens and insulin-like growth factor-1. In most cases, this was associated with an increase in perceived physical and psychological well-being.

It is well documented that there is a decline in reproductive functions during aging in rats of both sexes *(11,12)*. Many changes in neural, pituitary, and gonad functions may contribute to the aging of the reproductive system. In the central nervous system, age-related changes in the concentration of GnRH in the preoptic area and hypothalamus have been reported in the rat. In aging male rats, GnRH content appears to be depressed in the preoptic area as well as the median eminence *(13–15)*. In the female rat, Wise and Ratner *(13)* observed lower concentrations of GnRH in the medial basal hypothalamus of middle-aged animals, which were in constant estrous compared with concentrations observed in young cyclic rats. Thus, the gene expression of GnRH at the cellular level has not been correlated with aging. Recently, we have shown that in young adult male rats, DHEA administration induced a decrease in GnRH mRNA in intact and

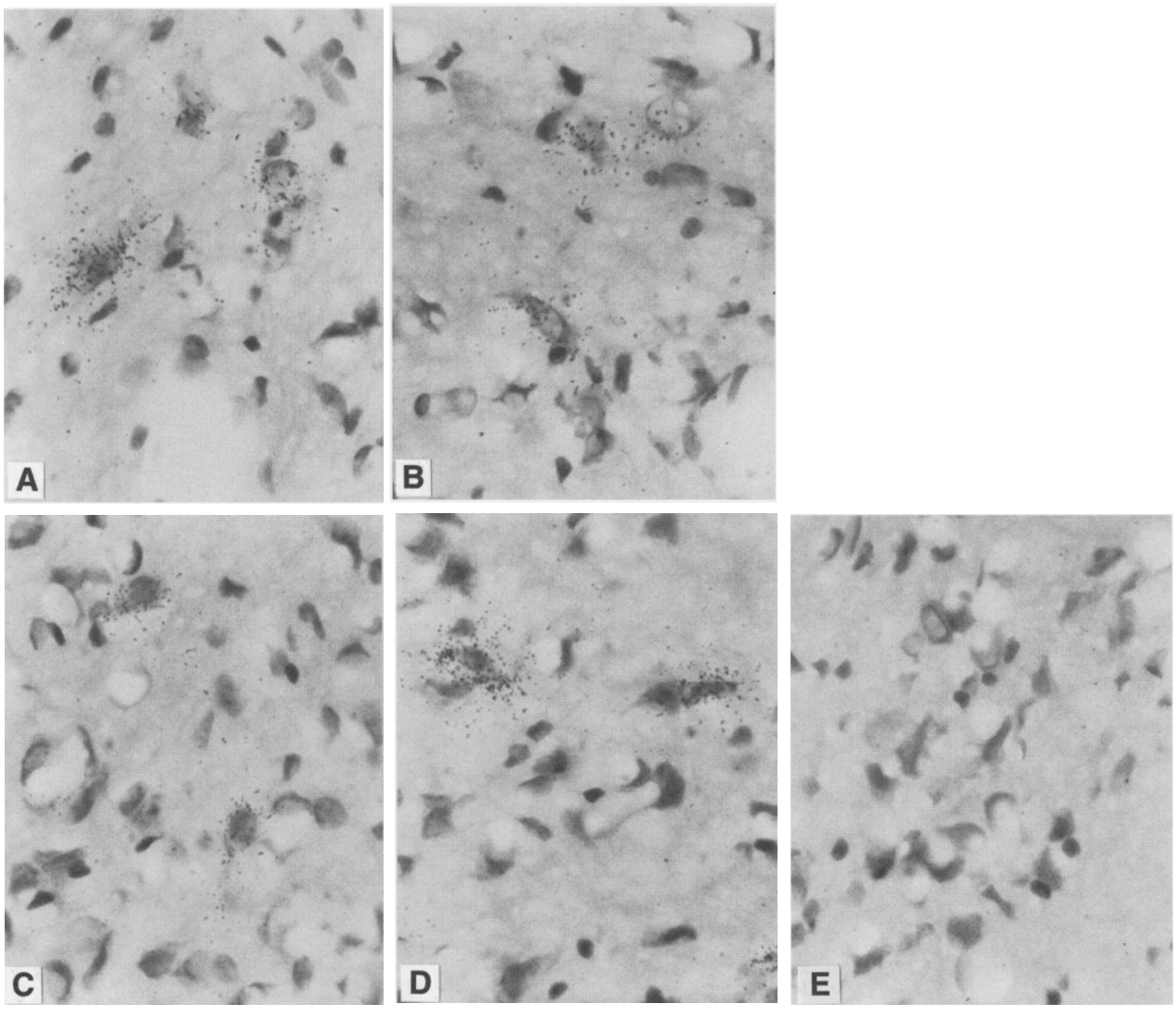

Fig. 1. Light microscope autoradiograph illustrating the hybridization signal in the medial preoptic area of young **(A)** and aged **(B)** male rats. Silver grains are concentrated over a few neurons. **(C)** Young animals treated with DHEA. **(D)** Aged animals treated with DHEA. **(E)** Control section from a young untreated male rat hybridized with a radiolabeled sense oligomer. No labeled neurons can be observed. The background level represented by a few dispersed grains is very low (×800).

orchiectomized animals, whereas, in young adult females, DHEA produced an increase in GnRH mRNA in both intact and ovariectomized animals *(16)*.

Since DHEA appears to restore some of the changes occurring during aging in humans and rodents *(10,17–20)*, it seemed of interest to determine the influence of aging on GnRH gene expression, as well as the effects of DHEA treatment on the changes in GnRH gene expression which might be detected in aged rats of both sexes.

Results

Light microscopic observation of the autoradiograms indicated that a strong hybridization signal could be obtained after 8 d of exposure (Figs. 1 and 3). The specificity of the signal could be established by the following findings:

1. Labeled cells have the same localization as that reported by immunocytochemical staining of GnRH *(21,22)*;
2. Hybridization with a sense oligomer produced no label ing in adjacent sections (Fig. 1E);
3. Pretreatment with RNase completely prevented any labeling following hybridization with the labeled antisense probe (data not shown). Moreover, using a combination of immunocytochemistry and in situ hybridization, we have previously shown that hybridization signal could be detected only in neurons containing immunoreactive GnRH *(23)*. Neurons expressing GnRH mRNA were observed by decreasing order of intensity in the medial preoptic area, anterior hypothalamus, diagonal band of Broca, and ventral septal area. The highest number of labeled cells (30 in a single 10-μ section) was observed in the area of the organum vasculosum of the lamina terminalis (OVLT).

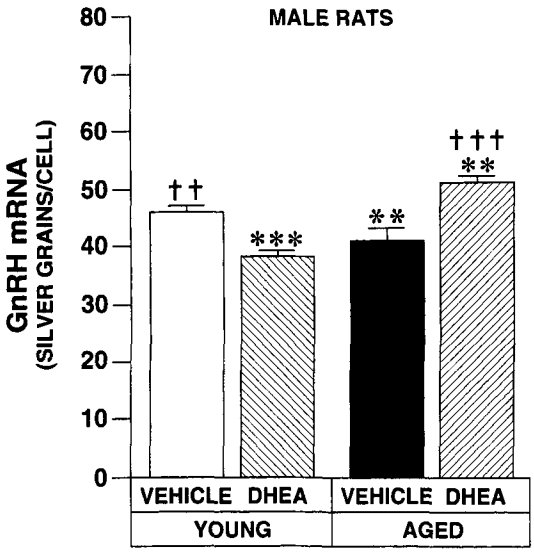

Fig. 2. Effect of DHEA on GnRH mRNA levels as measured by the number of grains overlying labeled neurons in the young and aged male rat. ***$p < 0.001$, **$p < 0.01$, vehicle-treated young animals vs all other experimental groups; +++, $p < 0.001$, ++, $p < 0.01$, vehicle-treated old animals vs all other groups.

As indicated in Table 1, in animals of both sexes neither aging nor DHEA treatment could significantly modify the number of labeled cells in the area of the OVLT. In the male rat, administration of DHEA to young animals induced a 18% reduction in the mean number of grains overlying the labeled neurons (Figs. 1 and 2). In aged males, the hybridization signal was 10% lower than that observed in young animals. In aged animals, DHEA treatment induced a 22% increase over the levels observed in vehicle-treated old animals, completely restoring the mRNA levels when compared to the levels found in young vehicle-treated rats.

In the young female rat, GnRH mRNA levels were evaluated at the diestrous 1 stage of the estrous cycle when GnRH gene expression is at its lowest level *(24,25)*. As illustrated in Figs. 3 and 4, DHEA induced an 11% increase in GnRH mRNA levels. In the aged females, the GnRH mRNA levels appeared to be reduced by 28% compared to the levels observed in young females. DHEA administration to aged female rats induced a 35% increase in the amount of mRNA and then completely reversed the influence of aging, the levels observed corresponding to 97% of the values observed in vehicle-treated young females.

Discussion

In the present experiments, quantitative *in situ* hybridization was used to study the influence of aging and the effects of DHEA treatment on GnRH mRNA levels in individual neurons in the brain of young and aged rats of both sexes. The results obtained demonstrate for the first time that in aged animals, the GnRH gene expression was decreased, the effect being more marked in female (28%) than in male (10%) rats. They are in agreement with previous reports indicating that there is a decline in GnRH content in the preoptic area and the median eminence in aging male rats *(13,15)* and in the medial basal hypothalamus of middle-aged female rats in constant estrous *(12,13)*.

By immunocytochemistry, it has been also demonstrated that in the median eminence there was a decrease in staining in aging animals of both sexes *(11)*. Moreover, it has been shown that in an "in vitro" superfusion system, GnRH release from male rat mediobasal hypothalamus was reduced in old rats compared to the release observed in young animals in basal conditions as well as following stimulation by norepinephine or naloxone *(26)*. These previous results together with the present data strongly suggest that in the rat, there is an age-related decline in both the synthesis and release of GnRH which may point to the involvement of the hypothalamus in reproductive senescence.

In young animals, the inhibitory influence of DHEA in the male as well as the stimulatory effects of the steroid observed in the female confirm our previous observations on the effects of DHEA on GnRH mRNA in intact and castratred rats *(27)*. The dimorphism observed in the effects of DHEA may be related to different conversion rates of DHEA into dihydrotestosterone (DHT) and estradiol in brain. In fact, it has already been shown that DHT administration depressed GnRH mRNA levels in rats of both sexes *(23)* and that a short-term estradiol treatment of female rats induced an increase in GnRH gene expression *(24,28)*. The important observation that the decrease in GnRH gene expression which might be directly involved in the loss of reproductive functions in aged animals can be totally reversed by the administration of DHEA during 2.5 d clearly indicates that this steroid can restore the GnRH neuronal activity. The mechanism of action of DHEA in the increase of GnRH gene expression is still unknown. It might be related to a restoration of the levels of estrogens and androgens in the hypothalamus and other brain structures. Since DHEA and DHEAS exert an antagonist activity on GABAA receptors *(29)*, it might be also considered that this action of the steroids could interfere with the inhibitory influence of the GABAergic system on GnRH gene expression. We have recently demonstrated that a variety of $GABA_A$ receptor agonists can strongly inhibit GnRH gene expression *(27,30)*.

The present results also strongly suggest that increase in GnRH neuronal activity might play a role in the beneficial effects of DHEA in different systems in aged rats. Among these effects, there is the improvement of memory induced by DHEA in middle-aged and old animals *(8)*. Although the mechanism involved in the action of DHEA on memory is unknown, it might be related to an increase in GnRH neuronal activity, since high levels of GnRH

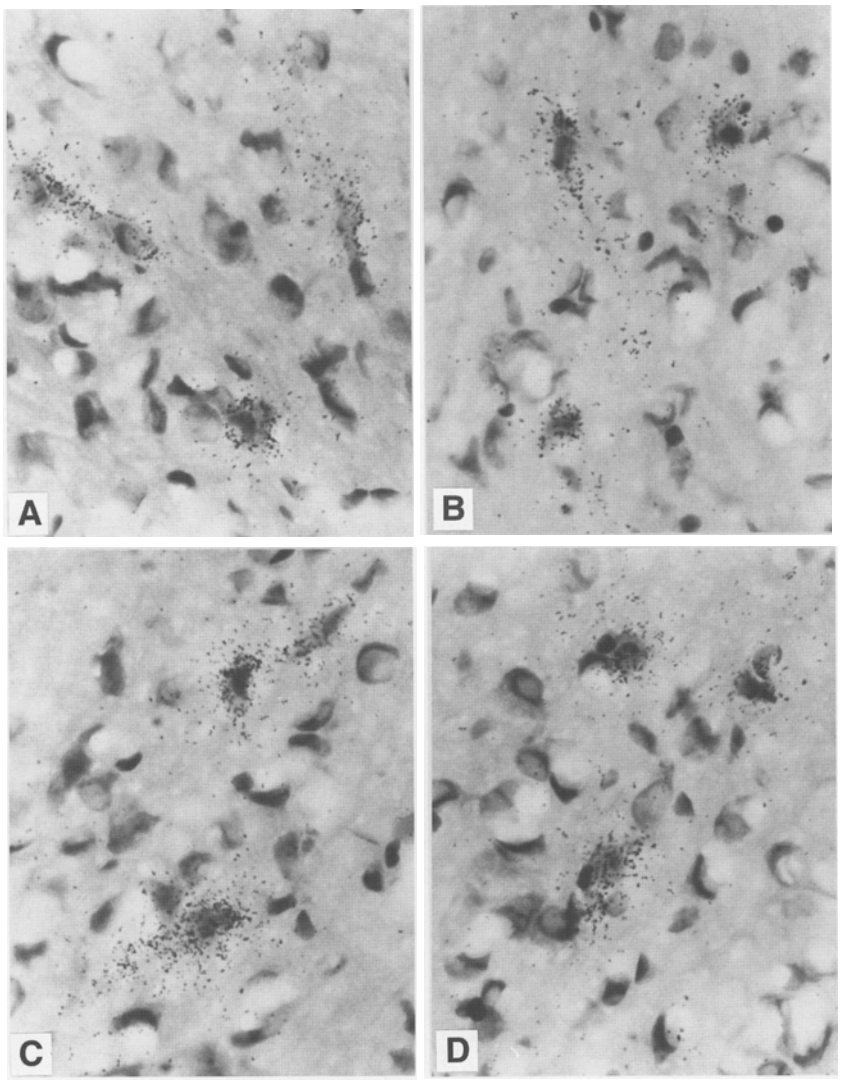

Fig. 3. Light microscope autoradiograph illustrating the hybridization signal in the medial preoptic area of young **(A)** and aged **(B)** female rats. Silver grains are concentrated over a few neurons. **(C)** Young animals treated with DHEA. **(D)** Aged animals treated with DHEA (×800).

receptors have been found in the hippocampus *(31)*, a brain structure that plays a key role in memory process. Very recently *(32)* have demonstrated that administration of a GnRH analog could improve working memory in aged female rats without affecting sex hormone plasma levels.

DHEA has also been shown to prevent and reverse some age-related defects in immunoprotection *(20)* and to also to prevent or stop the progression of some cancers *(18,33)* in the rat. Since immunodeficiency might be involved in the development of cancer during aging, it is possible that there is some relationship between cancer development and immune deficiency. In relation to the protective effect of DHEA on immune system, it has been very recently demonstrated that DHEA is an effective vaccine adjuvant in elderly humans *(34)*. GnRH might also be involved in the restoration in the immune system since treatment of aging female and male rats (16–17 mo old) with a potent GnRH agonist reversed the age-related decrease in thymus weight *(9)*. This treatment also restored thymic structure, which had completely disappeared in old rats, leaving only thymic remnants. It is then possible that the action of DHEA on the immune system might be completely or partially mediated by GnRH.

In conclusion, it then appears that aging induces a decrease in GnRH gene expression. This effect can be completely reversed by a short-term treatment with DHEA, supporting the hypothesis that DHEA might play role in the prevention and/or improvement of some deficits associated with aging through stimulation of GnRH synthesis.

Materials and Methods

Animals

Young (50–54 d old) and old (18 m of age) rats (Sprague-Dawley) of both sexes were housed in a light (14 h of light/

Table 1
Estimated Number of Labeled Neurons Per Rat in the Area of the OVLT (Mean + SEM)

Group	*Neurons*
Male Rats Young	205 ± 31
Young treated with DHEA	214 ± 22
Old	208 ± 26
Old treated with DHEA	201 ± 30
Female Rats Young	220 ± 19
Young treated with DHEA	212 ± 26
Old	230 ± 28
Old treated with DHEA	225 ± 19

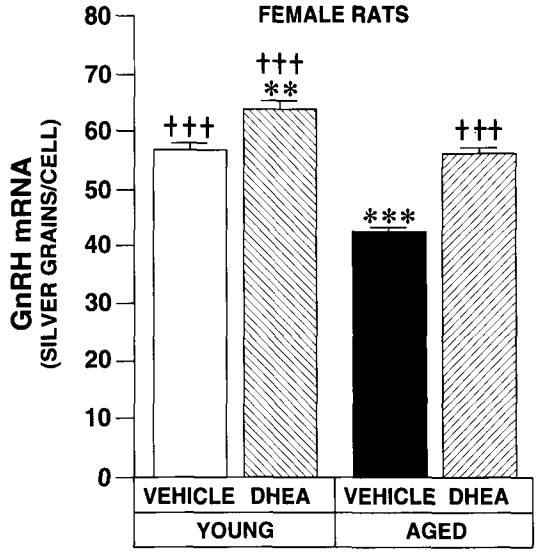

Fig. 4. Effect of DHEA on GnRH mRNA levels as measured by the number of grains overlying labeled neurons in the young and aged female rat. ***$p < 0.001$, **$p < 0.01$, vehicle-treated young animals vs. all other experimental groups; +++, $p < 0.001$, vehicle-treated old animals vs all other groups.

day, lights on at 0500 h) and temperature-controlled (22 ± 1°C) environment. They received Purina rat chow (Ralston-Purina, St. Louis, MO) and tap water ad libitum. In young females, vaginal smears were monitored daily, and only those exhibiting regular 4-d estrous cycle were included in the study. They were perfused on the day of diestrus 1 between 9.00 and 10.00 h.

Treatments

Four groups (6 animals/group) of male rats as well as four groups of female rats (6 animals/group) were used. Two groups of young animals of each sex (50–54 d old) received 5 s. c. injections of DHEA (12 mg/kg, body wt) at internals of 12 h. Aged rats of both sexes (18 mo of age) received a similar treatment. Control animals received vehicle (1% gelatin [V/V], 0.9% NaCl, and 5% ethanol) instead of DHEA. The animals were killed 6 h after the last injection. They were anesthetized with Ketalar (100 mg/kg, body wt) plus Xylosine (100 mg/mL/kg body wt) and then perfused for histological procedures as described below.

In Situ *Hybridization*

Brains were fixed by vascular perfusion with 200 mL of 4% (w/v) paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). They were then quickly frozen. Because it is now well established that, in the rat brain, the vast majority of GnRH-producing neuronal cell bodies are located in the preoptica area and anterior hypothalamus *(21,27)*, 10-µm thick coronal sections were serially cut through an area extending from the preoptic area to the anterior hypothalamus. The sections were then mounted onto gelatin- and poly-L-lysine-coated slides.

In situ hybridization was performed as described previously *(35)*. The probe chosen was a synthetic deoxyribonucleotide complementary to the GnRH coding region of the rat cDNA (bases 102–149) described by *(36)*. This probe was synthesized in our laboratory using the Biosearch DNA synthesizer and purified on a 15% polyacrylamide/8 *M* urea preparative sequencing gel. It was labeled on the 3'-end with (^{35}S)dATP (1000 µC/mmol; Amersham Co., Oakville, Ontario, Canada) and terminal deoxynucleotidyl transferase (Boeringher Mannheim, Montreal, Canada) to a specific activity of about 5000 µCi/mmol. The sections were prehybridized for 1 h in buffer A containing 50% (v/v) formamide, 5X SSPE (1X SSPE being 0.18 *M* NaCl, 10 m*M* NaH_2PO_4, pH 7.4, 1 m*M* EDTA), 0.1% (w/v) sodium dodecyl sulfate, 0.1% (w/v) BSA, 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylo-pyrrolindone, and 20 µm poly(A)/mL. Hybridization was carried out for 24 h at 42°C in buffer B (buffer A containing 4% [w/v] dextran sulfate) containing the labeled probe in a saturating concentration (10^7 cpm/section). After hybridization, sections were rinsed at room temperature for 2 h in 2X SSC (1X SSC being 0.15 *M* NaCl and 0.015 *M* sodium citrate, pH 7.0), 2 h in 1X SSC, 1 h in 0.5X SSC, and 1 h in 0.5X SSC at 37°C followed by one rinse of 1 h in 0.5X SSC at room temperature. Finally, the sections were dehydrated and coated with liquid photographic emulsion (Kodak NTB-2). After 8 d of exposure, the sections were processed and stained with hematoxylin and eosin. To assess the specificity of the hybridization signal, consecutive sections were alternatively hybridized with the labeled oligonucleotide probe encoding GnRH and a labeled sense oligomer directed to the complementary DNA strand. As an additional control, sections from each group were treated with pancreatic RNase A (20 µg/mL) (Boeringher Mannheim, Montral, Canada) for 30 min at room temperature before prehybridization.

Quantification of GnRH mRNA

Localization of neurons expressing GnRH mRNA as well as the count of grains per labeled neuron obtained

after hybridization with the ^{35}S-labeled probe were performed in a random order by an observer unaware of the treatment received. A neuron was considered specifically labeled if the number of grains overlying the cell exceeded five times the background count. For each experimental group, the mean number of silver grains per cell was calculated from at least 250 ± 10 cells from 6 animals/group. Cell count of labeled neurons was also performed using 7 sections/animal through the OVLT. For each animal, the total number of labeled cells detected in the 7 sections was used for statistical analysis ($n = 6$). Statistical significance was determined according to the multiple range test of Duncan-Kramer *(37)*.

References

1. Corpas, E., Harman, M., and Blackman, M. R. (1993). *Endocr. Rev.* **14,** 20–39.
2. Migeon, C. J., Killer, A. R., Lawrence, B., and Sheppord, T. H. (1957) *J. Clin. Endocrinol. Metab.* **17,** 1051–1060.
3. Orentreich, N., Brind, J. L., Vogelman, J. H., Andres, R., and Baldwin, H. (1992). *J. Clin. Endocrinol. Metab.* **75,** 1002–1004.
4. Thomas, G., Frenoy, N., Legrain, S., Sebag-Lanoe, R., Baulieu, E. E., and Debuire, B. (1994). *J. Clin. Encrinol. Metab.* **79,** 1273–1276.
5. Baulieu, E. E. (1996). *J. Clin. Endocrinol. Metab.* **181,** 3147–3151.
6. O'Malley, B. W. (1984). *J. Clin. Invest.* **74,** 307–312.
7. Cleary, M. P., Seidenstat, R., Tanner, R.H., and Schwartz, A. G. (1982). *Proc. Soc. Exp. Biol. Med.* **171,** 272–284.
8. Flood, J. F. and Roberts, E. (1988). *Brain Res.* **448,** 178–181.
9. Marchetti, B., Guarello, V., Morale, M. C., Bartoloni, G., Rinti, F., Palumbo, G., Farinello, Z., Cordaro, S., and Scopagnirin, V. (1989). *Endocrinology* **125,** 1037–1043.
10. Morales, A. J., Nolan, J. J., Nelson, J. C., and Yen, S. S. C. (1994). *J. Clin. Endocrinol. Metab.* **78,** 1360–1367.
11. Hoffmann, G. E. and Sladek, J. R. (1980). *Neurobiol. Aging* **1(1),** 27–37.
12. Wise, P. M., Scarbrough, K., Larson, G. H., Lloyd, J. M., Weiland, N. G., and Chiu, S. (1991). *Frontiers in Neuroendocrinol.* **12,** 323–356.
13. Wise, P. M. and Ratner, A. (1980). *Neuroendocrinology* **30,** 15–19.
14. Dorsa, D. M., Smith, E. R., and Davidson, J. M. (1984). *Neurobiol. Aging* **(5),** 115–120.
15. Gruenewal, D. A. and Matsumoto, A. M. (1991). *Endocrinology* **129,** 2442-2450.
16. Li, S., de Yebenes, E. G., and Pelletier, G. (1995). *Peptides* **16,** 425–430.
17. Gordon, G. B., Bush, D. E., and Weisman, H. F. (1988). *J. Clin. Invest.* **82,** 712–720.
18. Schwartz, A. G., Whitcomb, J. W., Nyce, J. W., Lewbart, M. L., and Pashko, L. L. (1988). *Adv. Cancer Res.* **51,** 391–424.
19. McIntosh, M. K. and Berdanier, C. D. (1991). *J. Nutr.* **121,** 2037–2043.
20. Daynes, R. A. and Araneo, B. A. (1992). *Aging Immun. Infect. Dis.* **3,** 135–154.
21. Pelletier, G. (1980). In: *Advances in Sex Hormone Research.* Thomas, J. A., and Singhal, R. L. (eds.). Urban and Schwarzenberg, Baltimore, pp. 197–211.
22. Silverman, A. J., Jhamandas, J. and Renaud, L. P. (1987). *J. Neurosci.* **7,** 2312–2319.
23. Toranzo, D., Dupont, E., Simard, J., Labrie, C., Couët, J., Labrie, F., and Pelletier, G. (1989). *Mol. Endocrinol.* **3,** 1748–1756.
24. Park, O. K., Gugneja, S., and Mayo, K. E. (1990). *Endocrinology* **127,** 365–372.
25. Gore, A. C. and Roberts, L. L. (1995) *Endocrinology* **136,** 889–896.
26. Jarjour, L. T., Handelsman, D. J., and Serdloff, R. S. (1986). *Endocrinology,* **119(3),** 1113–1117.
27. Li, S., Garcia de Yebenes, E., and Pelletier, G. (1995) *Peptides* **16,** 425–430.
28. Kim, K., Lee, B. J., Park, Y., and Cho, W. K. (1989). *Mol. Brain. Res.* **6,** 151–158.
29. Majweska, M. D. (1995). *Ann. NY Acad. Sci.* **774,** 111–120.
30. Vincens, M., Li, S. Y., and Pelletier, G. (1994). *Eur. J. Pharmacol.* **260,** 157–162.
31. Badr, M., Marchetti, B., and Pelletier, G. (1989). *Dev. Brain Res.* **45,** 179–184.
32. Alliot, J., Nauton, P., and Bruhat, M. A. (1993). *Psychoneuroendocrinology* **18(8),** 543–550.
33. Li, S. and Pelletier, G. (1993). *Neuroendocrinology* **58,** 136–139.
34. Araneo, B., Dowell, T., Woods, M. L., Daynes, R., Judd, M., and Evans, T. (1995). *Ann. NY Acad. Sci.* **774,** 232–248.
35. Li, S. and Pelletier, G. (1992). *Endocrinology* **131,** 395–399.
36. Adelman, J. P., Mason, A. J., Hayflick, J. S., and Seeburg, P. H. (1986). *Proc. Natl. Acad. Sci. USA* **83,** 179–183.