Endocrine, *vol. 9, no. 1, 33–37, August 1998* 0969–711X/98/9:33–37/$9.25

Responsiveness of Tuberoinfundibular Dopaminergic Neurons to 5-Hydroxytryptophan

Effects of Aging

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Serotonin is known to stimulate prolactin secretion by decreasing tyrosine hydroxylase (TH) activity in the tuberoinfundibular dopaminergic (TIDA) neurons. However, the effects of aging on the responsiveness of TIDA neurons to serotonin are not known. An effective way to increase serotonergic activity is to administer 5-hydroxytryptophan (5-HTP), a serotonin precursor. The present study was done to investigate the effects of 5-HTP on TIDA neuronal activity in aging animals. Middle-aged (10–12 mo), old (18–20 mo), and very old (22–24 mo) female Sprague-Dawley rats were bilaterally ovariectomized. Ten days later, they were injected iv with 50 mg/kg body wt of 5-HTP or the vehicle for 5-HTP (PBS-HCl). Twenty minutes later, *m*-hydroxybenzylhydrazine (NSD), a DOPA decarboxylase inhibitor, was administered. Ten minutes later, the animals were killed, and tyrosine hydroxylase (TH) activity was determined by measuring L-DOPA accumulation in the stalk median eminence by HPLC-EC. In all three groups, administration of 5-HTP increased serum prolactin levels significantly. In control middle-aged rats, TH activity (L-DOPA pg/μg protein) was 33.0 ± 5.6. Treatment with 5-HTP decreased TH activity by 60%. Similarly, 5-HTP treatment decreased TH activity by 52 and 56% in 18- to 20- and 22- to 24-mo-old rats, respectively, compared to the control rats. The magnitudes of the 5-HTP-induced decreases in TH activities in middle-aged, old, and very old rats were not different from each other. These results indicate that TIDA neuronal responsiveness to serotonin does not change with age and that 5-HTP is capable of stimulating PRL release even in very old rats.

Key Words: Aging; 5-hydroxytryptophan; TIDA, PRL.

Received December 10, 1997; Revised February 12 and March 23, 1998; Accepted April 17, 1998.

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Introduction

Prolactin secretion is under tonic inhibitory control of the hypothalamus, especially the hypothalamic dopaminergic system. Dopamine (DA) from the tuberoinfundibular dopaminergic neurons (TIDA) is a major prolactin-inhibiting factor *(1)*. TIDA neurons have their cell bodies in the arcuate nucleus and their terminals in the median eminence *(2,3)*. DA released from these terminals reaches the pituitary lactotrophs through the hypothalamo-hypophyseal portal vessels and inhibits PRL secretion *(1,2)*. In addition to tonic inhibitory regulation by the TIDA neurons, PRL secretion is also stimulated by many hypothalamic factors, including serotonin.

Serotonin, its precursor 5-hydroxytryptophan, serotonin uptake inhibitors, and serotonin agonists have all been shown to stimulate PRL secretion *(4)*. Since pituitary lactotrophs are not directly affected by serotonergic agents, serotonergic stimulation of PRL release is thought to be brought about by an indirect mechanism *(4)*. There is evidence that serotonin-induced increase in PRL secretion might involve stimulation of a prolactin-releasing factor, as well as inhibition of DA release into the portal blood *(5,6)*. Recently, we provided evidence that central administration of serotonin decreased both tyrosine hydroxylase (TH) catalytic activity and TH messenger ribonucleic acid signal levels in the hypothalamus of female rats, indicating that serotonin stimulates PRL release through inhibition of DA release from the TIDA neurons *(7)*.

It is known that the ability of TIDA neurons to produce DA is greatly reduced in aging animals *(1,8–10)*. In contrast, serotonergic activity of the hypothalamus has been shown to increase with age *(8,11)*. Since TIDA neurons may be involved in serotonin-induced increase in PRL secretion, it is possible that the responsiveness of TIDA neurons to serotonin may also be altered in aging animals. However, the effects of aging on serotonin-induced stimulation of PRL have not been investigated. The aim of the present study was to measure the effects of 5-hydroxytryptophan (5-HTP), a serotonin precursor, on PRL secretion during aging and to correlate changes in tyrosine hydroxylase activity in the stalk median eminence with changes in PRL secretion.

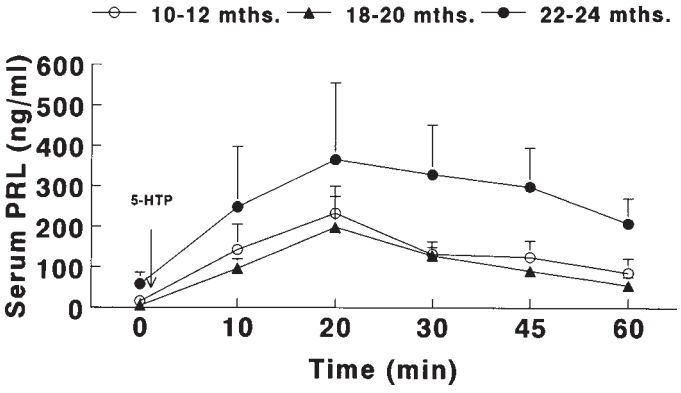

Fig. 1. Effects of 5-HTP on serum PRL. Regularly cycling 10- to 12-mo-old ($n = 5$), persistent estrous 18- to 20-mo-old ($n = 6$), and persistent diestrous 22- to 24-mo ($n = 5$) rats were used in the experiment. They were ovariectomized bilaterally. After collection of a pretreatment blood sample (0 min) through a jugular catheter, they were administered iv with 50 mg/kg body weight of 5-HTP. Posttreatment blood samples were collected at 10, 20, 30, 45, and 60 min. Serum PRL levels at 10, 20, 30, 45, and 60 min after 5-HTP administration were significantly higher ($p < 0.05$) compared to the pretreatment levels (0 min) in all three age groups.

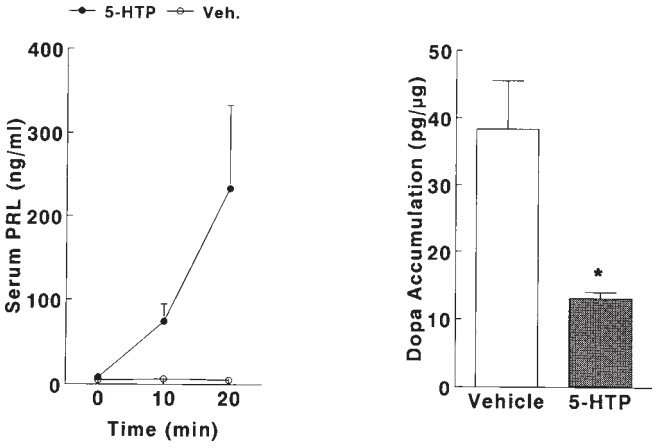

Fig. 2. Effects of 5-HTP ($n = 6$) or its vehicle ($n = 6$) on serum PRL and TH activity in the SME of 10- to 12-mo-old rats. After collecting the 20-min blood sample, rats were administered m-hydroxybenzylhydrazine (NSD), a DOPA decarboxylase inhibitor. Ten minutes later, they were sacrificed, and the SME was dissected out and TH activity was measured in terms of L-DOPA accumulation using HPLC. *Significantly different from controls ($p < 0.05$). Serum PRL levels at 10 and 20 min after 5-HTP administration were significantly ($p < 0.05$) higher compared to the pretreatment levels (0 min).

Results

Effects of 5-HTP on Serum PRL Levels in Aging Animals

Serum PRL concentrations (mean ± SE; ng/mL) following administration of 5-HTP in various age groups are shown in Fig. 1. The mean serum PRL concentrations in 10- to 12-mo-old rats before administration of 5-HTP were 15.9 ± 4.6. Injection of 5-HTP increased PRL concentrations significantly within 10 min (142.3 ± 62.9), which reached maximum concentrations of 232.5 ± 66.1 at 20 min. After this, PRL levels started to decline, but at 60 min were still significantly higher (85.8 ± 35.5, $p < 0.05$) than the basal levels. A similar profile in PRL release was observed in 18- to 20-mo-old rats after administration of 5-HTP. Compared to the 10- to 12-mo-old and 18- to 20-mo-old rats, basal PRL concentrations in 22- to 24-mo-old rats were significantly higher (57.8 ± 29.3, $p < 0.05$). In these animals, 5-HTP produced similar increases in PRL concentrations at 10 min (247.6 ± 149.7) and at 20 min (364.6 ± 190.3); PRL levels remained significantly ($p < 0.05$) elevated at 60 min.

Effects of 5-HTP or Vehicle on Serum PRL Levels and TH Activity in the SME

The effects of 5-HTP on serum PRL levels and on TH activity in the SME of 10- to 12-mo-old rats are shown in Fig. 2. Serum PRL concentrations before treatment in the control and 5-HTP-treated groups were not different from each other (5.1 ± 3.1 and 8.2 ± 1.7, respectively). Treatment with the vehicle did not change serum PRL concentrations, but administration of 5-HTP increased serum PRL concentrations significantly to 233.2 ± 99.6 within 20 min ($p < 0.05$). Administration of 5-HTP also resulted in a significant reduction in the accumulation of L-DOPA (13.0 ± 1.0 pg/μg) in the SME when compared to that in the control group (38.3 ± 7.2, $p < 0.05$).

In 18- to 20-mo-old rats, basal PRL concentrations before treatment in the control and 5-HTP-treated groups were not significantly different from each other (Fig. 3). As in 10- to 12-mo-old rats, administration of 5-HTP increased serum PRL concentration significantly from 41.6 ± 15.1 to 101.4 ± 38.0 within 20 min ($p < 0.05$). Similarly, administration of 5-HTP produced a significant decrease in L-DOPA accumulation (pg/μg) in the SME (13.2 ± 3.3) when compared to that in the vehicle-treated rats (27.1 ± 4.3, $p < 0.05$).

Administration of the vehicle alone did not change serum PRL concentrations in 22- to 24-mo-old rats (Fig. 4). As in the other age groups, administration of 5-HTP increased PRL concentrations from 88.0 ± 50.1 to 472.7 ± 52.5 within 20 min ($p < 0.05$). Treatment with 5-HTP also resulted in a marked decrease in L-DOPA accumulation in the SME (13.3 ± 2.9) when compared to that in the control rats (24.5 ± 2.9; $p < 0.05$).

Discussion

This study provides evidence that 5-HTP, a serotonin precursor, stimulates PRL secretion in middle-aged and old animals. Our findings also indicate that 5-HTP-induced increase in PRL secretion is accompanied by a decrease in TIDA neuronal activity, and this effect is not lost in aging animals. The 5-HTP-induced increases in PRL secretion were similar in 10- to 12-, 18- to 20-, and 22- to 24-mo-old rats. Even though the basal levels of PRL were significantly higher in 22- to 24-mo-old rats compared to those in the

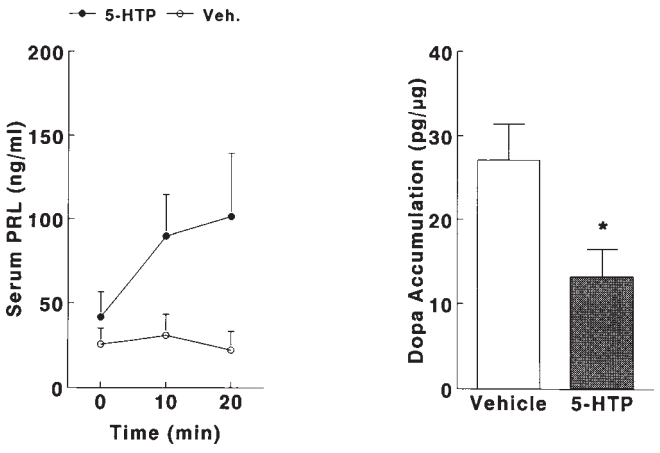

Fig. 3. Effects of 5-HTP ($n = 6$) or its vehicle ($n = 5$) on serum PRL and TH activity in the SME of 18- to 20-mo-old rats. *See* Fig. 2 legend for details. *Significantly different from controls ($p < 0.05$). Serum PRL levels at 10 and 20 min after 5-HTP administration were significantly ($p < 0.05$) higher compared to the pretreatment levels (0 min).

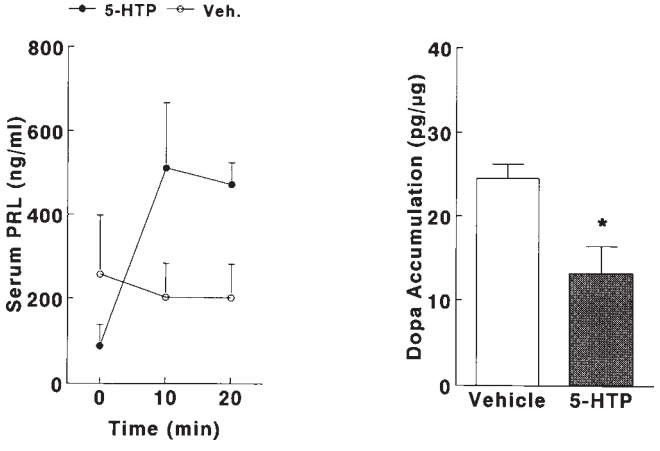

Fig. 4. Effects of 5-HTP ($n = 7$) or its vehicle ($n = 6$) on serum PRL and tyrosine hydroxylase (TH) activity in the SME of 22- to 24-mo-old rats. *See* Fig. 2 legend for details. *Significantly different from controls ($p < 0.05$). Serum PRL levels at 10 and 20 min after 5-HTP administration were significantly ($p < 0.05$) higher compared to the pretreatment levels (0 min).

other groups, 5-HTP still produced a significant increase in these animals.

PRL secretion is primarily under the inhibitory regulation of DA from the TIDA neurons *(1,2)*. DA from these neurons enters the hypothalamo–hypophyseal portal blood and acts directly on the lactotrophs to inhibit the secretion of PRL *(1,2)*. It is now known that PRL secretion is affected by many factors in the hypothalamus, including serotonin *(2)*. The central serotonergic system has been shown to have a stimulatory role in the secretion of PRL *(2,4)*. Drugs that can increase brain serotonin levels, such as serotonin precursors, serotonin uptake inhibitors, and serotonin agonists, have all been shown to stimulate PRL secretion *(4)*. Conversely, drugs that inhibit or decrease brain serotonergic activity have been shown to block PRL surges produced by estrogen, and those induced by suckling and pregnancy *(4,12–18)*. Thus, it is not surprising that administration of 5-HTP, a serotonin precursor, increases PRL secretion. Although it is known that serotonin can affect PRL secretion in young animals, it is not known whether aging results in a change in the responsiveness of neural mechanisms to serotonergic input. This study provides evidence that this regulation is intact in aging animals.

The serotonin precursor, 5-HTP, used in this study has been used by several investigators to increase serotonin levels *(4,19,20)*. The effects of 5-HTP on other body functions have been described elsewhere *(21)*. The mechanism by which serotonin increases PRL secretion is still controversial. Since serotonergic agents that stimulate PRL secretion do not affect pituitary lactotrophs, serotonin-induced increase in PRL is thought to occur by an indirect mechanism *(4)*. There is evidence that serotonin-induced increase in PRL might involve a prolactin-releasing factor *(5)*. Serotonin has also been shown to increase the release of several brain peptides, such as thyrotropin-releasing hormone, vasoactive intestinal peptide, neurotensin, and β-endorphin, all of which are known to stimulate PRL secretion *(22–24)*. Serotonin could also produce its stimulatory effect on PRL secretion by affecting TIDA neuronal activity *(2)*. It has been shown that central administration of serotonin reduced DA levels in the hypophyseal portal blood while increasing PRL levels *(6)*. On the contrary, studies have also shown that serotonin can stimulate PRL secretion even after DA synthesis has been inhibited or after blockade of DA receptors *(25)*. However, recently we provided evidence that central administration of serotonin resulted in a significant decrease in TH activity and TH mRNA in the TIDA neurons *(7)*. TH is the rate-limiting enzyme in the synthesis of DA. Since central administration of serotonin decreases TH mRNA and TH activity in the TIDA neurons, the resulting decrease in DA levels could be responsible for an increase in PRL secretion. Further support for this mechanism of action comes from the fact that the arcuate nucleus, where the cell bodies of the TIDA neurons are located, receives serotonergic innervation from the raphe nuclei *(25–28)* and that serotonin containing axons have been found in close proximity to DA cell bodies in the arcuate nucleus *(29,30)*. In the present study, 5-HTP treatment resulted in maximum increases in PRL secretion within 20 min, and produced a significant decrease in TH activity in the stalk median eminence. This suggests that 5-HTP stimulates PRL secretion at least partially by inhibiting TIDA neuronal activity.

The principal focus of this study was responsiveness of the TIDA neurons to 5-HTP in aging animals. Aging is marked by changes in central and neuroendocrine functions *(8)*. It also produces marked changes in the activity of the TIDA neurons *(1,9,10,31)*. There is evidence that aging

might reduce responsiveness of the TIDA neurons to PRL *(32)*. However, it is not clear whether responsiveness of the TIDA neurons to 5-HTP is altered during aging. It has been shown that although hypothalamic serotonergic activity increases with aging, the dopaminergic activity decreases concurrently *(8,11)*. It is not known whether these two changes are related. It is possible that the increased serotonergic activity observed in aging animals might increase its inhibitory effect on TIDA neurons and decrease DA levels. To test whether DA neurons retain their responsiveness to serotonin as the rat ages, 5-HTP was administered exogenously. A marked decrease in TH activity was observed in all the age groups after administration of 5-HTP providing support to the hypothesis that TIDA neurons remain responsive to serotonergic input with advancing age. Moreover, the decrease in TH activity after 5-HTP administration was correlated with an increase in PRL levels in these animals. The basal levels of PRL were high in 22- to 24-mo-old rats. Given the observation that an increase in serotonergic activity occurs in the hypothalamus of aging animals *(8,11)*, it is possible that this naturally occurring increase in serotonergic activity promotes an increase in PRL secretion in aging animals by inhibition of TIDA neurons.

The increase in PRL levels observed after 5-HTP administration in 10- to 12-mo-old animals was similar to that observed previously in young (3- to 4-mo-old) animals *(2,4)*. The response in terms of PRL levels within 20 min after administration of 5-HTP was much greater in the 10- to 12-mo-old animals (about 28-fold increase) when compared to the 18- to 20-mo-old (about twofold increase) and 22- to 24-mo-old (about fivefold increase) animals. However, the changes in TH activity in the stalk median eminence were of similar magnitude in all the three age groups studied. Although the reasons for this difference in PRL responses are not clear, other brain chemicals that could be involved in the serotonin-induced increase in PRL *(5,22–24)* and other factors, such as age, reproductive status, and hormonal milieu of the animals, could have contributed to this phenomenon *(1,2,8,11)*.

In summary, the results from this study indicate that 5-HTP stimulates PRL secretion even in aging animals, which have high basal levels of PRL. Our data also provide evidence that 5-HTP-induced increase in PRL release is at least partially mediated through a decrease in TIDA neuronal activity and that this mechanism is not lost in aging animals.

Materials and Methods

Animals

Seven- to 8-mo-old retired female Sprague-Dawley rats (SASCO, Omaha, NE) were housed in air-conditioned (23 ± 2°C) and light-controlled (lights on between 0500 and 1900 h) rooms, and were provided with rat chow and water ad libitum (Kansas State University, Manhattan, KS). When they reached the ages of 10–12, 18–20, or 22–24 mo, their estrous cycles were monitored by daily examination of vaginal smears for a period of 3 wk. About 60% of the 10- to 12-mo-old rats exhibited regular estrous cycles, 60–70% of the 18- to 20-mo-old rats exhibited persistent estrus and about 80% of the 22- to 24-mo-old rats exhibited persistent diestrus. In the 10- to 12-mo group, rats that showed two regular estrous cycles were used in the third cycle. Persistent estrous (18–20 mo) and persistent diestrous (22–24 mo) rats were selected based on continuous estrous or diestrous smears for a period of at least 2 wk. After determining the stage of the estrous cycle, the rats were ovariectomized bilaterally and were given a rest period of 10 d before using them in this experiment.

Jugular Catheterization

On the morning of the experiment, rats were implanted with jugular catheters. Details of the jugular catheterization procedure have been described previously *(33)*. Briefly, the catheter, made out of Silastic tubing, was inserted into the right jugular vein under light ether anesthesia. The free end of the catheter was passed underneath the skin and was exteriorized near the base of the skull. It was rinsed with heparinized saline (10 U/mL) and was used for infusion of 5-HTP or its vehicle, *m*-hydroxybenzylhydrazine (NSD), and for blood sampling.

Treatment

In experiment 1, 10- to 12-, 18- to 20-, and 22- to 24-mo-old ovariectomized rats were injected iv. with 50 mg kg/body weight of 5-HTP in 1 mL of 0.5 *M* HCl/0.5 mL PBS (v/v). Serial blood samples were collected at 0, 10, 20, 30, 45, and 60 min after administration of 5-HTP. In experiment 2, similar groups of rats were injected iv with the same amount of 5-HTP or its vehicle. Serial blood samples were collected at 0, 10, and 20 min after administration of 5-HTP or the vehicle. The samples were refrigerated overnight, and the serum was separated by centrifugation. The serum samples were stored at –20°C until PRL determination by RIA. After collecting the 20-min sample, the rats were injected iv with 25 mg/kg body weight of *m*-hydroxybenzylhydrazine (NSD), a DOPA decarboxylase inhibitor to prevent conversion of L-DOPA to dopamine. Ten minutes later, the rats were sacrificed and their brains were removed carefully, and the stalk median eminence (SME) was dissected out with a pair of fine scissors under a stereomicroscope as described before *(34)*.

Measurement of TH Activity

The SME was homogenized in 60 μL of 0.1 *M* $HClO_4$ and centrifuged in a refrigerated centrifuge for 10 min to precipitate the protein. L-DOPA concentrations in the supernatant were determined by HPLC-EC. The pellet was dissolved in 25 μL of 0.5 *N* NaOH. PBS (75 μL) was added to it to bring the volume to 100 μL. These samples were stored at –20°C until they were assayed for protein content

by the Bradford method using a Bio-Rad (Bio-Rad, Hercules, CA) protein assay kit.

HPLC-EC

The details of the high-performance liquid chromatography with electrochemic detection (HPLC-EC) procedure have been described previously *(9,10,33,35)*. The HPLC system (Kansas State University) consisted of a rheodyne injector, LC-6A pump (Shimadzu, Columbia, MD), LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN), C-R4A Chromatopac integrator (Shimadzu), and a CTO-6A column oven (Shimadzu), which maintained a C18 reverse-phase, 5-μm particle size, 250-mm long analytical column (Bioanalytical Systems) under a constant temperature of 37°C. The mobile phase consisting of monochloroacetic acid (14.15 g/L), octanesulfonic acid (0.3 g/L), EDTA (0.25 g/L), sodium hydroxide (4.675 g/L), and acetonitrile (3.5%) in pyrogen-free water and was pumped by the LC-6A pump at a flow rate of 1.7 mL/min. The pH of the mobile phase was 3.1. The mobile phase was filtered and degassed with the use of the Milli-Q water-purification apparatus. The LC-4B detector had a sensitivity of 1 nA full scale, and the potential of the working electrode was 0.65 V with respect to an Ag/AgCl reference electrode. Fifty microliters of the supernatant along with 25 μL of internal standard (dihydroxybenzylhydrazine) were injected into the HPLC system for determination of L-DOPA concentrations, which were expressed in terms of pg/μg protein.

Radioimmunoassay (RIA)

Serum PRL concentrations (ng/mL) were determined using a double-antibody RIA as described before *(33,35)*. PRL label was obtained from New England Nuclear (Boston, MA), and PRL antibody and standards were obtained from NIDDK (Bethesda, MD). The reference preparation, rPRL-RP-3, had a potency of 30 IU/mg. The assay had a sensitivity of <10 pg.

Statistical Analysis

The data on the effects of 5-HTP on serum prolactin concentrations were analyzed using one-way repeated measures analysis of variance followed by Fisher's least-significant difference test. The data on the effects of 5-HTP on TH activity were analyzed using one-way analysis of variance followed by Fisher's least-significant difference test.

Acknowledgments

This work was supported by a grant AG 11561 to J. L. V. from the National Institutes of Health. We thank Shawn Taylor and Joseph Francis, Neuroendocrine Research Laboratory, Kansas State University, Manhattan, KS 66506, for their technical assistance in this study. Preliminary results from this study were presented in the annual meeting of the Endocrine Society, 1997.

References

1. Meites, J. (1982). *Neuroendocrinology* **34,** 151–156.
2. Ben-Jonathan, N., Arbogast, L. A., and Hyde, J. F. (1989). *Prog. Neurobiol.* **33,** 399–477.
3. Bjorklund, A., Flack, B., Nobin, A., and Stenevi, U. (1974) In: *Neurosecretion: The Final Neuroendocrine Pathway.* Knowles, F. and Vollarath, L. (eds.). Springer Verlag, New York, p. 209.
4. Tuomisto, J. and Mannisto, P. (1985). *Pharmacol. Rev.* **37,** 249–332.
5. Clemens, J. A., Roush, M. E., and Fuller, R. W. (1978). *Life Sci.* **22,** 2209–2214.
6. Pilotte, N. S. and Porter, J. C. (1981). *Endocrinology* **108,** 2137–2141.
7. Mathisen, J. R., Arbogast, L. A., and Voogt, J. L. (1992). *J. Neuroendocrinolo.* **4,** 631–639.
8. Meites, J. (1991). *Acta. Endocrinol. Copenh.* **125,** 98–103.
9. MohanKumar, P. S., ThyagaRajan, S., and Quadri, S. K. (1997). *Brain Res. Bull.* **42,** 265–271.
10. ThyagaRajan, S., MohanKumar, P. S., and Quadri, S. K. (1995). *Brain Res.* **689,** 122–128.
11. Steger, R. W., DePaolo, L. V., and Shepherd, A. M. (1985). *Neurobiol. Aging* **6,** 113–116.
12. Arey, B. J. and Freeman, M. E. (1989). *Endocrinology* **124,** 878–883.
13. Jahn, G. A. and Deis, R. P. (1987). *J. Endocrinol.* **11,** 367–374.
14. Mistry, A. M. and Voogt, J. L. (1989). *Endocrinology* **125,** 2875–2880.
15. Mistry, A. M. and Voogt, J. L. (1990). *Life Sci.* **47,** 693–701.
16. Pan, J.-T. and Gala, R. R. (1987). *Endocrinology* **120,** 2070–2077.
17. Pan, J.-T. and Gala, R. R. (1988). *Life Sci.* **42,** 1869–1874.
18. Pan, J.-T. and Wang, P. S. (1989). *Neuroendocrinology* **49,** 281–285.
19. Hyde, J. F. (1992). *Brain Res.* **573,** 204–208.
20. Li, X. M., Perry, K. W., and Fuller, R. W. (1996). *J. Pharm. Pharmacol.* **48,** 825–828.
21. Müller, E. E. and Nistico, G. (1988). In: *Brain Messengers and the Pituitary*. Academic, San Diego.
22. Krulich, L., Vijayan, E., Coppings, R. J., Giacheti, A., McCann, S. M., and Mayfield, M. A. (1979). *Endocrinology* **105,** 276–283.
23. Shimatsu, A., Kato, Y., Matsushita, N., Katakami, H., Yanaihara, N., and Imura, H. (1982). *Endocrinology* **111,** 338–340.
24. Smythe, G. A., Bradshaw, J. E., Cai, W. Y., and Symons, R. G. (1982). *Endocrinology* **111,** 1181–1191.
25. Krulich, L., Coppings, R. J., Giachetti, A., McCann, S. M., and Mayfield, M. A. (1980). *Neuroendocrinology* **30,** 133–138.
26. Beaudet, A. and Descarries, L. (1979). *Brain Res.* **160,** 231–243.
27. Descarries, L. and Beaudet, A. (1978) In: *Cell Biology of Hypothalamic Neurosecretion.* Vincent, J. D. and Kordon, C. (eds.). National Center for Scientific Research, Paris, pp. 135–153.
28. Saavedra, J. M. (1977). *Fed. Proc.* **36,** 2134–2141.
29. Bosler, O., Joh, T. H., and Beaudet, A. (1984). *Neurosci. Lett.* **48,** 279–285.
30. Steinbusch, H. W. M. (1981). *Neuroscience* **6**, 557-618.
31. Voogt, J. L., Arbogast, L. A., Quadri, S. K., and Andrews, G. A. (1990). *Mol. Brain Res.* **8,** 55–62.
32. Reymond, M. (1990). *Neuroendocrinology* **52,** 490–496.
33. MohanKumar, P. S., ThyagaRajan, S., and Quadri, S. K. (1995). *Endocrinology* **135,** 119–126.
34. Arbogast, L. A. and Voogt, J. L. (1991). *Endocrinology* **128,** 997–1005.
35. MohanKumar, P. S., MohanKumar, S. M. J., Quadri, S. K., and Voogt, J. L. (1997). *Brain Res. Bull.* **42,** 435–441.