Neurobiology of Aging, Vol. 6, pp. 107–111, 1985. © Ankho International Inc. Printed in the U.S.A. 0197-4580/85 $3.00 + .00

Synergistic Effects of Estrogen and Serotonin-Receptor Agonists on the Development of Pituitary Tumors in Aging Rats[1]

RICHARD F. WALKER

Department of Anatomy and Sanders-Brown Research Center on Aging, University of Kentucky Medical Center Lexington, KY

AND

RALPH L. COOPER[2]

Department of Psychiatry and Center for the Study of Aging and Human Development Duke University Medical Center, Durham, NC

Received 25 January 1983

WALKER, R. F. AND R. L. COOPER. *Synergistic effects of estrogen and serotonin-receptor agonists on the development of pituitary tumors in aging rats.* NEUROBIOL AGING 6(2) 107–111, 1985.—The purpose of this study was to determine if pituitary 5-HT levels change as a function of age or endocrine state, and further if such changes are associated with pituitary pathology. Middle-aged constant estrous (CE) rats had larger ($p<0.05$) pituitary glands containing more ($p<0.05$) serotonin (5-HT) than those from young females or comparably aged, irregularly cycling rats. Ovariectomy lowered pituitary 5-HT content in middle aged CE rats. In contrast, pituitary weight and 5-HT content were increased in young rats of both sexes bearing subcutaneous, steroid-containing capsules that produced elevated levels of serum estradiol 17β. However, exogenous estrogen failed to raise pituitary 5-HT concentrations since pituitary weight increased more than 5-HT levels, even though the total amount of amine was significantly increased ($p<0.05$) compared with controls. These findings suggest that pituitary 5-HT increases during aging regardless of ovarian status and in addition, that total 5-HT content of the gland is increased further in hyperestrogenic states such as CE. Since pituitary adenomas occur more frequently in aged CE rats than in diestrous females or males, it was of interest to determine if 5-HT contributes to the tumorogenic effect of estrogen. Thus, the 5-HT receptor agonists zimelidine or quipazine were administered to ovariectomized rats bearing estrogen containing capsules. Rats treated with drugs had larger pituitaries containing more tumors than those receiving the steroid alone. However, these effects were dependent upon estrogen since pituitary pathology did not increase when ovariectomized rats were given 5-HT neuroleptics without the steroid. It is concluded that 5-HT levels in the pituitary increase during aging and in the presence of estrogen may contribute to the frequent occurrence of pituitary tumors in old female rats.

Aging Pituitary tumors Estrogen Serotonin

THE incidence of pituitary adenomas in rats increases during aging, with greatest numbers occurring in females [9, 10, 17]. The majority of tumors derive from chromophobes and are most often prolactin secreting [22,25]. Although the reason(s) for a sex difference in the incidence of pituitary tumors is not established conclusively, it probably results from differences in exposure to sex steroids. Estrogen stimulates pituitary hypertrophy, hyperplasia and tumorogenesis [3,16] whether or not the pituitary resides in the sella turcica, suggesting a direct steroid effect upon lactotrophs [13]. Thus, the higher incidence of pituitary adenomas in constant estrous (CE) rats is not surprising as these females generally experience prolonged periods of exposure to endogenous estrogen.

In addition to a direct effect on the pituitary, changes in estrogen secretion also alter CNS monoamine metabolism which could further contribute to the growth of pituitary tumors. For example, dopamine turnover and content of the

[1]This research was supported by grants from the National Institute on Aging (AG 02876; RFW and AG 00566; RLC), American Federation for Aging Research, Inc. (AFAR) and the McDowell Cancer Network.

[2]Present Address: Reproductive Toxicology Branch, Environmental Protection Agency, Research Triangle Park, NC 27711.

median eminence is lower than normal in female rats with pituitary tumors. This change occurs in females with pituitary tumors associated with advanced age, as well as in younger ones exposed chronically to estrogen [21]. Age-related changes in pituitary dopamine content have also been reported [5]. In contrast to dopamine, serotonin (5-HT) metabolism increases with age in rats of both sexes [23,26]. 5-HT stimulates prolactin secretion [11,14] in part, by direct action on pituitary cells [1] that possess a specific uptake system for the indolamine [8]. Collectively, these findings suggest that prolonged stimulation of the pituitary by estrogen when hypothalamic dopamine metabolism is depressed and 5-HT metabolism is increased promotes the development of lactotroph adenomas in aging female rats. Thus, the specific aims of the present study were to determine: (1) if 5-HT content of the pituitary is elevated in aged CE rats, (2) if pituitary 5-HT increases in young rats after prolonged exposure to estrogen and (3) if the incidence of pituitary tumor development is altered by drugs affecting 5-HT metabolism or its receptors.

METHOD

Female Long-Evans hooded rats purchased from Charles River Labs (Wilmington, MA) were used in this study. Upon arrival, the rats were housed in the Sanders-Brown Research Center on Aging animal facility for at least one month. Standard lighting (14 hr light:10 hr dark) and temperature (22±2°) were maintained automatically. Throughout the experiment all animals were allowed unrestricted access to food and water.

In a preliminary study, hypothalamic tissue taken from 7 rats with pituitary tumors was analysed for 5-HT concentrations. Since serotoninergic projections to the pituitary have been identified [18] it was of interest to determine if pituitary tumors are associated with metabolic changes in the brain having the potential to alter hypophyseal 5-HT. Blocks of hypothalamic tissue were dissected as previously described, sonicated in 10 volumes of 0.1 N perchloric acid containing ascorbic acid and parygline (10^{-3} M each) and assayed for 5-HT by radioenzymatic methods [4,27].

Subsequent studies were designed to determine if changes in pituitary 5-HT were related to the occurrence of hypophyseal tumors in aging rats. Two weeks prior to these studies, daily vaginal smears were obtained from all rats in order to identify young females (3–4 months old) with regular four-day vaginal cycles, middle-aged females with irregular vaginal cycles (i.e., 4–8 days in duration) and middle-aged females (10–12 months old) in constant estrus (CE = constant vaginal cornification). The vaginal smear patterns present in the middle-aged females are associated with the termination of their reproductive lifespan. After the two-week observation period, the rats were assigned to one of the experimental groups described below.

Experiment 1: Pituitary Serotonin: Effect of age

This study compared pituitary 5-HT content and concentration in young and middle-aged female rats representing different reproductive conditions. Middle-aged rats with irregular cycles or CE and young females with regular 4-day estrous cycles were decapitated between 1400–1500 hr. The pituitaries were removed rapidly, their neural lobes dissected free, and the adenohypophyses frozen on dry ice. Afterwards, the anterior pituitary glands were weighed and sonicated for 15 seconds in 10 volumes of 0.1 N perchloric acid, centrifuged and the supernatant frozen at −80°C until being assayed for 5-HT content by radioenzymatic methods.

TABLE 1

ANTERIOR PITUITARY 5-HT CONTENT IN RATS OF DIFFERENT AGE AND REPRODUCTIVE STATUS

Group	N	Anterior Pituitary Weight (mg ± SEM)	Anterior Pituitary 5-HT (pg + SEM) Content	Concentration
Young				
Estrus	9	14.3 ± 0.77	1973 ± 410	137.6 ± 28
Diestrus	6	13.5 ± 0.89	1710 ± 349	126.5 ± 25
Middle-Aged				
Diestrus	6	14.1 ± 0.95	2323 ± 386	164.8 ± 27
Constant Estrus	7	17.8 ± 1.14*	3394 ± 840†	190.6 ± 47

*Pituitaries significantly ($p<0.05$) heavier than all other groups.
†Pituitary serotonin content significantly ($p<0.05$) greater than two young groups.

Experiment 2: Pituitary Serotonin: Effect of Estrogen

The purpose of this experiment was to determine if estrogen alters pituitary 5-HT levels. In addition, this experiment tested whether or not the steroids' effect on pituitary 5-HT content was sex-specific.

Twelve-month-old constant estrous rats were ovariectomized (OVX) under ether anesthesia and housed in pairs for a two-week recovery period. Controls were anesthetized and laparotomies were performed without ovariectomy. During recovery, vaginal smears were taken from the control animals to determine the effect of ether and laparotomy on ovarian function. Only females that remained CE after surgery were included in the control group. Both OVX and control CE rats were killed 30 days after surgery. Pituitary weights and 5-HT levels were then determined. A similar analysis of estrogen's influence on pituitary 5-HT was also performed in young rats. However, at the time of ovariectomy, these animals received subcutaneous implants of Silastic tubing containing 5 mm or 2 mm of estradiol 17β [12] to determine the effects of pharmacologic as well as physiologic levels of the steroid. Controls were ovariectomized and received empty Silastic capsules. Thirty days later rats were decapitated, and pituitary weights and 5-HT levels were determined.

To determine whether or not the effect of estrogen on pituitary 5-HT is sex-specific the following study was done in 4–6 month-old male rats. The males were castrated under ether anesthesia. Two weeks later they received subcutaneous estrogen implants (5 mm) or empty Silastic capsules, and were returned to their cages for 30 days. Thereafter, they were decapitated, their pituitaries removed, weighed and assayed for 5-HT

Experiment 3: Influence of Serotonin Neuroleptics on Pituitary Tumorogenesis in Estrogen-Treated Rats

The purpose of this experiment was to determine whether

TABLE 2
EFFECT OF ESTROGEN ON PITUITARY WEIGHT AND 5-HT CONTENT

Group	N	Weight	Pituitary 5-HT (pg + SEM) Content	Concentration
Young Females				
OVX	5	13.6 ± 0.54	1983 ± 359	145.8 ± 26
OVX + Estrogen				
(5 mm)	6	36.3 ± 1.55†	3120 ± 415*	85.1 ± 11*
(2 mm)	6	29.2 ± 1.06†	2780 ± 510	95.2 ± 17
Middle-Aged Females				
Constant Estrus	7	17.8 ± 1.14	3394 ± 840	190.6 ± 47
OVX (previously CE)	6	15.2 ± 0.95	2619 ± 610	172.3 ± 40
Young Males				
Castrated	6	14.4 ± 0.61	1629 ± 439	113.1 ± 30
Castrated + Estrogen				
(5 mm)	6	27.3 ± 1.01†	2754 ± 480*	100.6 ± 18

*Significantly different ($p<0.05$) than gonadectomized control.
†$p<0.01$.

or not drugs that alter serotonergic activity also alter the tumorogenic effect of chronic estrogen exposure. Young rats were ovariectomized and received estrogen-containing (5 mm) or empty Silastic capsules as described above. The capsules were removed and replaced with new ones at two-week intervals for a period of three months. At the time of ovariectomy, these females were divided into five groups and given bi-weekly subcutaneous injections of either (1) p-chlorophenylalanine (PCPA, Sigma, St. Louis, MO; 150 mg/kg), (2) 5-hydroxytryptophan (5-HTP Sigma, 50 mg/kg), (3) zimelidine (Astra Pharm., Worcester, MA; 20 mg/kg), (4) quipazine (Miles Labs, Elkhart, IN; 2 mg/kg) or (5) saline. PCPA blocks 5-HT synthesis and was given to determine if it would inhibit the hypertrophic effect of estrogen on the pituitary. 5-HTP is a precursor of 5-HT that enhances its synthesis. 5-HTP, like the 5-HT receptor agonists zimelidine and quipazine, was given to determine if drugs that increase serotonergic stimulation also enhance the tumorogenic effect of estrogen. After three months the rats were sacrificed, their pituitaries weighed and examined to determine the incidence and size of tumors as well as 5-HT levels in each treatment group.

A description of the microscaled method for 5-HT determination [19] using serotonin-N-acetyl transferase partially purified from *Drosophila* [15] was reported previously [4,27]. Pituitary 5-HT content and concentration were expressed as pg/anterior lobe and pg/mg tissue wet weight, respectively. Differences ($p<0.05$) in pituitary 5-HT content or concentration between groups were determined using analysis of variance and Duncan's multiple range test.

Plasma estrogen in ovariectomized rats bearing Silastic capsules containing estradiol 17β was analysed by radioimmunoassay [2]. Estrogen levels (>35 pg/ml) resembling those found in aged-CE rats were produced with 2 mm capsules while the larger ones produced supraphysiological (>100 pg/ml) values.

RESULTS

Data from the preliminary study showed a correlation between increased brain 5-HT and pituitary tumors. Hypothalamic 5-HT concentration (2.6±0.37 ng/mg wet wgt.) was significantly ($p<0.001$) higher in aged rats with macroscopic pituitary tumors than in rats of comparable age but without tumors (1.2±0.09 ng/mg wet weight).

Pituitary Studies 1: Effect of Age on Pituitary Weight and Serotonin Levels

Mean pituitary weights increased with age although differences between the middle-aged, irregularly cycling rats and their young controls were not significant. Pituitaries obtained from CE rats were significantly larger ($p<0.05$) than those from the other groups regardless of age (Table 1).

The data presented in Table 1 also show that mean pituitary 5-HT content and concentration were highest in aged CE rats. However, only the difference between pituitary 5-HT content in these rats and young cycling rats killed on the day of vaginal diestrus was significant ($p<0.05$). Although mean pituitary 5-HT levels in young rats sacrificed at vaginal estrus was higher than the mean pituitary 5-HT content of young rats sacrificed at diestrus, the differences were not statistically significant. Pituitaries from 12-month-old rats that were cycling and sacrificed during diestrus contained 5-HT levels comparable to young rats.

Experiment 2: Effect of Estrogen on Pituitary Serotonin Content

As seen in Table 2, pituitary size and 5-HT content were elevated ($p<0.01$ and $p<0.05$, respectively) resembling old rats when young ovariectomized rats were treated chronically with supraphysiological doses of estrogen. Physiological levels of plasma estrogen did not produce effects as great as the higher dose. Unlike the intact CE rats, pituitary 5-HT

TABLE 3

EFFECTS OF 5-HT RECEPTOR AGONISTS AND ESTROGEN ON PITUITARY HYPERTROPHY AND TUMOROGENESIS IN 4-MONTH-OLD, OVARIECTOMIZED RATS

Treatment	N	Pituitary Weight (mg ± SEM)	Number of Rats with Macroadenomas
OVX*	4	12.0 ± 0.82†	0
Estrogen	5	36.6 ± 3.95	0
Estrogen + PCPA	6	32.2 ± 2.87	0
Estrogen + 5-HTP	7	38.4 ± 3.54	0
Estrogen + Zimelidine	7	78.6 ± 18.00‡	3
Estrogen + Quipazine	5	53.2 ± 13.60	1
Zimelidine*	4	14.2 ± 0.71†	0
Quipazine*	4	12.6 ± 0.69†	0

*Animals had empty Silastic capsules implanted subcutaneously.

†The pituitary weight of these three groups was significantly smaller ($p<0.05$) than those groups receiving estrogen implants.

‡Significantly larger ($p<0.05$) than all other groups except estrogen + Quipazine.

concentration was significantly reduced ($p<0.05$) by exogenous estrogen. Similar changes in pituitary size and 5-HT levels occurred in castrated male rats that were implanted with estradiol capsules. Thus, the effect of estrogen on these parameters is not sex specific.

Experiment 3: Effect of 5-HT and Estrogen on Pituitary Tumor Development

The data in Table 3 show that the hypertrophic effect of estrogen upon the pituitary was enhanced in rats receiving concomittant treatment with serotonin receptor agonists. Rats receiving combined treatment with estrogen and zimelidine had heavier pituitaries ($p<0.05$) containing more macroadenomas than did rats treated with estrogen alone. Neither of the serotonin receptor agonists significantly altered pituitary 5-HT levels. Mean pituitary weight and 5-HT levels in estrogen-treated rats receiving 5-HTP were not significantly different from those of their controls (5-HT content; 3120±415 vs. 3671±378 respectively). Mean pituitary weight from PCPA treated rats was lower than their controls but the drug failed to significantly alter the hypertrophic effect of estrogen. On the other hand pituitary 5-HT content in these animals were significantly depressed ($p<0.02$; 1322±179 vs. 3120±415).

None of the 5-HT neuroleptics altered pituitary weight or the frequency of adenomas when given without estrogen (Table 3).

DISCUSSION

The preliminary findings of the study showing elevated hypothalamic 5-HT levels in rats with pituitary tumors do not confirm a "cause and effect" relationship. However, the prolactin stimulating effect of 5-HT [11,14] suggests that hypothalamic changes in the metabolism of this amine during aging have the potential to alter lactotroph function. Another potential relationship between hypothalamic amines and pituitary function was previously described, involving reciprocal changes in dopamine and prolactin in aging rodents [21]. Since a diencephalic serotoninergic projection to the pituitary has been described [18], increased hypophyseal 5-HT content in aged rats may reflect altered states of hypothalamic 5-HT metabolism. Furthermore, this effect may contribute to the hypertrophic effect of estrogen on the pituitary. This hypothesis is supported by the fact that pituitaries from middle-aged CE females contained significantly more 5-HT than those from irregularly cycling rats of the same age. Thus, serotonin levels in the pituitary increase spontaneously during aging and the rise in pituitary 5-HT can be further enhanced by ovarian state. However, treatment of young rats with estrogen produced changes in pituitary 5-HT that only partially mimic the effects of age. Although chronic exposure to high and low doses of estrogen caused pituitary hypertrophy and increased 5-HT content, the concentration of pituitary 5-HT fell in response to the steroid. It is possible that this difference from the intact, aging animal results from a differential rate of pituitary hypertrophy and 5-HT accumulation after estrogen treatment. Perhaps pituitary weight increases more rapidly than 5-HT accumulates causing concentrations of the amine to drop even though its content increases. Furthermore, serotoninergic neural innervation of the pars distalis is lacking [18]. Instead, prominent 5-HT projections to the pars intermedia and pars nervosa probably control levels of the amine found in the anterior lobe. Since no synthetic relationship exists between the number or size of cells in the pars distalis and adenohypophyseal 5-HT levels, expression of the amine in terms of unit weight (mg) may not be meaningful. Instead the absolute number of molecules found in the pituitary during aging and with estrogen treatment may reflect a true increase which could have significant physiologic implications.

This study also showed that 5-HT modulates the tumorogenic effect of estrogen on the rat's pituitary. Prolonged stimulation of serotonin receptors by quipazine and zimelidine accelerated the hypertrophic effect of estrogen on the pituitary. Larger pituitaries and more macroadenomas occurred in rats bearing estrogen capsules and given zimelidine or quipazine, than in rats treated with estrogen alone. However treatment with 5-HT receptor agonists failed to cause pituitary hypertrophy in rats bearing empty Silastic capsules suggesting that estrogen is necessary for the effect of 5-HT on the pituitary. These observations may explain, in part, why aged male rats have a lower incidence of pituitary tumors than females despite the fact that they experience similar alterations in CNS 5-HT metabolsim [5].

PCPA and 5-HPT treatment did not alter the effect of estrogen on pituitary weight. Although 5-HTP did not change pituitary 5-HT content, PCPA partially lowered it. Whether or not a higher dose of these drugs would have modified the tumorogenic effects of estrogen remains to be determined. However comparable changes in pituitary 5-HT have been reported to occur in response to treatment with similarly acting 5-HT neuroleptics [20]. The doses and treatment schedule chosen in the present study were based on our concern that higher doses of these drugs might have also affected CNS catecholamine function. In any event, the differences between the effects of the synthesis modifiers (PCPA and 5-HTP) and receptor agonists (zimelidine and quipazine) may relate to the degree to which they affect serotonin sensitive cells in the pituitary. The synthesis modulating drugs can be controlled to some extent presynaptically. Since the effect of 5-HTP treatment has been

shown to be controlled by end product inhibition [17], the amount of 5-HT arriving at the pituitary after treatment with this drug may not be excessive. Similarly, pituitary enlargement in the presence of PCPA demonstrates the hypertrophic effect of estrogen that is independent of 5-HT. In contrast to PCPA and 5-HTP, zimelidine and quipazine probably escaped the influence of synthesis regulating mechanisms by directly affecting postsynaptic receptors. Under these circumstances, 5-HT stimulation of pituitary cells previously sensitized by estrogen likely enhanced tumor growth.

In conclusion, the development of pituitary tumors in aging female rats seems to be secondary to changes in the reproductive system, especially those neuroendocrine events that lead to constant estrus and the chronic elevation of serum estrogen. The greater incidence of lactotroph adenomas in the female as compared to the male can be explained, in part, by the action of endogenous estrogen directly upon the gland, and indirectly by altering dopamine and 5-HT levels in the pituitary. These findings may have clinical relevance since drugs such as bromocryptine which are currently used to regress lactotroph adenomas in humans [24] possess weak antiserotonergic activity [6] in addition to their dopamine agonistic effects. Perhaps, in the future the use of drugs that are strong 5-HT receptor antagonists in addition to being dopamine agonists will prove beneficial to the management of patients with certain types of pituitary tumors.

REFERENCES

1. Akabori, A., S. Araki and T. Tamada. The effect of DL-5-hydroxytryptophan (5-HTP) on plasma prolactin in pituitary stalk sectioned rats. *Endocrinol Jpn* **28**: 541–545, 1981.
2. Blake, C. A., R. J. Scaramuzzi, J. Hilliard and C. H. Sawyer. Circulating levels of pituitary gonadotropins and ovarian steroids in rats after hypothalamic deafferentation. *Neuroendocrinology* **12**: 86–97, 1973.
3. Casanueva, F., D. Cocchi, V. Locatelli, C. Flauto, F. Zambotti, G. Bestetti, G. L. Rossi and E. Muller. Defective central nervous system dopaminergic function in rats with estrogen-induced pituitary tumors, as assessed by plasma prolactin concentrations. *Endocrinology* **110**: 590–599, 1982.
4. Cooper, R. L. and R. F. Walker. Enzymatic-isotopic assays for the measurement of catecholamines and serotonin. In: *Methods in Enzymology: Neuroendocrine Peptides*, edited by P. M. Conn. New York: Academic Press, 1983, pp. 483–493.
5. Demarest, K. T., K. E. Moore and G. D. Riegle. Dopaminergic neuronal function, anterior pituitary dopamine content and serum concentrations of prolactin, luteinizing hormone and progesterone in the aging female rat. *Brain Res* **247**: 347, 1982.
6. Fluckiger, E. Ergot alkaloids and the modulation of hypothalamic function. In: *Endocrinie Physiology*, edited by B. Cox, I. D. Morris and A. H. Weston. London: MacMillan Press Ltd, 1978, pp. 137–159.
7. Hamon, M., S. Bourgoin, Y. Morot-Gaudry and J. Glowinski. End product inhibition of serotonin synthesis in the rat striatum. *Nature New Biol* **237**: 184–187, 1972.
8. Johns, M. A., E. C. Aznuta and D. T. Krieger. Specific *in vitro* uptake of serotonin by cells in the anterior pituitary of the rat. *Endocrinology* **110**: 754–760, 1982.
9. Kovacs, K., E. Horwath, R. G. Ilse, C. Ezrin and D. Ilse. Spontaneous pituitary adenomas in aging rats. *Bietrage zur Pathologie* **161**: 1–16, 1977.
10. Kroes, R., J. M. Gabris-Berkvens, T. deVries and J. H. J. van Nesselrooy. Histopathological profiles of Wistar rat stock including a survey of the literature. *J Gerontol* **36**: 259–279, 1981.
11. Lawson, D. M. and R. R. Gala. The interaction of dopaminergic and serotonergic drugs on plasma prolactin in ovariectomized estrogen-treated rats. *Endocrinology* **98**: 42–47, 1976.
12. Legan, S. J., G. A. Coon and F. J. Karsh. Role of estrogen as initiator of LH surges in the ovariectomized rat. *Endocrinology* **18**: 50–56, 1975.
13. Lloyd, H. M., J. D. Meares and J. Jacobi. Early effects on stilboestrol on growth hormone and prolactin secretion and pituitary mitotic activity in the male rat. *J Endocrinol* **58**: 227, 1973.
14. Lu, K. H. and J. Meites. Effects of serotonin precursors and melatonin on serum prolactin release in rats. *Endocrinology* **93**: 153–155, 1973.
15. McCaman, M., R. McCaman and J. Stetsler. A rapid radioenzymatic assay for dopamine and N-acetyldopamine. *Anal Biochem* **96**: 175–180, 1979.
16. McEuen, C. S., H. Selye and J. B. Collip. Some effects of prolonged administration of oestrogen in rats. *Lancet* **230**: 775, 1963.
17. Meites, J., and C. S. Nicoll. Adenohypophysis prolactin. *Annu Rev Physiol* **28**: 57–88, 1966.
18. Mezey, E., C. Leranth, M. Brownstein, E. Friedman, D. Krieger and M. Palkovits. On the origin of serotonergic input to the intermediate lobe of the rat pituitary. *Brain Res* **294**: 231–237, 1984.
19. Saavedra, J. M., M. Brownstein and J. Axelrod. A specific and sensitive enzymatic-isotope microassay for serotonin in tissues. *J Pharm Exp Ther* **186**: 508–515, 1973.
20. Saland, L. C., W. G. Dail and E. Reyes. Effects of p-chloroamphetamine, a serotonin-depleting drug, on the median eminence and pituitary pars intermedia. *J Neurobiol* **11**: 577–589, 1980.
21. Sarkar, D. K., P. E. Gottschall and J. Meites. Damage to hypothalamic dopamine neurons is associated with development of prolactin secreting tumors. *Science* **218**: 684, 1982.
22. Saxton, J. A. and J. B. Graham. Chromophobe adenoma-like lesions of the rat hypophysis. *Cancer Res* **4**: 168–175, 1955.
23. Simpkins, J. W., G. P. Mueller, H. H. Huang and J. Meites. Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats. Possible relation to gonadotropin secretion. *Endocrinology* **100**: 1672–1678, 1977.
24. Sobrinho, L. G., M. C. Nunes, C. Calhaz-Jorge, J. C. Mauricio and M. A. Santos. Effect of treatment with bromocryptine on the size and activity of prolactin producing pituitary tumors. *Acta Endocrinol* **96**: 24–29, 1981.
25. Sto, A., P. Moy, H. Kaunitz, K. Kortwright, S. Clarke, J. Furth and J. Meites. Incidence and character of the spontaneous pituitary tumors in strains CR and W/FU male rats. *J Natl Cancer Inst* **49**: 701–711, 1972.
26. Walker, R. F. Serotonin circadian rhythm as a pacemaker for reproductive cycles in the female rat. In: *Progress in Psychoneuroendocrinology*, edited by F. Brambilla, G. Racagni and D. de Wied. Amsterdam: Elsevier/North Holland Biomedical Press, 1980, pp. 591–599.
27. Walker, R. F., D. W. Friedman and A. Jimenez. A modified enzymatic-isotopic microassay for serotonin (5-HT) using 5HT-N-Acetyltransferase partially purified from *Drosophila*. *Life Sci* **33**: 1915–1924, 1983.