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AGING AND THE HYPOTHALAMO-PITUITARY-TESTICULAR AXIS IN THE RAT

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Summary—Several experiments have been performed in order to clarify the mechanisms through which aging in male rats brings about profound modifications of the neuroendocrine system (reduced pulsatile secretion of LH and FSH, decreased serum levels of gonadotropins and testosterone, etc.).

(1) It has been found that the number of mu opioid receptors decreases significantly in the hypothalami of old male rats; the substitution therapy with testosterone is ineffective in increasing the number of mu opioid receptors. These data suggest that the decrease of hypothalamic mu opioid receptors is not due to a decline of serum testosterone levels, but appears to be an independent phenomenon.

(2) K opioid receptors increase significantly in the amygdala and in the thalamus of old male rats. These results show that aging, in addition to mu receptors, affects also the number of K receptors in selected areas of the brain. The increase of the number of K receptors in the amygdala might have some bearing on the decrease of serum gonadotropins observed in aged rats, since the amygdala is involved in the nervous circuitry influencing the hypothalamo-pituitary-gonadal axis.

(3) The study of the release of LHRH from the hypothalamus of old male rats with an *in vitro* perfusion system shows that the release of the hormone is comparable in young and old animals, both in basal and in K^+ stimulated conditions. These results indicate that the hypothalamus of old male rats retains the capacity of releasing LHRH both in basal and in stimulated conditions.

(4) It has been observed that the number of LHRH receptors at the level of the anterior pituitary is significantly reduced in old male rats. This finding might explain the low serum levels of gonadotropins and testosterone in aged rats, due to a lack of an adequate response of the pituitary to hypothalamic LHRH.

INTRODUCTION

It is becoming increasingly clear that aging exerts profound influences on the hypothalamo-pituitary-gonadal axis of the rat. In particular, it has been observed that old male rats exhibit a decrease of serum testosterone, LH, and FSH [1, 2]. Moreover, the pulsatile secretion of LH is altered in old male rats [3, 4]. These alterations have been attributed either to a degradation of the neurons synthesizing and releasing LHRH, or to age-linked modifications of the synthesis, the metabolism and the release of the brain neurotransmitters (e.g. serotonin, catecholamines, opioids, etc.), which control LHRH secretion [5–8]. Alterations of the feedback mechanisms controlling gonadotropin release have also been reported to occur in old animals [1, 2]. The present paper will summarize the most recent findings of this laboratory on:

(1) the modifications of brain opioid receptors of the mu and K type induced by aging;

(2) the effects of aging on the capability of the hypothalamus of male rats to release LHRH;

(3) the changes induced by aging on the binding characteristics of LHRH receptors in the anterior pituitary.

I. MODIFICATIONS OF BRAIN OPIOID RECEPTORS OF THE MU AND K TYPE INDUCED BY AGING

It is known that the effects of the naturally occurring opioids (met-enkephalin, leu-enkephalin, β-endorphin, dynorphin, etc.) are exerted through the interaction with specific binding sites; different classes of opioid receptors (named respectively mu, K, delta, etc.) have been described [9]. Very little information is available on possible age-induced changes of the number and of the binding characteristics of brain opioid receptors [10, 11]. The experiments here to be described have been designed in order to analyze whether the binding capability of brain mu and K receptors is modified by age in the male rat.

These two types of receptors were investigated, since the majority of the data available indicates that mu and K receptors are responsible for the control of LHRH and gonadotropin secretion [12–15].

In a first experiment, the concentration of mu receptors in the hypothalami of male rats of 2 and 22 months of age has been studied. In this experiment, it was also investigated whether the administration of exogenous testosterone might modify the number of mu binding sites in the hypothalamus of old animals. Dihydromorphine has been used as the *in vitro* specific ligand. Serum levels of testosterone, prolactin, LH and FSH in the young and old animals have also been evaluated. Table 1 shows that serum testosterone levels are significantly decreased in old rats, a phenomenon which can be reversed by the implantation of silastic capsules filled with testosterone in the implanted animals, serum testosterone

Table 1. Effect of aging and of testosterone (T) administration on serum levels of LH, FSH, prolactin and T in male rats

Age (months)	LH (ng/ml) NIH S-20	FSH (ng/ml) NIADDK-RP-2	Prolactin (ng/ml) NIADDK-RP-3	Testosterone (ng/ml)
2	1.38 ± 0.16* (10)†	7.83 ± 0.39 (10)	5.07 ± 0.74 (9)	2.59 ± 0.83 (9)
22	0.98 ± 0.17 (11)	6.43 ± 0.61 (11)	11.78 ± 2.47 (10)	0.87 ± 0.30‡ (9)
22 + T	0.58 ± 0.09‡ (10)	3.63 ± 0.35‡§ (10)	9.46 ± 2.50 (10)	1.53 ± 0.37 (9)

*Values are means ± SE.
†Number of animals in parentheses.
‡Significant vs 2-month-old animals ($P < 0.05$).
§Significant vs 22-month-old animals ($P < 0.05$).

levels, even if not restored to the values found in young animals, were not significantly different from those present in the young controls. Table 1 also shows that age induces a small decrease of serum LH and FSH and an increase of serum prolactin. The implantation of silastic capsules containing testosterone brought about a significant decrease of serum FSH; serum LH was decreased even if not significantly so. Serum prolactin remained unchanged.

It is apparent from Fig. 1 that the concentration of opioid receptors binding dihydromorphine decreases significantly at hypothalamic level between 2 and 22 months of age. The data also indicate that the administration of the dose of testosterone able to increase serum testosterone levels towards normal and to inhibit gonadotropin secretion (see Table 1) does not bring back to normal the number of opioid receptors in the hypothalami of 22-month-old animals. The decrease of the number of mu receptors was not accompanied by any change of the affinity constant.

The present results agree, in general, with those reported in the only other study in which dihydromorphine binding has been studied in the brain of old male rats. Messing *et al.*[11] have found the number of sites binding this ligand to be lower in the frontal poles, anterior cortex and striatum of 26-month-old than in the corresponding structures of young male rats. In the study of Messing *et al.*[11] no age-linked changes of the number of dihydromorphine receptors were found to occur in the thalamus, amygdala and midbrain. The same authors also reported that the decline in the receptor concentration in the frontal poles is accompanied by a significant increase in the affinity of dihydromorphine for its receptors, a phenomenon which was not found in the present experiments. The reason for the minor discrepancies between the study by Messing *et al.*[11] and the data here reported probably resides in the fact that different regions of the brain have been analyzed. Moreover, it must be pointed out that Messing *et al.*[11] used F344 rats, while the present study was performed on Sprague–Dawley animals; finally, methodological differences may also be involved.

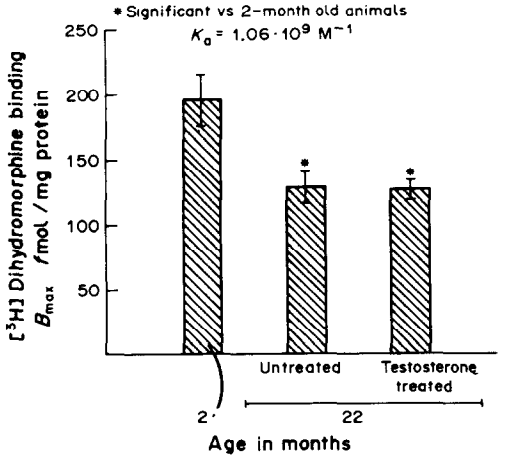

Fig. 1. Effect of aging and of testosterone replacement on the number of hypothalamic mu opioid receptors in male rats.

One might hypothesize that the age-linked decrease of the number of brain opioid receptors here reported might be due to the decline of serum testosterone levels occurring in old male rats. It is still controversial whether serum testosterone may influence the number of brain opioid receptors in young animals. Hahn and Fishman [16, 17] have reported that orchidectomy increases by a factor of 2 the number of saturable cerebral binding sites for naltrexone and naloxone, and that testosterone replacement decreases their number to control levels. On the contrary, Diez and Roberts[18], Cicero *et al.*[19] and Olasmaa *et al.*[20] have found that neither castration nor the subsequent androgen replacement therapy is able to modify the binding capacity of the whole brain and of the hypothalamus for dihydromorphine, leu-enkephalin, an enkephalin analogue, naloxone and naltrexone. Whatever the effects of testosterone on the number of brain opioid receptors in young animals, the present findings clearly show that the administration of exogenous testosterone does not re-establish to normal the number of opioid receptors at hypothalamic level in old animals. These data suggest that the decline of

the number of opioid receptors observed with increasing age is not due to the decreased availability of testosterone. On the basis of the present findings, the age-linked decrease of the number of the brain receptors binding dihydromorphine in the hypothalamus appears to an amonomous phenomenon, it is possible that the age-linked decrease in the number of hypothalamic opioid receptors reported in this paper plays a role in inducing the deterioration of the function of the hypothalamo-pituitary-gonadal axis observed in normal male rats. Many experiments suggest that brain opioids play an important role in the control of LHRH secretion [21].

In a second experiment, the binding characteristics of K receptors have been evaluated in selected areas of the brain of animals aged either 2 or 19 months.

The areas considered were the hypothalamus, the amygdala, the hippocampus, the thalamus, the corpus striatum, the mesencephalon, the frontal poles, and the anterior and posterior cerebral cortex. The ligand selected was Bremazocine, which was used after having protected mu and delta receptors with dihydromorphine and d-ala-d-leu-enkephalin, respectively. The results obtained are depicted in Fig. 2. It appears that the number of K receptors in the different areas investigated is extremely variable in young animals. High concentrations of K receptors have been found (in descending order) in the hypothalamus, in the striatum, in the mesencephalon and in the amygdala. The number of K receptors in the thalamus, frontal poles, hippocampus, and anterior and posterior cerebral cortex appears to be much lower. In these last structures the density of K receptors appears to be very similar. Age does not seem to influence the number of K binding sites in most of the areas considered. However, in the amygdala and in the thalamus the number of K receptors was significantly increased in aged animals; such an increase was not accompanied by any change in the affinity constant.

To the authors' knowledge, no data are available in the literature supporting this finding. On the basis of the fact that the amygdala has been repeatedly shown to be involved in the control of gonadotropin secretion (see Refs. [22] and [23] for reviews), one might be tempted to speculate that the change of the number of opioid K receptors occurring in this structure might be of importance in explaining the modifications of gonadotropin secretion occurring in old animals. However, there is no indication so far that the thalamus might be involved in the control of gonadotropin secretion. It is then possible that the reported increase of thalamic K opioid receptors might be correlated with changes of behavioral phenomena (pain, etc.) occurring in old animals.

II. EFFECTS OF AGING ON THE CAPABILITY OF THE HYPOTHALAMUS OF MALE RATS TO RELEASE LHRH

The present experiments were designed in order to test whether the hypothalamus of old male rats is able to release LHRH *in vitro*, in a manner similar to that of the hypothalamus of young animals. To this purpose, the mediobasal hypothalami (MBH) of 18-month-old male rats have been perfused *in vitro*, and the release of LHRH has been measured in the effluent, both in basal conditions and after the exposure to a depolarizing stimulus (K^+ 110 mM). The concentration of K^+ used in the present experiments has been previously reported to release LHRH and other hypothalamic hormones from the incubated or perfused rat hypothalamus [24]. The MBH of young adult (6-month-old) male rats of the same strain served as controls.

The results obtained show that the mean spontaneous output of LHRH (before any K^+ stimulus was applied) from perfused MBH was 6.58 ± 0.7 pg/ml in young and 6.93 ± 1.3 pg/ml in the old rats. These two values were not statistically different.

Figure 3 provides the results of a representative experiment obtained perfusing the MBH's

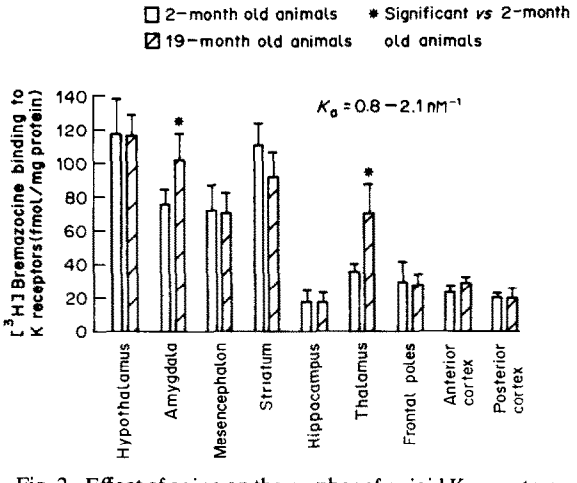

Fig. 2. Effect of aging on the number of opioid K receptors in discrete brain regions of male rats.

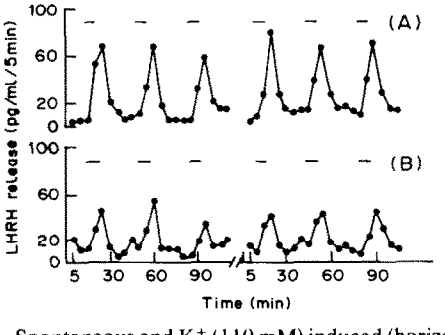

Fig. 3. Spontaneous and K^+ (110 mM) induced (horizontal bars) LHRH release from a single perfused mediobasal hypothalamus of a young (A) and an old (B) male rat.

of old and young rats. It is apparent that the application of the first K^+ stimulus to the MBH of young animals results in a very clear and significant enhancement of LHRH release in the effluent (panel A). The first increase of LHRH above basal level was observed to occur in the sample collected 5 min after the initiation of the K^+ stimulus; a further increase was observed 10 min after the application of the K^+ stimulus (i.e. 5 min after the termination of the delivery of K^+). The LHRH values returned to basal (pre-stimulation) levels 20 min following the beginning of the stimulation, and remained to low levels up to the time at which the next K^+ stimulus was applied. It is obvious from Fig. 3 (panel A) that the K^+ stimulus induced quantitatively similar LHRH responses each time it was applied. Figure 3 (panel B) shows the results obtained while studying the responses of the MBHs of old animals to K^+ stimuli. The results are very similar to those obtained in young animals; however, the responses to the K^+ stimulus seem to be somewhat smaller.

Figure 4 provides the delta of the LHRH hypersecretion induced by K^+ stimuli in all the experiments performed in young and old animals. It is clear that in young animals: (a) all K^+ pulses are followed by an increase of LHRH release; (b) there is a tendency toward a small decrease of LHRH release at the 3rd stimulation; this decrease appeared to be statistically significant vs the first response when a two-way analysis of variance was applied, but not with another statistical test (one-way analysis of variance); (c) after 1 h interruption of the K^+ stimulus the LHRH-induced pulses are similar to those obtained in the first part of the experiment. It is clear from Fig. 4 also that the MBHs of old animals respond with a good LHRH release to each K^+ stimulus. Here again, a tendency towards a decrease of the response is observed at the 3rd stimulus: this decrease appeared to be statistically significant vs the first response when a two-way analysis of variance was applied, but not with another statistical test (one-way analysis of variance). Responses identical to those obtained at the beginning of the experiment were found to occur also after the 60-min interruption. The increases of LHRH secretion obtained at each stimulus from the MBH of both young and old animals were not statistically different from each other, when analyzed by repeated measures by two-way analysis of variance.

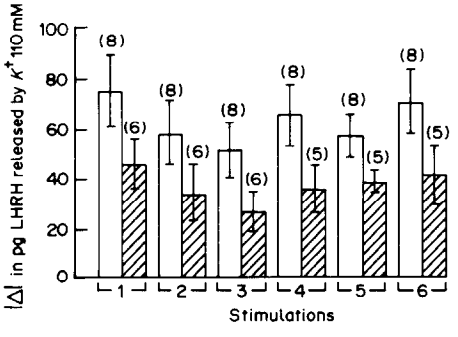

Fig. 4. Effect of K^+ (110 mM) on LHRH release from the mediobasal hypothalamus of young (□) and old (▨) male rats.

At the end of the experiment, the LHRH content of the perfused MBHs was 5212.9 ± 696 pg/MBH in young animals, and 2721.9 ± 705 pg/MBH in old animals. These two values were not statistically different from each other, and from those of the MBHs of non-perfused animals. Also the content of LHRH of non-perfused MBHs of young and old rats were not different at the statistical analysis (5259.7 ± 660 pg/MBH in young rats and 5017.5 ± 1701 pg/MBH in old animals).

The present results show first of all that the basal secretion of LHRH from the perfused MBH of young and old male rats is quantitatively similar. Moreover, the MBHs of young and old animals are capable of responding over a prolonged period of time to the administration of K^+ stimuli with repeated bursts of LHRH hypersecretion. It is interesting to note that, from a quantitative point of view, the MBHs of old animals respond to the K^+ stimulus in a fashion similar to that found in young-animals; even if the responses to K^+ of the hypothalami of old animals appear to be somewhat lower than those of the hypothalami of young-animals, the differences recorded were not statistically significant with any of the tests utilized. It also emerges from the data that the LHRH content of the MBH is similar in young and old animals, both before and after the perfusion period. This appears of particular interest: it is known that castration usually induces a decrease of intrahypothalamic LHRH stores [25, 26], and that old male rats have decreased levels of circulating testosterone [1, 2, 4, 27]. It appears then that the age-linked decline of serum testosterone does not induce the same effect produced by castration in young-animals.

In conclusion, the present results suggest that the LHRH-releasing machinery of the hypothalamus of the old male rat is not substantially different from that of young-animals. On the basis of the present findings, it may be suggested that the alterations of the function of the hypothalamo-pituitary complex observed in the aged male rat are not due to an intrinsic age-related defect of the LHRH synthesizing neurons. It is then possible that the aging of the "central" reproductive system is brought about by the age-linked alterations of the neurotransmitter systems controlling LHRH *in vivo* [1, 5–8].

III. CHANGES INDUCED BY AGING ON THE BINDING CHARACTERISTICS OF LHRH RECEPTORS IN THE ANTERIOR PITUITARY

It has been reported that the pituitary of old male rats is less responsive than that of young-animals to the administration of exogenous LHRH [28]. The present experiments have been designed to analyze

whether this phenomenon might be linked to age-induced changes of the number of LHRH receptors in the anterior pituitary gland. The binding characteristics of an LHRH analog D - ser - $(TBu)^6$ - des - Gly^{10} - LHRH - ethylamide (Hoe 766) to the pituitary membrane preparations derived from male rats of 2 and 19 months of age have been studied. In addition, the amounts of LHRH stored in the MBH have been measured in the same animals. Figure 5 illustrates the results obtained. It is clear that a significant decrease of the number of LHRH receptors occurs in the anterior pituitary of aged male rats, while the hypothalamic content of LHRH does not change with age. These data confirm first of all that the hypothalamic LHRH content does not change with age (see Section II). The finding that the number of binding sites for LHRH at the level of the anterior pituitary decreases in old animals may explain why in old male rats the release of LH and FSH is reduced.

The observation that pituitary LHRH receptors are decreased in old male rats is not supported by a previous finding by Sonntag *et al.*[28], who found the number of LHRH receptors to be similar in young and old rats. The apparent discrepancy might reside in methodological differences: Sonntag *et al.*[28] assessed the number of LHRH receptors starting from pituitary pools; in addition their data were based only on inhibition curves followed by Scatchard analysis. On the contrary, in the present experiments, single pituitaries were independently analyzed, on the basis of the saturation and inhibition curves followed by single point assays. It is obvious that this approach is more precise, and gives a better evaluation of the parameter under study.

The decrease of LHRH receptors in the pituitary of old rats was an unexpected phenomenon. As previously mentioned, old animals have a decreased secretion of testosterone [1, 2, 4, 27]. It is known that

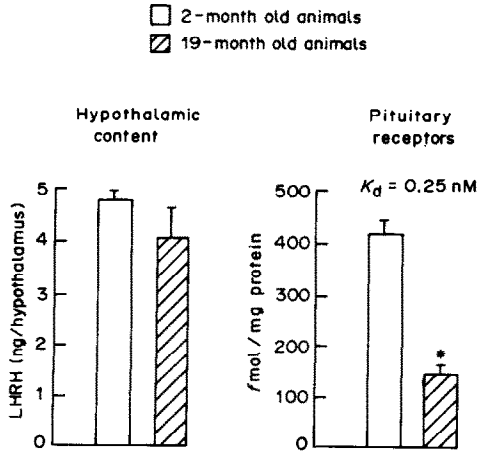

Fig. 5. Effect of aging on hypothalamic LHRH content and on anterior pituitary [^{125}I]LHRH analog (HOE 766) binding sites in male rats. *Significant vs 2-month-old animals.

castration induces an increase of LHRH receptors in the pituitaries of animals of both sexes [30]. On the basis of these data, one might have expected an increase rather than a decrease of pituitary LHRH receptors in old animals. Obviously, this was not the case. Several interpretations may be put forward to explain the results presented here. The most obvious one is that LHRH receptors in the pituitary of old animals decrease because of the described changes in LHRH pulsatility occurring in old animals [3, 4]. It is known that a physiological pulsatility of LHRH is necessary to maintain pituitary LHRH receptors within a normal range [31–35].

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REFERENCES

1. Meites J.: Changes in neuroendocrine control of anterior pituitary function during aging. *Neuroendocrinology* **34** (1982) 151–156.
2. Steger R. W., Sonntag W. E., Van Vugt D. A., Forman L. J. and Meites J.: Reduced ability of naloxone to stimulate LH and testosterone release in aging male rats: possible relation to increase in hypothalamic met^5-enkephalin. *Life Sci.* **27** (1980) 747–753.
3. Karpas A. E., Bremner W. J., Clifton D. K., Steiner R. A. and Dorsa D. M.: Diminished luteinizing hormone pulse frequency and amplitude with aging in the male rat. *Endocrinology* **112** (1983) 788–792.
4. Steiner R. A., Bremner W. J., Clifton D. K. and Dorsa D. M.: Reduced pulsatile luteinizing hormone and testosterone with aging in the male rat. *Biol. Reprod.* **31** (1984) 251–258.
5. Simpkins J. W., Mueller G. P., Huang H. H. and Meites J.: Evidence for a depressed catecholamine and enhanced serotonin metabolism in aging male rat: possible relation to gonadotropin secretion. *Endocrinology* **100** (1977) 1672–1678.
6. Demarest K. T., Riegle G. D. and Moore K. E.: Characteristics of dopaminergic neurons in the aged male rats. *Neuroendocrinology* **31** (1980) 222–227.
7. Estes K. S. and Simpkins J. W.: Age-related alterations in catecholamine concentrations in discrete preoptic area and hypothalamic regions in the male rat. *Brain Res.* **194** (1980) 556–560.
8. Ponzio F., Calderini G., Lomuscio G., Vantini G., Toffano G. and Algeri S.: Changes in monoamines and their metabolite levels in some brain regions of aged rats. *Neurobiol. Aging* **3** (1982) 23–29.
9. Lord J. A. H., Waterfield A. A., Huges J. and Kosterlitz H. W.: Endogeneous opioid peptides: multiple agonists and receptors. *Nature, Lond.* **267** (1977) 495–499.
10. Messing R. B., Vasquez B. J., Spiehler V. R., Martinez J. L., Jr, Jensen R. A., Rigter H. and McGaugh J. L.:

[^{3}H]Dihydromorphine binding in brain regions of young and aged rats. *Life Sci.* **26** (1980) 921–927.
11. Messing R. B., Vasquez B. J., Samaniego B., Jensen R. A., Martinez J. L., Jr and McCaugh J. L.: Alterations in dihydromorphine binding in cerebral hemispheres of aged male rats. *J. Neurochem.* **36** (1981) 784–787.
12. Schultz R., Wilhelm A., Pirke K. M., Gramsch C. and Hertz A.: β-Endorphin and dynorphin control serum luteinizing hormone levels in immature female rats. *Nature, Lond.* **294** (1981) 757–759.
13. Marko M. and Romer D.: Inhibitory effect of a new opioid agonist on reproductive endocrine activity in rats of both sexes. *Life Sci.* **33** (1983) 233–240.
14. Leadem C. A. and Kalra S. P.: Effects of endogenous opioid peptides and opiates on luteinizing hormone and prolactin secretion in ovariectomized rats. *Neuroendocrinology* **41** (1985) 342–352.
15. Panerai A. E., Petraglia F., Sacerdote P. and Genazzani A. R.: Mainly mu-opiate receptors are involved in luteinizing hormone and prolactin secretion. *Endocrinology* **117** (1985) 1096–1099.
16. Hahn E. F. and Fishman J.: Changes in rat brain opiate receptor content upon castration and testosterone replacement. *Biochem. biophys. Res. Commun.* **90** (1979) 819–823.
17. Hahn E. F. and Fishman J.: Castration affects male rat brain opiate receptor content. *Neuroendocrinology* **41** (1985) 60–63.
18. Diez J. A. and Roberts D. L.: Evidence contradicting the notion that gonadal hormones regulate brain opiate receptors. *Biochem. biophys. Res. Commun.* **108** (1982) 1313–1319.
19. Cicero T. J., Neumann K. S. and Meyer E. R.: Testosterone does not influence opiate binding sites in the male rat brain. *Life Sci.* **33** (1983) 1231–1239.
20. Olasmaa M., Limonta P., Maggi R., Dondi D., Martini L. and Piva F.: Further evidence that gonadal steroids do not modulate brain opiate receptors in male rats. *Endocr. Jap.* **34** (1987) 415–423.
21. Kalra P. S.: Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. In *Frontiers in Neuroendocrinology* (Edited by W. F. Ganong and L. Martini). Raven Press, New York, Vol. IX (1986) pp. 31–75.
22. Borrell J., Piva F. and Martini L.: Adrenergic inputs to the amygdala and the control of gonadotrophin release. *Acta endocr., Copenh.* **90** (1979) 385–393.
23. Piva F., Borrell J., Limonta P., Gavazzi G. and Martini L.: Cholinergic inputs to the amygdala and the control of gonadotrophin release. *Acta endocr., Copenh.* **93** (1980) 1–6.
24. Hartter D. E. and Ramirez V. D.: The effects of ions, metabolic inhibitors, and colchicine on luteinizing hormone-releasing hormone release from superfused rat hypothalami. *Endocrinology* **107** (1980) 375–382.
25. Shin S. G. and Howitt C. J.: Effect of castration on LH and LHRH in the male rat. *J. Endocr.* **65** (1975) 447–450.
26. Kalra P. S. and Kalra S. P.: Modulation of hypothalamic LHRH levels by intracranial and subcutaneous implants of gonadal steroids in castrated rats: effect of androgen and estrogen antagonists. *Endocrinology* **106** (1980) 390–397.
27. Kinoshita Y., Higashi Y., Winters S. J., Oshima H. and Troen P.: An analysis of the age-related decline in testicular steroidogenesis in the rat. *Biol. Reprod.* **32** (1985) 309–314.
28. Miller A. E. and Riegle G. D.: Temporal patterns of serum luteinizing hormone and testosterone and endocrine response to luteinizing hormone releasing hormone in aging male rats. *J. Gerontol.* **37** (1982) 522–528.
29. Sonntag W. E., Forman L. J., Fiori J. M., Hylka V. W. and Meites J.: Decreased ability of old male rats to secrete luteinizing hormone (LH) is not due to alterations in pituitary LH-releasing hormone receptors. *Endocrinology* **114** (1984) 1657–1664.
30. Clayton R. N. and Catt K. J.: Gonadotropin-releasing hormone receptors: characterisation, physiological regulation, and relationship to reproductive function. *Endocr. Rev.* **2** (1981) 186–209.
31. Young L. S., Speight A., Charlton H. M. and Clayton R. N.: Pituitary gonadotropin-releasing hormone receptor regulation in the hypogonadotrophic hypogonadal (hpg) mouse. *Endocrinology* **113** (1983) 55–61.
32. Garcia A., Schiff M. and Marshall J. C.: Regulation of pituitary gonadotropin-releasing hormone receptors by pulsatile gonadotropin-releasing hormone injections in male rats. Modulation by testosterone. *J. clin. Invest.* **74** (1984) 920–928.
33. Naik S. I., Young L. S., Charlton H. M. and Clayton R. N.: Pituitary gonadotropin-releasing hormone receptor regulation in mice. I: Males. *Endocrinology* **115** (1984) 106–113.
34. Garcia A., Herbon L., Barkan A., Papavasiliou S. and Marshall J. C.: Hyperprolactinemia inhibits gonadotropin-releasing hormone (GnRH) stimulation of the number of pituitary GnRH receptors. *Endocrinology* **117** (1985) 954–959.
35. Duncan J. A., Barkan A., Herbon L. and Marshall J. C.: Regulation of pituitary gonadotropin-releasing hormone (GnRH) receptors by pulsatile GnRH in female rats: effects of estradiol and prolactin. *Endocrinology* **118** (1986) 320–327.