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Changes in the Pituitary and Plasma LH, Hypothalamic LHRH and Pituitary Response to LHRH in Aging Swiss Albino Mice

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In the present study changs in the secretion of hypothalamus and pituitary are reported in aging mice. Hypothalamic Luteinizing hormone releasing hormone (LHRH) and pituitary and plasma Luteinizing hormone (LH) were estimated by radioimmunoassay (RIA) in aging male and female (10 to 20 months) mice at 2 months age interval. In addition, changes in pituitary response to LHRH were evaluated in these age groups by in vitro incubation of isolated pituitary cells with synthetic LHRH (250 ng). While plasma LH gradually decreased in both the sexes, the pituitary LH remained nearly unchanged in female up to 20 months age, whereas it increased in male after 14 months of age. Hypothalamic LHRH and pituitary response to LHRH too, declined during aging in both males and females but more prominently in females at 14 months age. Decrease in hypothalamic LHRH and pituitary responsiveness appear to be responsible for decline in plasma LH which in turn causes a decrease in gonadal activity.

Key Words: Swiss albino mice, Luteinizing hormones, Luteinizing hormone releasing hormone, Aging, Pituitary response

Introduction

Aging in female rodent is associated with disappearance of reporductive cycles. Rat and mice (over 8 months of age), show gradual lengthening of estrus cycle eventually showing a state of persistant estrus or repeated pseudopregnancy and finally anestrus (Wise 1983, Felicio et al. 1984). In constant estrus rats, serum LH and FSH levels are similar to estrus level in young cycling rats but pituitary LH is low and FSH and prolactin are high. The hormone levels in repeatedly pseudopregnant rats are comparable with young rats in diestrus. In anestrus rats, serum LH and FSH are low (Meites et al. 1978, Riegle & Miller 1978). However Parkening et al. (1982), reported higher plasma FSH and LH in aged female mice. In male rats, decrease in serum LH, FSH and testoserone has been reported at 24 months age (Gruenewald et al. 1992). Takase et al. (1989) reported significant decline in LH at 14 months in male mice.

Hypothalamic LHRH is low in aged anestrus rats (Steger et al. 1979) and white-footed mouse (Steger et al. 1980). The increase in serum LH after LHRH administration is also less in aged rats and mice (Riegle & Miller 1978, Parkening et al. 1982). Aging hypothalamo-hypophyseal complex is primarily responsible for reproductive failure in aged female mice (Parkening et al. 1985).

In view of lack of detailed and systematic information on the changes in the levels of hormones and pituitary response to LHRH in aging laboratory rodents, the present work was done using Swiss albino mice.

Materials and Methods

Swiss albino mice of both sexes were kept under standard laboratory conditions. Five to ten animals of each sex and age group (10 to 20 months at 2 month age intervals) were autopsied by decapitation and immediately the blood was collected in heparinized tubes and centrifuged for 10 to 15 minutes at 1000 rpm. The plasma was stored at -20°C till assayed for LH. Pituitary and hypothalamus were carefully dissected under cold and immediately preserved in cold acetone and stored at -20°C.

Pituitary and plasma LH were estimated by RIA. LHRH content in the hypothalamus was measured from the amount of LH released by isolated pituitary cells (isolation by using collagenase). The pituitary response to LHRH was studied by the potency of its isolated cells to *in vitro* release of LH in the presence of a fixed concentration of LHRH (250 ng/ml). LH released in the medium was estimated by RIA. The data was analysed statistically by students' 't' test.

Results

Female mice

The pituitary LH remained nearly unchanged till 20 months. A significant decrease in plasma LH was observed at 16 months which further decreased and reached a very low level at 20 months age. Hypothalamic LHRH declined significantly and reached a very low level at 20 months age. Similarly pituitary response to LHRH decreased signifiantly from 14 months and became very low at 20 months (table 1, figures 1,3).

Male mice

Pituitary LH remained unchanged up to 14 months but suddenly increased at 16 months and still further at 20 months. Plasma LH too, remained high till 10 months but started decreasing from 12 months and became very low at 20 months. Hypothalamic LHRH started decreasing from 12 months and became further low at 20 months age. Pituitary response to LHRH also continued to decrease till 20 months (table 2, figures 2,3).

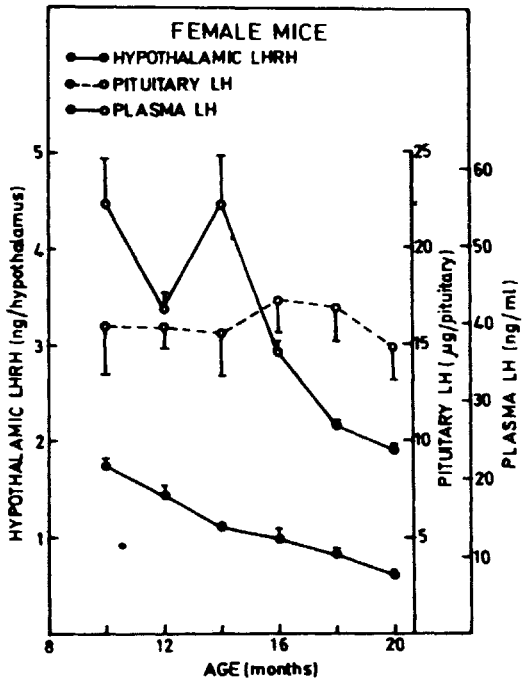

Figure 1 Hypothalamic LHRH and pituitary and plasma LH in aging female mice. Vertical bars indicate standard error or means of eight to ten animals in each age group

Discussion

The present study shows that there is a gradual decline of the hypothalamo-pituitary functioning with increasing age in the mice. The plasma LH starts decreasing after 14 months age in female which is similar to that in aged female rats (Miller & Riegle 1978). In male, the plasma LH starts decreasing after 10 months and becomes very low at 20 months of age. These results are in confirmation with those of Takase et al. (1989) and Gruenewald et al. (1992). The decline in

Table 1. *Hypothalamic LHRH, pituitary and plasma LH and pituitary sensitivity to LHRH in aging female mice*

Age (months)	Hypothalamic LHRH (ng/hypothalamus)	Pituitary LH (μg/pituitary)	Plasma LH (ng/ml)	Pituitary weight (mg)	Pituitary Sensitivity (based on ng LH released/pituitary)
10	1.76 ± 0.03 (7)[a]	16.0 ± 2.5	55.9 ± 55.7[d]	2.7 ± 0.18	223 ± 12
12	1.45 ± 0.08 (8)	15.9 ± 1.1	42.1 ± 2.1	2.95 ± 0.09	219 ± 3
14	1.1 ± 0.02 (6)	15.6 ± 2.2	55.8 ± 6.1	2.6 ± 0.08	173 ± 5[g]
16	1.0 ± 0.1 (9)[b]	17.3 ± 1.7	36.6 ± 1.4[e]	2.35 ± 0.08	152 ± 7
18	0.83 ± 0.07 (7)	16.9 ± 1.8	27.0 ± 0.4	2.8 ± 0.59	117 ± 5
20	0.63 ± 0.03 (6)[c]	14.8 ± 1.6	23.8 ± 0.6[f]	2.5 ± 0.14	84 ± 15[h]

The number of animals used in parenthesis
'p' values
a vs b < 0.001 e vs f < 0.05
b vs c < 0.001 g vs h < 0.01
d vs e < 0.001

Table 2. *Hypothalamic LHRH, pituitary and plasma LH and pituitary sensitivity to LHRH in aging male mice*

Age (months)	Hypothalamic LHRH (ng/hypothalamus)	Pituitary LH (μg/pituitary)	Plasma LH (ng/ml)	Pituitary weight (mg)	Pituitary Sensitivity (based on ng LH released/pituitary)
10	3.80 ± 0.25 (8)	5.7 ± 0.6	44.0 ± 1.7[f]	2.0 ± 0.02	212 ± 11[i]
12	3.25 ± 0.25[a](7)	No samples*	34.5 ± 0.9[g]	No samples	
14	No samples*	4.4 ± 0.04[c](6)	29.5 ± 0.5	2.1 ± 0.35	187 ± 4
16	1.01 ± 0.02(8)	8.0 ± 0.7[d]	27.9 ± 2.3	No samples	
20	0.56 ± 0.02[b](8)	12.1 ± 0.5[e]	16.4 ± 0.9[h]	2.5 ± 0.2	140 ± 9[j]

* Samples were spoiled during storage. 'p' values a vs b < 0.005 f vs g < 0.001
The number of animals used given in parenthesis., c vs d < 0.005 g vs h < 0.001
d vs e < 0.001 i vs j < 0.001

plasma LH could be mainly due to decreased pituitary LH release. This also explains increased level of LH in the pituitary of aging males. Plasma LH decline is accompanied by a simultaneous decrease in the content of hypothalamic LHRH and low pituitary responsiveness to LHRH. Decline in hypothalamic LHRH at higher age has been reported in the rat (Steger et al. 1979) and white footed mouse (Steger et al. 1980). Low hypothalamic LHRH may be due to its decreased synthesis or reduced storage capacity. Riegle and Meites (1976) have reported that hypothalamus of aged rats contains sufficient releasing factors to stimulate *in vitro* pituitary LH release, but during aging there is decrease in factors (biogenic amines) which regulate its release *in vivo*. Physiologically, active decapeptide was reported to accumulate in the terminal regions of median eminence which shows that there is failure of adequate release of LHRH from neurovascular terminals which contribute to reproductive decline in aging female rats (Rubin et al. 1984).

Decline in pituitary response at higher age has been reported in C57 BL/6 strain of mice (Riegle et al. 1977, Wise & Ratner 1980b). The changes in the responsiveness of the pituitary may be due to decline in the gonadal steroids output. In aged mice,

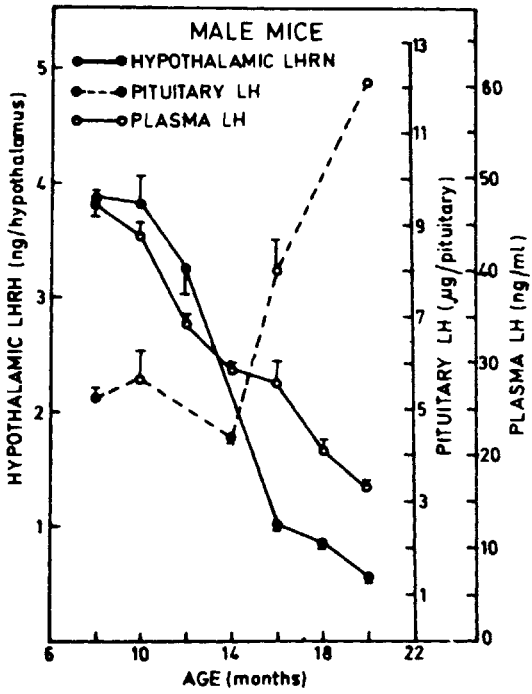

Figure 2 Hypothalamic LHRH and pituitary and plasma LH levels in aging male mice. M S E is indicated by long bars

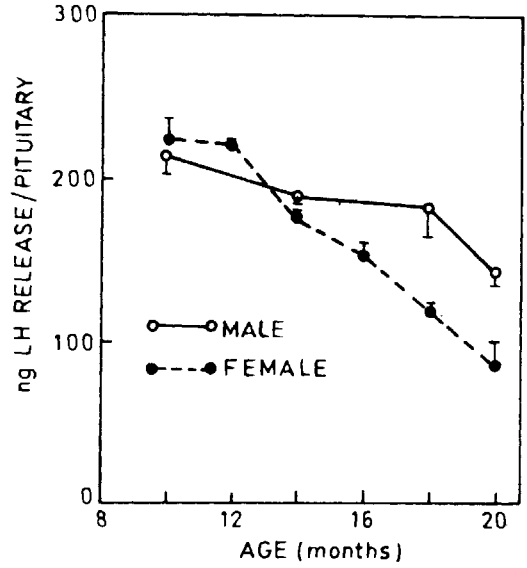

Figure 3 Changes in pituitary response to LHRH (based on LH release per pituitary) in aging male and female mice. M S E is indicated by long bars

with prolonged diestrus, high progesterone and low estrogen levels have been reported (Wise & Ratner 1980a). It is also known that the effect of estrogen on LH secretion changes with age in mice (Mobbs et al. 1985a). In rats, the progesterone level is markedly increased (Gruenewald et al. 1992) which suppresses GnRH (Melrose & Gross 1987) and gonadstrophins secretion (Harris Rice et al. 1982) and eventually the male reproductive function as shown in guinea pigs (Connolly et al. 1988).

This aging involves a chain of factors (Nelson et al. 1987) by which neuroendocrine activity regulating the hypothalamo-hypophyseal-gonadal axis may either be impaired or suppressed.

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