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Morphological and Hormonal Studies Related to Ageing Changes of Hypothalamo-pituitary Gland in Rabbits

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Abstract

Background The hypothalamic - pituitary axis is an auto-regulating system that realizes a tight integration among the endocrine, nervous and immune systems.

Objective To identify anatomical and microscopical age changes of hypothalamus-pituitary gland.

Methods Eighteen female rabbits are divided into 3 groups, group A (4-6 months), group B (12-24 months) and group C (36-60 months). The rabbits are sacrificed and dissection of hypothalamus, pituitary gland is done, anatomical position, their weight, measurements and histological study of their sections regarding the number of different cell types.

Results The weight and number of cells in different parts of hypothalamus and pituitary gland are negatively correlated with age.The serum thyroid stimulating hormone (TSH), growth hormone (GH), prolactin hormone (PRL), and adrenocorticotrophic hormone (ACTH) hormones almost remain unchanged while the level of leutinizing hormone (LH) and follicular stimulating hormone (FSH) were increasing with age, and the prolactin hormone level primarily increases with age then decreases with advancing age.

Conclusion The weight of the Hypothalamus and pituitary gland is primarily increased before age of 24 months, and then it started to decrease. The effects of aging inversely on the function and structure of hypothalamus and pituitary gland.

Key words Aging, hypothalamus, pituitary, HPA axis

Introduction

Epithelial cells of pituitary gland are of 2 types, the chromophobes and the granule containing chromophils which can be further subdivided into acidophil cells and basophil cells [(1-3)]. Posterior lobe is an extension of the brain, composed primarily of nerve fibers (axons) which originate from nerve cell bodies in the hypothalamus to the pars.

The aging is biophysical and biochemical changes of cell matter, physico-chemical changes of cell structure, and the gradual loss of the cell capacity for reproduction and regeneration [(4)]. Endocrine system was thought to play a critical role in aging[(5,6)] . The intension of this study is to detect changes in hormone levels during age progress of hypothalamus and pituitary gland.

Methods

Eighteen female rabbits (Oryctolaguscuniculus), local breed growing their ages between 4 months and 5 years are used in this study. They were divided according to age into 3 groups, six rabbits in each group. The three groups are:

1. Group A: their age ranged between 4-6 months (control group).
2. Group B: their age ranged between 1-2 years.
3. Group C: their age ranged between 3-5 years.

The animals are sacrificed by using intensive dose of chloroform in sealed glass box.Skull cap was removed then the brain was removed in one piece. Dissection of hypothalamus by making a horizontal cut through interventricularforamen (Figure 1).

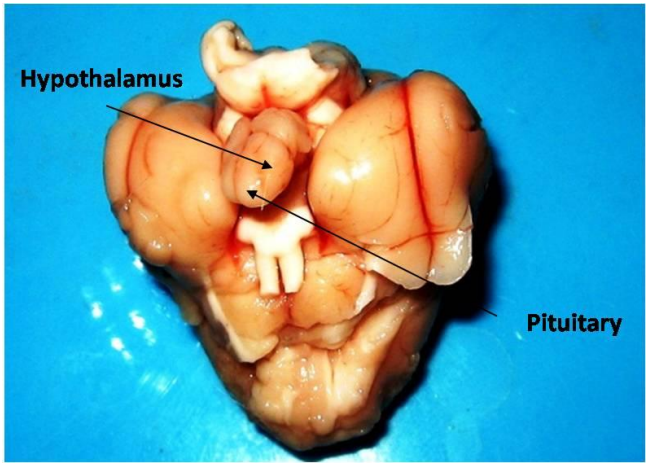

Figure 1. Location of the brain parts (pituitary and hypothalamus)

The weight of each individual gland is measured by an electronically weighing scale. Histological Preparation for Light Microscopy [8] is performed by Fixation of the specimens is made by using 10% isotonic formalin saline for 24-48 hours. Routine staining of sections is performed by using H&E stains.

The standard counting unit is expressed per microscopic field, by micrometer calibrated ocular lens. The numbers of cells are counted in twenty microscopic fields for each specimen and then the mean values of these numbers were calculated.

The hormonal measurements are performed by radioimmunoassay (RIA) method, using the Mini VIDAS instrument (BioMeriux 69280-France model). Then serum is obtained and kept at -20^{o}C in a deep freezer some days in order to use it for measuring the level of LH, FSH, PRL, GH, TSH and ACTH. The SPRs is used for measuring each of them. Specific strip contains 10 wells, the first well for serum sample. Insert the strip and SPRs of each hormone inside the Mini VIDAS instrument and then initiate the radioimmuno-assay technique [9, 10].

Results are analyzed statistically using correlation analysis and t tests between different age groups.

Results

Weight of hypothalamus:

The mean of total Hypothalamus weights are 0.03±0.01, 0.05±0.01 and 0.04±0.01, for group A, B and C, respectively. This reveals a non significant differences ($p>0.05$) are existed between B and C group in comparison with control group (A) (Table 1).

Weight of pituitary gland:

The Weight of Pituitary gland is 0.0085±0.0073, 0.03±0.005 and 0.025±0.01 in groups A, B and C, respectively. There is increase in weight of pituitary gland in groups B in comparison with control group (A) with statically high significant difference ($p<0.01$). There is also increase in weight of pituitary gland in groups C in comparison with control group (A) with statically significant difference ($p<0.05$) (Table 1).

Table 1.Weight of different Glands types

Age group	Gland weight mean±SD	
	Pituitary	Hypothalamus
A	0.0085±0.0073	0.03±0.01
B	0.03±0.01**	0.05±0.01^{ns}
C	0.025±0.005*	0.04±0.01^{ns}

*= P<0.05, **= P<0.01, ^{ns} = not significant (comparison of B and C groups with group).

Number of Supraopticand paraventricularcells of hypothalamus gland

The mean values of the number of supraoptic cells are 15.6±4.39, 22.0±3.94 and 10.2±3.54 of A, B and C. There is a highly significant difference between age group B in comparison with control group (A) p<0.01, and a significant reduction in number of supraoptic cells in age groups C and control group (A) p<0.05 (Table 2).

Table 2. Number of cells in Hypothalamus Gland

Group	Hypothalamus Gland mean±SD	
	supraoptic	paraventricular
A	15.60±4.39	17.80±4.21
B	22.00±3.94**	24.40±4.51**
C	10.20±3.54*	12.00±1.79*

*= P<0.05, **= P<0.01 (comparison of B and C groups with group A).

The mean values of the number of paraventricularcells (Figure 2) are 17.8±4.21, 24.4±4.51 and 12.0±1.79 in age group A, B and C. There is a highly significant difference between age group B in comparison with control group (A) p<0.01, and a significant reduction in number of cells in age groups C in comparison with control group (A) p<0.05 (Table 2).

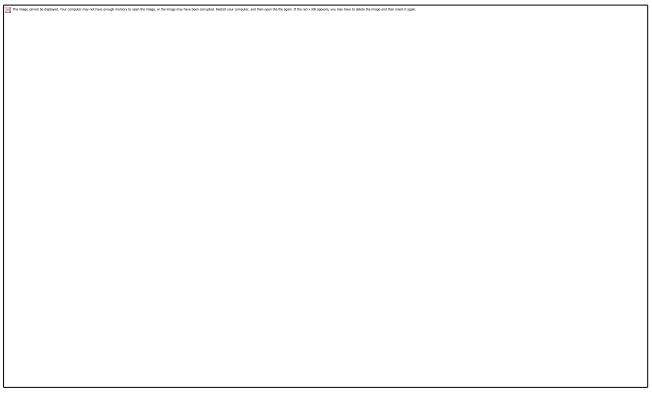

Figure 2.Hypothalamus, shows paraventricular nucleus, neurons and tract fibers of the nucleus (H&E,400X).

Number of the Acidophil, Basophile and Chromophope cells in Pituitary Glands

The mean values of the Acidophil are 14.2±1.64, 23.0±6.0 and 07.2±1.92 in age groups A, B and C, respectively. These results reveals an increase in number of Acidophilsin group B(young adult) in comparison with control group (A), with a significant difference (p<0.05) and a decrease in number of Acidophils in group C in comparison with control group (A), which shows a significant difference (p<0.05) (Table 3).

The mean values of Basophil cells number were 44.0±3.94, 55.0±7.91 and 21.8±6.76 in groups A, B and C, respectively. these results showed B group(young adult) reveals an increase in number of basophile cells in comparison with control group (A), with a significant difference (p<0.05) and a decrease in number of basophile in group C (aged group) in comparison with control group (A), with a high significant difference (p<0.01) (Table 3).

The mean values of cells numbers of chromophope are 10.2±4.32, 14.6±6.73 and 05.8±1.48 in age groups A, B and C, respectively. these results showed B group(young adult) reveals an increase in number of chromophope cells in comparison with control group (A), with a significant difference (p<0.05) and a decrease in number of chromophope in group C (aged group) in comparison with control group (A), with a significant difference (p<0.05) (Table 3).

Table 3. Number of cells in pars distalis of Pituitary gland

Group	Type of cell mean±SD		
	Acidophil	Basophil	Chromophobe
A	14.20±1.64	44.0±3.94	10.2±4.32
B	23.0±6.0*	55.00±7.91*	14.6±6.73*
C	07.2±1.92*	21.80±6.76**	05.8±1.48*

*= P<0.05, **= P<0.01 (comparison of B and C groups with group A).

Hormones measurement in age groups

The mean values of ACTH in different age groups are 0.056±0.018, 0.060 ± 0.016 and 0.053± 0.017 nmol/L in groups A, B and C, respectively. these results showed group B(young adult) reveals an increase in mean values of ACTH in comparison with control group (A), with a non significant difference (p>0.05) and a decrease in mean values of ACTH in group C (aged group) in comparison with control group (A), with a non significant difference (p>0.05) (Table 4).

The mean values of LH in different age groups are increased as following, 1.22±0.01, 1.38±0.02 and 1.62±0.02 nmol/L in groups A, B and C, respectively. These results showed that group B(young adult) reveals an increase in mean values of LH in comparison with control group (A), with a significant difference (p<0.05) and aincrease in mean values of LH in group C (aged group) in comparison with control group (A), with a significant difference (p<0.05) (Table 4).

The mean values of FSH in different age groups are increased as following, 2.5±0.361, 3.5±1.1 and 5.6± 0.1 nmol/L, in groups A, B and C, respectively. These results showed that group B(young adult) reveals an increase in mean values of FSH in comparison with control group (A), with a significant difference (p<0.05) and aincrease in mean values of FSH in group C (aged group) in comparison with control group (A), with a high significant difference (p<0.01) (Table 4).

The mean values of PRL in different age groups are 1.61±0.02, 1.69±0.01 and 1.6±0.01 noml/L in groups A, B and C, respectively. These results showed group B(young adult) reveals an increase in mean values of PRL in comparison with control group (A), with a significant difference (p<0.05) and a decrease in mean values of PRL in group C (aged group) in comparison with control group (A), with non significant difference (p>0.05) (Table 4).

The mean values of GH in different age groups are 1.95±0.1, 1.97±0.02 and 1.92±0.02 nmol/L in groups A, B and C, respectively. these results showed that group B(young adult) reveals an increase in mean values of GH in comparison with control group (A), with a non significant difference (p>0.05) and a decrease in mean values of GH in group C (aged group) in comparison with control group (A), with non significant difference (p>0.05) (Table 4).

The mean values of TSH in different age groups are increased as following, 2.4±0.2 nmol/L, 3.6±0.436nmol/L and 6.1±0.1nmol/L in age groups A, B and C, respectively. These results showed group B(young adult) reveals an increase in mean values of TSH in comparison with control group (A), with a significant difference (p<0.05) and aincrease in mean values of TSH in group C (aged group) in comparison with control group (A), with a high significant difference (p<0.01) (Table 4).

Table 4. Level of Hormones in nmol/ml of different age group

Hormone	Age Group		
	A	B	C
LH	1.22±0.01	1.38±0.02*	1.62±0.02*
FSH	2.5±0.361	3.5±1.1*	5.6±0.1*
PRL	1.61±0.02	1.69±0.01*	1.6±0.1^{ns}
GH	1.95±0.01	1.97±0.02^{ns}	1.92±0.02^{ns}
TSH	2.4±0.2	3.6±0.436*	6.1±0.1**
ACTH	0.056±0.018	0.06±0.016 ^{ns}	0.053±0.017^{ns}

*= P<0.05, **= P<0.01, ^{ns}= non significant (comparison of B and C groups with group A).

Discussion

Weight of Hypothalamus gland

The specimens reveal that there is a non-significant differences in group B and C then the weight decreases beyond that age this finding was in disagreement with Zietz[11], Treier[12], Oliver [13]and Calogero[14].

Concerning the weight of Pituitary gland also underwent early increment in young and then revealed a decrease in weight in aged group(C)

and this is due to reduction of cell number and this result agreed with Saxton[15], who found that there is a reduction of weight and cells number in pituitary in old rodents compared to young, also Stein[16], Wolfe[17], Payne[18], Hanke[7] and Oliver [13]are got same results.

Hypothalamus Glands

The number of cells in the supra optic nucleus was increased in early (group B) but it decreased in later months (group C) and the paraventicular cells undergo same variation in number of cells according to age, also the cell number of paraventricular nucleus is more than supra optic nucleus. These findings may be due to death of cells of the hypothalamic nuclei without replacement of dead cells in aged animal which lead to defiantly and these findings are in agreement with Zietz[11], Treier[12] and Calogero[14].

Pituitary gland

The number of different cells of pars distalis of pituitary gland during early months of age, increased in number but later beyond the age of 24 months. These cells start to decrease in number and basophil cells form the predominant type (60-65%) and the chromophope is the least cell number (15-16%). These findings may be due to difficulty in replacing the dead cells in aged animal and these finding are in agreement with Saxton[15], Stein[16], Wolfe[17], Hanke[7] and Payne[18].

The level of PRL hormone tend to increase during the early months of life then the level was reduced later after the age 24 months and this result is in agreement with Saxton [15] who found that in rabbits with pituitary glands ,serum PRL levels are increased in rabbits of 25-29-month-old, in comparison to rabbits of 15-24 months old female rabbit and then shows a decline of PRL level with further aging. This rise and decline during aging correlated with changes in PRL cell volume, density.

In present study, the serum concentration of thyroid stimulating hormone increases modestly. The variations in TSH concentration between young and elderly subjects are statistically high significant ($P<0.01$) between group C and control group. In contrast, studies reported the decreased in the level of TSH in the elderly men but not in women Olsson[19]. Others found that TSH decreased in aging women [10].

The level of GH has no correlation with age progress in the current study. This disagrees with studies that suggested the onset of aging might be under the control of GH, since primary importance of GH is in the regulation of growth during the maturation process, [20].

In the current study the level of ACTH remain unchanged throughout age. Aging of the anterior pituitary's capacity to ACTH would have significance because of this hormone's mediating role in mobilizing the body's energy reserves in stressful situations. There are however, apparently no age effects on the concentration of ACTH in the blood and these results are agreed with Blichert[21] and disagreed with Thieme[22] who found that ACTH level is positively correlated with age. The HPA axis would become less resilient with age in responding to stimulations.

The current study reveal that the level of FSH hormone is positively correlated with age and this result is in agreement with Tatone[23] and Chand [24] who stated that High levels of Follicle-Stimulating Hormone are indicative of situations where the normal restricting feedback from the gonad is absent, leading to an unrestricted pituitary FSH production. Whereas this is normal in women leading up to and during post menopause, it is abnormal during the reproductive years.

The current study reveal positive correlation between the level of LH hormone and age, just like the FSH hormone and this result is in agreement with Ahmed[25] who stated that there was a significant progressive increase in FSH levels as early as age 29-30 years which was continued throughout the 30s and become more

marked in the early 40s. The Increase In basal FSH (and later LH) may represent the earliest endocrine marker of reproductive aging and can be used as hormonal markers to counsel patients as to the likelihood of their reproductive potential[25].

References

1. RomanesGJ. Cunningham's manual of practical anatomy. 15th ed. Oxford, Oxford Medical Publication, 1997; p. 264-289.
2. Halász B. Anatomy-functional histological notes on hypothalamo-pituitary adrenal axis of rodents. *Neuroendocrinology* 1993; 57: 1196-1207.
3. Stevens A, Low JS. Human Histology, 2nd edition. New York: Mosby publisher 1997; p. 131-132.
4. Tissenbaum HA, Guarente L. Model organisms as a guide to mammalian aging. *Dev Cell* 2002; 2: 9-19.
5. Silver AJ, Guillen CP,Kahl MJ. Effect of aging on human body. *J Am Geriatric Soc*. 1995; 41: 211-213.
6. Neepa Y, Gloria D, Cathy S, et al. Role of thyroid hormones in human and laboratory animal reproductive health. *Birth Defect Res* 2003; 68: 479-491.
7. Hanke HH, Charippee HA. The anatomy and cytology of the pituitary gland of the golden hamster. *Anat Rec* 1994; 102: 123-139.
8. Johan DB, Alan S. Theory and Practice of Histological Techniques. 2nd edition. Langman Group Limited, London, 1982; p. 49-56.
9. Braverman LE, Utiger RD. Werner and Ingbar's, the thyroid. A fundamental and clinical text. 8th edition, Lippincott Williams &Wilkins. 2000; p. 20-37.
10. Fathzadeh M, Seyedna H, Khazali H, et al. Epidemiological study of T4, T3 and TSH mean concentrations in four Iranian populations. *Iranian J Publ Health* 2005; 34(1): 74-79.
11. Zietz B, Hrach S, Scholmerich J, et al. Differential age-related changes of hypothalamus-pituitary-adrenal axis hormones in healthy women and men. *ExperClinEndocrinolDiab*2001; 109(2): 93-101.
12. Treier M, Rosenfeld MG. The hypothalamic-Pituitary axis: co-development of two organs. *CurrOpin Cell Biol* 1996; 8: 833-43.
13. Oliver O, Tony FD, Hanjoon M, et al. Physiological features of aging persons. *Am MedAsso*2003; 138: 1068-1076.
14. Calogero AE, Gallucci WT, Gold PW, et al. Multiple feedback regulatory loops upon rat hypothalamic corticotropin-releasing hormone secretion. Potential clinical implications. *J Clin Invest* 1988; 82: 767-774.
15. Saxton JA, Green HSH. Age and sex differences in hormone content of rabbit hypophysis. *Endocrinology*, 1989; 24: 494-502.
16. Stein KFE, Caldwell, Peters J. Age changes in the anterior pituitary gland of the rabbits. *Anat Rec* 1992;84: 523-26.
17. Wolfe JM, Bryan WR, Wright AW. Histologic observation on the anterior pituitary of old rats with particular reference to the spontaneous appearance of pituitary adenomata. *Am J Cancer* 2001; 67: 321-326.
18. Payne F. The cellular picture of anterior pituitary of normal fowls from embryo to old age. *Anat Rec* 1999; 96: 77-91.
19. Olsson T, Viitanen M, Hagg E, et al. Hormones in "young" and "old" elderly: pituitary-thyroid and pituitary adrenal axes. *Gerontology* 1998; 35: 144-152.
20. Everitt AV, Burgess JA. Growth hormone and aging. In:EverittAV, Burgess A (eds.). Hypothalamus, pituitary and aging. Springfield, IL: C C Thomas 1996; p. 435-437.
21. Blichert-Toft M. Secretion of corticotrophin and somatotrophin by the senescent adenohypophysis in man. *ActaEndocrinologica (Supp.)* 1997; 195: 150-154.
22. Zietz B, Hrach S, Schölmerich J, Straub RH. Differential age-related changes of hypothalamus - pituitary - adrenal axis hormones in healthy women and men - role of interleukin 6. *ExpClinEndocrinol Diabetes* 2001;109(2):93-101.
23. Tatone CF, Amicarelli MC, Carbone P, et al. Cellular and molecular aspects of ovarian follicle ageing.*Hum Reprod Update* Mar. 2008; 14(2): 131-142.
24. ChandAL, Ooi GT, Harrison CA, et al. Functional analysis of the human inhibin(alpha) subunit variant A257T and its potential role in premature ovarian failure.*Hum Reprod*Dec. 2007; 22(12): 3241-3248.
25. Ahmed Ebbiary NA, LentonEA, Cooke ID. Hypothalamic-pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. *ClinEndocrinol (Oxf.)*1994 Aug;41(2):199-206.

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