Clinica Chimica Acta 499 (2019) 149–153

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Role of apelin/APJ system in hypothalamic-pituitary axis

Na Yang[a], Tianping Li[a], Jun Cheng[b], Qinhui Tuo[c], Jian Shen[d,*]

[a] Office of Educational Administration, Hunan Polytechnic of Environment and Biology, Hengyang 421005, China
[b] Institute of Pharmacy and Pharmacology, Hunan Province Cooperative innovation Center for Molecular Target New Drugs Study, Hengyang Medical College, University of South China, Hengyang 421001, China
[c] Hunan university of Chinese medicine, Changsha, 410208 Hunan, China
[d] Intensive care unit, Hengyang No.1 Peoples Hospital, Hengyang 421001, Hunan, China

ARTICLE INFO

Keywords:
Apelin
APJ
Hypothalamus
Pituitary

ABSTRACT

Apelin and its G protein-coupled receptor APJ are specifically expressed in endocrine organs.

As well known, the hypothalamus is the regulatory center of endocrine activity, which combined with the pituitary and other gonads form regulatory axes involved in endocrine function. Evidence to date has shown that the apelin/APJ system plays an important role in mediating these axes, such as food intake, acute stress, steroid release, as well as, an anti-depressant-like activity. Here we review the effect of the apelin/APJ system on hypothalamic-pituitary-thyroid, hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes. Although the apelinergic system exerts a positive effect on these axes, there are contradictory reports on the role of apelin in endocrine disease caused by hypothalamic-pituitary disorders. Thus, as research continues to evolve we expect that apelin-related drugs can be used as a treatment for clinical diseases resulting from hypothalamic-pituitary dysfunction.

1. Introduction

In 1993 , O'Dowd et al. identified a novel G protein-coupled receptor in a human gene, named APJ. In their study, they found that APJ receptors were widely expressed in the following rat brain tissues, such as striatum, hippocampus, cerebellum and cortex [1]. Besides, APJ was also detected in the white matter region of the human brain [2]. These findings indicate that endogenous ligands of the APJ receptor may play a pivotal role in the transmission of information in the brain. However, little is known about endogenous ligand for APJ receptor for a long time. It was not until that Tatemoto and colleagues established a cell model that can express the APJ receptor, which used as a bait to catch its corresponding ligand. Finally, they isolated a ligand for the APJ receptor from the stomach extract of cattle and was given the name apelin. Moreover, they also found that the precursor protein of apelin is composed of 77 amino acids, which can be cleaved into different subunits by endopeptidase. Among them, apelin-13 and apelin-17 have the most obvious activity [3]. In addition, the N-terminus of apelin can also be modified by glutamate to form pGlu-apelin-13, which has been reported to have the most active against APJ receptor in vitro [4].

The endocrine system consists of many endocrine organs, including the hypothalamus, pituitary, thyroid gland, adrenal gland, islet, ovary, etc. All these glands play a crucial role in the regulation of the body's nerves and fluids. A growing number of studies have shown that apelin and APJ specific distributed in central gland. A study by O'Carroll et al. reported that B78/apj, a homologue of the human APJ receptor in rat, was striking distribution in the cells from the anterior pituitary, hypothalamic paraventricular nucleus as well as the supraoptic nucleus [5]. Similar findings have been found in De et al. research [6]. In addition, apelin/APJ system also exists in peripheral gland organs, like thyroid, adrenal gland, islet, ovary [7,8]. Herein, we explore the interrelationship between the apelin/APJ system and endocrine organs.

A mounting number of researches have reported that endocrine system play a vital role in regulating the physiological and pathological processes of the body, i.e. food and water intake, stress response, immunologic homeostasis [9–11]. So far, the endocrine system is mainly regulated by three signal axes, which are the hypothalamic-pituitary-thyroid axis (HPT), the hypothalamic-pituitary-adrenal axis (HPA), the hypothalamic-pituitary-gonadal axis (HPG), respectively. In recent years, apelin/APJ system is reported to participate in the regulation of endocrine signals, which affect the biological functions, such as food intake, acute stress, and antidepressant-like actions [12,13].

2. The role of apelin in hypothalamus–pituitary-thyroid axis

HPT is an important axis for maintaining the level of thyroid

* Corresponding author.
E-mail address: narlalove66@163.com (J. Shen).

https://doi.org/10.1016/j.cca.2019.09.011
Received 29 August 2019; Received in revised form 11 September 2019; Accepted 12 September 2019
Available online 13 September 2019

hormone in the body. Studies have found that the thyrotropin-releasing hormone (TRH) was secreted by the hypothalamic paraventricular nuclei (PVN), which is the initial state of activating HPT axis [14]. Once activated, TRH enhances the secretion of thyroid-stimulating hormone (TSH) from the pituitary, which undergoes blood circulation and ultimately enhances the synthesis and secretion of thyroid hormone (TH). Of note, when the TH reaches a certain level in the blood, the expression of thyrotropin-releasing hormone receptor (TRHR) gene can be activated, thereby inhibiting the production of TSH [15]. It is reported that apelinergic system is involved in regulating HPT axis.

An investigation on immunohistochemical analysis of apelin in lizards revealed that apelin was localized in endothelial cells of the thyroid gland [7]. Gürel et al. showed that the levels of apelin were much higher in patients with hypothyroidism and hyperthyroidism compare with the health subjects [16]. In contrast, apelin levels was found to lower in patients with subclinical hypothyroidism (SCH) compare with the control group, while treatment of 12-weeks of levothyroxine, the levels of apelin increased significantly [17]. In addition, addition of apelin and thyroxin (T4) would play a protective role in (propylthiouracil) PTU-induced hypothyroid rats [18]. In conclusion, there is still little known about the reasons for this contradictory results, we still need more evidences to identify the relationship between apelin and HPT axis (Table.1).

3. The role of apelin in the hypothalamic-pituitary-adrenal axis

HPA axis is mainly consist of three parts, which are important locations for hormone production. It is reported that PVN can secrete antidiuretic hormone (ADH) and corticotropin releasing hormone(CRH, also named CRF) , furthermore, the anterior pituitary gland is found to release adrenocorticotropic hormone (ACTH), in addition, adrenal cortex can synthesize glucocorticoids under the influence of ACTH. Therefore, the mutual regulation of these hormones is involved in biological functions such as the control of stress responses, energy storage and consumption [19,20]. Apelin is reported to play a regulatory role in HPA axis. Taheri et al. demonstrated that exogenous administration of apelin-13 (10 nmol) significantly increased the levels of ACTH and corticosterone in plasma at 30 min compare with the control group. Besides, apelin-13 also stimulated the release of CRH and vasopressin (AVP) from hypothalamic explants [21]. Moreover, addition of apelin-17greatly improved the release of ACTH in an ex vivo perifusion system of anterior pituitaries [22]. Newson et al. showed that pGlu-apelin-13 (1 mg/kg i.c.v.) supplementation induced the increasing of ACTH and corticosterone (CORT), on the contrary, pretreatment of alpha-helical CRF (9–41) (a type of CRF receptor antagonist) or in mice with loss of AVP V1b receptors function, the effects of these two hormones would diminish. These data indicating that apelin appeared to play an important role in regulating HPA function via both CRF and AVP [23]. When injection of lipopolysaccharide (LPS) to APJ knockout (APJ KO) male mice, Newson et al. demonstrated that the level of ACTH has markedly impared compared with wildtype mice, however, the female APJ KO has no effect. They infer the reason may be related to gender specific with the regulation of APJ. In addition, hypoglycemia significantly increased ACTH and did not increase in APJ KO male mice. Therefore, all their findings indicate that APJ has gender specific in the process of activating HPA axis [12]. Additionally, studies have showed that HPA axis is associate with depression [24,25]. Recently, apelin is discovered to ameliorate chronic water-immersion restraint stress (WIRS)-induced depression-like phenotype through reducing nuclear translocation of hippocampal glucocorticoid receptor (GR) and increasing brain-derived neurotrophic factor (BDNF) expression [13]. Therefore, apelinergic is expected to become a potential target for regulating the HPA axis.

4. The role of apelin in the hypothalamic-pituitary-adrenal axis

4.1. The role of apelin in hypothalamic-pituitary-ovary axis

The ovaries are female reproductive organs, which mainly secreting estrogen (E), progesterone (P) and a small amount of androgen. As mentioned before, APJ receptor is abundantly expressed in rat ovarian corpus luteum (CL), furthermore, a reported from Shimizu et al. showed that APJ receptor in granulosa cells, as well as the APJ receptor and apelin in theca tissues, those were all expressed in the ovary of bovine [26]. All these findings suggest that apelin may contribute to regulate ovarian function. Shirasuna et al. demonstrated that the expression of apelin mRNA significantly increased from early to late CL, which later decreased in regressed CL. However, the expression of APJ mRNA enhanced from early to mid-CL, furthermore, it remained up-regulated in late and regressed CL. In addition, they also found prostaglandin F(2)

Table 1
Apelin/APJ is involved in hypothalamic-pituitary axis.

Hypothalamic-pituitary axis	Experiment models	Treatment	Pathways	Effects	References
Hypothalamic-pituitary-thyroid axis	Patients with SCH	Levothyroxine	↑Apelin	Protect	17
	PTU-induced hypothyroid rats	Apelin and T4	↑Heart rate ↑QRS voltage	Protect	18
Hypothalamic-pituitary-adrenal axis	Wistar rats	Apelin-13	↑ACTH ↑CORT	↑Water intake	21
	Mice	pGlu-apelin-13	↑CRF ↑AVP	↑ACTH ↑CORT	23
	APJ KO mice	LPS;Insulin-induced hypoglycaemia;FS stressors	↑ACTH ↑CORT	Protect	12
	SD rats	Apelin-13	TrkB-BDNF	Antidepressant	13
Hypothalamic-pituitary-ovary axis	Ovarian GC	Apelin	PI3/Akt	↑GC proliferation	28
	Co-cultures of Gc and Tc	Apelin	MAPK/ERK; Akt/PI3	↑Cell proliferation	29
	OVCAR-3 cells	Apelin	↑PPARg	↑Cell progression	36
	OVISE cells	Apelin	↑ P53	↑Cell growth	37
	Ovarian cancer model	Overexpression of APJ	↑STAT3	↑Cell aggressive	38
Hypothalamic-pituitary-testis axis	Rats	Apelin-13	↑LH secretion	↑Testosterone release	44

SCH: subclinical hypothyroidism; PTU: propylthiouracil; ACTH: adrenocorticotropic hormone; CORT: corticosterone; CRF: corticotropin releasing factor; AVP: vasopressin; LPS: lipopolysaccharides;
FS: forced swim; SD rats: Sprague–Dawley rats; GC: ovarian granulosa cells; Tc: theca; APJ KO mice: APJ.
knockout mice; OVCAR-3: epithelial ovarian cancer cell line; OVISE cells: ovarian clear cell carcinoma cell lines; PPARg: peroxisome proliferator-activated receptor g; STAT3: Signal transducers and activators of transcription; LH: luteinizing hormone;

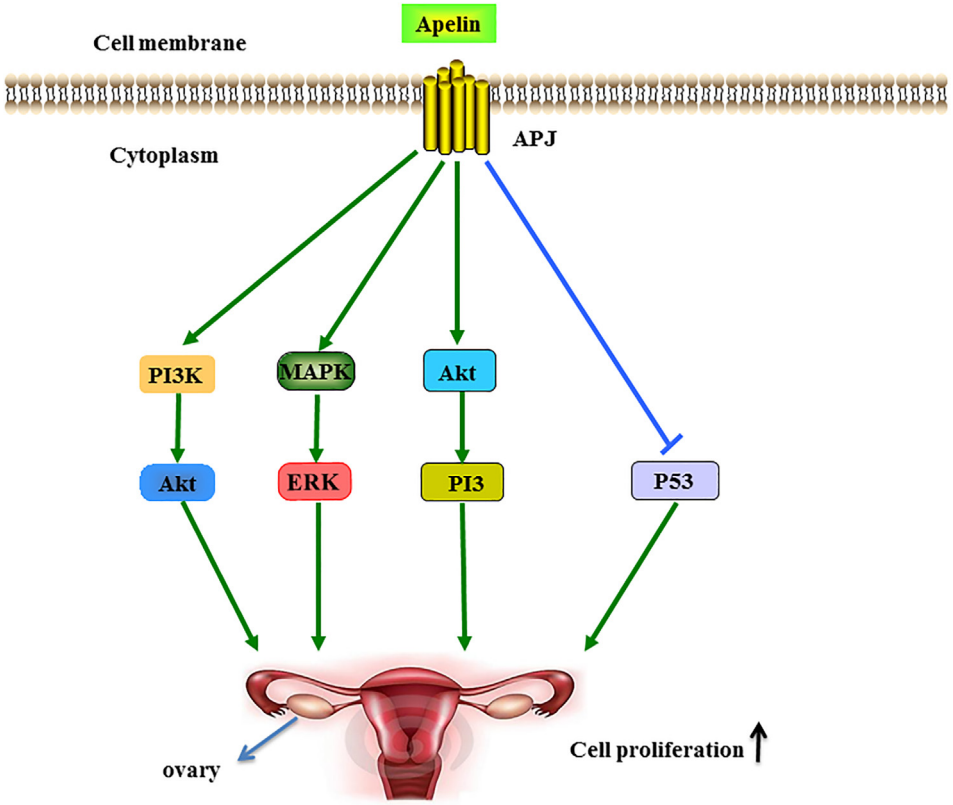

Fig. 1. Apelin/APJ system signal pathways in hypothalamic-pituitary-ovary axis.
Apelin promotes ovarian cells proliferation though PI3K/Akt, MAPK/ERK, Akt/PI3 signal pathways. On the other hand, apelin enhances the ovarian cancer cells proliferation by inhibition of P53.

(alpha) (PGF(2)(alpha)) was able to induce luteolysis, which stimulated the mRNA expression of apelin and APJ at 0.5-2 h and 2 h respectively. Notably, the apelin/APJ was blocked from 4 h during PGF(2)(alpha)-induced luteolysis. These results indicating that apelin/APJ not only participated in the maturation of follicle, but also an intrinsic regulator of corpus luteum formation [27]. Recently, apelin has been reported to involve in regulating the above process by activating a series of signaling pathways. Shuang et al. discovered that apelin up-regulated the proliferation of granulosa cells (GCs) by PI3/Akt signaling [28]. Rak et al. found that apelin enhanced ovarian cells proliferation via activating AMPK/ERK and Akt/PI3 singal pathways. Moreover, they also observed that apelin abolished IGF1- and follicle stimulating hormone (FSH)-induced steroid secretion [29]. Of note, addition of apelin to maturation medium, which contain IGF1 (10–8 M) but not FSH (10–8 M), it was observed that apelin attenuated the progression of meiotic at the germinal vesicle stage [30]. Thus, apelin has an important biological function in control of hypothalamic-pituitary-ovary axis.

Besides this, polycystic ovary syndrome (PCOS) is a syndrome of endocrine disorders induced by hypothalamic-pituitary-ovarian axis dysfunction, which characterized by sparse ovulation or anovulation, luteinizing hormone (LH) secretion, high androgen or insulin resistance, as well as polycystic ovary [31]. Researches have shown that apelin seems to be correlated to the progress of PCOS. It is reported that the levels of serum apelin in pateints with PCOS appeared to lower than that of the control subjects [32,33]. However, in other studies, researchers get the opposite results [34,35]. We speculate on the reasons for these different conclusions may result from the difference of study subjects. It seems that PCOS patients with higher apelin levels are complicated by obesity.

Ovarian tumors are one of the common malignant diseases of female reproductive organs, which often classified into three categories, epithelial ovarian tumors, granulosa cell tumors, germ cell tumors, respectively. Among them, epithelial cell tumors account for the vast majority. Hoffmann et al. indicated that the levels of APJ mRNA were higher in epithelial cancer cells compared with granulosa cell tumor cells. However, the expression and secretion of apelin were higher in granulocyte tumor cells than in other types of ovarian cancer. Moreover, they also found that BPA, an exogenous estrogen, which promoted the expression of apelin in epithelial ovarian cancer cell line OVCAR-3 by activating peroxisome proliferator-activated receptor g (PPARg) [36]. In addition, ovarian cancer can also be classified into several subtypes depending on the histotypes, including high-grade serous (HGSC), clear-cell (OCCC), endometrioid (EC), lowgrade serous (LGSC), as well as mucinous carcinoma. A study from the Yi et al. group showed that apelin much higher in OCCC than other types. Furthermore, apelin promoted the development of ovarian cancer via inhibiting the activation of P53 in OCCC tumors [37]. Recently, Neelakantan et al. demonstrated that the APJ expression of malignant cells in HGSC were significantly higher than that in normal ovarian epithelial cells. In their study, they also discovered that high APJ expression promoted the progression and metastasis of HGSC and shortened the survival period. The mechanism may be associate with activating the downstream STAT3 signal [38].

Besides, it is recent immunostaining showed that a strong expression of apelin in secretory phase of glandular cells during ectopic and ectopic endometrial [39]. Overall, these findings correlated with previous data suggested that apelin/APJ exert an important impact on hypothalamic-pituitary-ovary axis, and it might be an attractive novel therapeutic target for treating ovarian diseases (Fig.1).

4.2. *The role of apelin in hypothalamic-pituitary-testis axis*

The testis is an important reproductive organ in men. It is reported

that testosterone secretion by Leydig cells (LC) in the testis is essential for maintaining male physiological function, such as.

male genital differentiation, development of secondary sexual characteristics, as well as maintain spermatogenesis [40–42]. In mammals, testosterone is primarily regulated by the hypothalamus-.

pituitary. It is demonstrated that Gonadotropin-releasing hormone (GnRH) released from the hypothalamus, which stimulated the release of FSH and LH from the anterior pituitary. Subsequently, LH enters into the bloodstream to reach the testis, binds to the membrane receptors on the interstitial cells, and promotes the synthesis and secretion of testosterone. In addition, testosterone levels in plasma can also negatively regulate the secretion of LH, thereby maintaining the normal physiological concentration of testosterone in the body. In the past, the expression of apelin in testicular tissue has been confirmed [43]. Therefore, the effect of apelin on the testis has become a new focus for researchers. An investigation from Sandal et al. indicated that high-dose apelin-13 were lower in testosterone levels compared with the control subject. Moreover, histology demonstrated that the number of Leydig cells were significantly reduced in high doses of apelin-13 than that of other group. The mechanism may be related to the inhibition of LH release by apelin [44]. Thus, apelin may also play an important role in male infertility.

5. Conclusion and future directions

In conclusion, apelin/APJ system, as a novel activity regulator, is involved in the regulation of neurohumoral fluid in the endocrine system, especially the hypothalamic-pituitary axis. In this review, we summarize the expression of apelin and APJ in the hypothalamus, pituitary, thyroid, adrenal, ovarian, and testicular tissues. In addition, we further explored its effect on endocrine diseases by affecting these signal axes. It is worthy of our attention that the difference in apelin concentration and stimulation time may lead to conflicting results. The possible mechanism are related to the distribution of hormone tissues, the time and frequency of the pulses, as well as the state of the body. However, most of the results indicated that apelin may play a positive role in regulating the above hormones. Further data are also needed to support this idea. Therefore, targeting for diseases caused by hypothalamic-pituitary axis disorders, apelin may become a new treatment for such diseases.

Disclosure statement

The authors declare there are no competing financial interests exist.

Acknowledgments

This work is supported by grants from the National Natural Science Foundation of China (grant numbers: 81673722, 81600291), Hunan Provincial Natural Science Foundation (14JJ1024), and The Education Department of Hunan Province (16A156).

References

[1] B.F.H.M. O'Dowd, A. Chan, H.H. Heng, L.C. Tsui, J.L. Kennedy, X. Shi, A. Petronis, S.R. George, T. Nguyen, A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11, Gene 136 (1993) 355–360.
[2] M. Matsumoto, K. Hidaka, H. Akiho, S. Tada, M. Okada, T. Yamaguchi, Low stringency hybridization study of the dopamine D4 receptor revealed D4-like mRNA distribution of the orphan seven-transmembrane receptor, APJ, in human brain, Neurosci. Lett. 219 (1996) 119–122.
[3] K. Tatemoto, M. Hosoya, Y. Habata, R. Fujii, T. Kakegawa, M.X. Zou, et al., Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor, Biochem. Biophys. Res. Commun. 251 (1998) 471–476.
[4] M. Hosoya, Y. Kawamata, S. Fukusumi, R. Fujii, Y. Habata, S. Hinuma, et al., Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin, J. Biol. Chem. 275 (2000) 21061–21067.
[5] A.M. O'Carroll, T.L. Selby, M. Palkovits, S.J. Lolait, Distribution of mRNA encoding B78/APJ, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues, Biochim. Biophys. Acta 1492 (2000) 72–80.
[6] N. De Mota, Z. Lenkei, C. Llorens-Cortes, Cloning, pharmacological characterization and brain distribution of the rat apelin receptor, Neuroendocrinology 72 (2000) 400–407.
[7] M. De Falco, V. Fedele, T. Russo, F. Virgilio, R. Sciarrillo, S. Leone, et al., Distribution of apelin, the endogenous ligand of the APJ receptor, in the lizard Podarcis sicula, J. Mol. Histol. 35 (2004) 521–527.
[8] G.R. Pope, E.M. Roberts, S.J. Lolait, A.M. O'Carroll, Central and peripheral apelin receptor distribution in the mouse: species differences with rat, Peptides 33 (2012) 139–148.
[9] R.J. Perry, J.M. Resch, A.M. Douglass, J.C. Madara, A. Rabin-Court, H. Kucukdereli, et al., Leptin's hunger-suppressing effects are mediated by the hypothalamic-pituitary-adrenocortical axis in rodents, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 13670–13679.
[10] C. Wu, W.G. Gong, Y.J. Wang, J.J. Sun, H. Zhou, Z.J. Zhang, et al., Escitalopram alleviates stress-induced Alzheimer's disease-like tau pathologies and cognitive deficits by reducing hypothalamic-pituitary-adrenal axis reactivity and insulin/GSK-3beta signal pathway activity, Neurobiol. Aging 67 (2018) 137–147.
[11] I.I.Y. Matsumoto, T. Shimada, T. Aikawa, Brain mast cells act as an immune gate to the hypothalamic-pituitary-adrenal axis in dogs, J. Exp. Med. 194 (2001) 71–78.
[12] M.J. Newson, G.R. Pope, E.M. Roberts, S.J. Lolait, A.M. O'Carroll, Stress-dependent and gender-specific neuroregulatory roles of the apelin receptor in the hypothalamic-pituitary-adrenal axis response to acute stress, J. Endocrinol. 216 (2013) 99–109.
[13] T.T. Dai, B. Wang, Z.Y. Xiao, Y. You, S.W. Tian, Apelin-13 upregulates BDNF against chronic stress-induced depression-like phenotypes by ameliorating HPA Axis and hippocampal glucocorticoid receptor dysfunctions, Neuroscience 390 (2018) 151–159.
[14] A.N. Hollenberg, The role of the thyrotropin-releasing hormone (TRH) neuron as a metabolic sensor, Thyroid 18 (2008) 131–139.
[15] T.P. Segerson, J. Kauer, H.C. Wolfe, H. Mobtaker, P. Wu, I.M. Jackson, et al., Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus, Science 238 (1987) 78–80.
[16] A. Gurel, A. Dogantekin, Y. Ozkan, S. Aydin, Serum apelin levels in patients with thyroid dysfunction, Int. J. Clin. Exp. Med. 8 (2015) 16394–16398.
[17] H.Y. Yasar, M. Demirpence, A. Colak, L. Yurdakul, M. Zeytinli, H. Turkon, et al., Serum irisin and apelin levels and markers of atherosclerosis in patients with subclinical hypothyroidism, Arch .Endocrinol. Metab. 63 (2019) 16–21.
[18] Z. Akhondali, M. Badavi, M. Dianat, F. Faraji, Co-administration of Apelin and T4 protects inotropic and chronotropic changes occurring in hypothyroid rats, Arq. Bras. Cardiol. 105 (2015) 235–240.
[19] D. Wang, B. Xu, J. Wang, H. Wang, J. Guo, H. Ji, et al., Response of the maternal hypothalamus to cold stress during late pregnancy in rats, Brain Res. 1722 (2019) 146354.
[20] C.P. Elias, D.E. Antunes, M.S. Coelho, C.L. de Lima, N. Rassi, A.P.M. de Melo, et al., Evaluation of the hypothalamic-pituitary-adrenal axis in a case series of familial partial lipodystrophy, Diabetol. Metab. Syndr. 11 (1) (2019).
[21] S. Taheri, K. Murphy, M. Cohen, E. Sujkovic, A. Kennedy, W. Dhillo, et al., The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats, Biochem. Biophys. Res. Commun. 291 (2002) 1208–1212.
[22] A. Reaux-Le Goazigo, R. Alvear-Perez, P. Zizzari, J. Epelbaum, M.T. Bluet-Pajot, C. Llorens-Cortes, Cellular localization of apelin and its receptor in the anterior pituitary: evidence for a direct stimulatory action of apelin on ACTH release, Am. J. Phys. Endocrinol. Metab. 292 (2007) E7–15.
[23] M.J. Newson, E.M. Roberts, G.R. Pope, S.J. Lolait, A.M. O'Carroll, The effects of apelin on hypothalamic-pituitary-adrenal axis neuroendocrine function are mediated through corticotrophin-releasing factor- and vasopressin-dependent mechanisms, J. Endocrinol. 202 (2009) 123–129.
[24] R. Andreatini, Depression and the hypothalamic-pituitary-adrenal axis: increasing the scope, Acta Neuropsychiatr. 24 (2012) 1–3.
[25] J.H. Meador-Woodruff, J.F. Greden, L. Grunhaus, R.F. Haskett, Severity of depression and hypothalamic-pituitary-adrenal axis dysregulation: identification of contributing factors, Acta Psychiatr. Scand. 81 (1990) 364–371.
[26] T. Shimizu, N. Kosaka, C. Murayama, M. Tetsuka, A. Miyamoto, Apelin and APJ receptor expression in granulosa and theca cells during different stages of follicular development in the bovine ovary: involvement of apoptosis and hormonal regulation, Anim. Reprod. Sci. 116 (2009) 28–37.
[27] K. Shirasuna, T. Shimizu, K. Sayama, T. Asahi, M. Sasaki, B. Berisha, et al., Expression and localization of apelin and its receptor APJ in the bovine corpus luteum during the estrous cycle and prostaglandin F2alpha-induced luteolysis, Reproduction 135 (2008) 519–525.
[28] L.J.W. Shuang, P. Hongjuan, Y. Zhenwei, Effects of apelin on proliferation and apoptosis in rat ovarian granulosa cells, Clin. Exp. Obstet. Gynecol. 43 (2016) 409–413.
[29] A. Rak, E. Drwal, C. Rame, K. Knapczyk-Stwora, M. Slomczynska, J. Dupont, et al., Expression of apelin and apelin receptor (APJ) in porcine ovarian follicles and in vitro effect of apelin on steroidogenesis and proliferation through APJ activation and different signaling pathways, Theriogenology 96 (2017) 126–135.
[30] J. Roche, C. Rame, M. Reverchon, N. Mellouk, A. Rak, P. Froment, et al., Apelin (APLN) regulates progesterone secretion and oocyte maturation in bovine ovarian cells, Reproduction 153 (2017) 589–603.
[31] A.S.K. Dunaif, W. Futterweit, A. Dobrjansky, Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome, Diabetes 38 (1989) 1165–1174.

[32] C.Y. Chang, Y.C. Tsai, C.H. Lee, T.F. Chan, S.H. Wang, J.H. Su, Lower serum apelin levels in women with polycystic ovary syndrome, Fertil. Steril. 95 (2011) 2520–3 e1-2.
[33] S.Ö. Altinkaya, S. Nergiz, M. Küçük, H. Yüksel, Apelin levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome, Eur. J. Obstet. Gynecol. Reprod. Biol. 176 (2014) 168–172.
[34] K. Goren, N. Sagsoz, V. Noyan, A. Yucel, O. Caglayan, M.S. Bostanci, Plasma apelin levels in patients with polycystic ovary syndrome, J Turk Ger Gynecol Assoc. 13 (2012) 27–31.
[35] M. Olszanecka-Glinianowicz, P. Madej, M. Nylec, A. Owczarek, W. Szanecki, P. Skałba, et al., Circulating apelin level in relation to nutritional status in polycystic ovary syndrome and its association with metabolic and hormonal disturbances, Clin. Endocrinol. 79 (2013) 238–242.
[36] M. Hoffmann, E. Fiedor, A. Ptak, Bisphenol A and its derivatives tetrabromobisphenol A and tetrachlorobisphenol A induce apelin expression and secretion in ovarian cancer cells through a peroxisome proliferator-activated receptor gamma-dependent mechanism, Toxicol. Lett. 269 (2017) 15–22.
[37] Y. Yi, S.H. Tsai, J.C. Cheng, E.Y. Wang, M.S. Anglesio, D.R. Cochrane, et al., APELA promotes tumour growth and cell migration in ovarian cancer in a p53-dependent manner, Gynecol. Oncol. 147 (2017) 663–671.
[38] D. Neelakantan, S. Dogra, B. Devapatla, P. Jaiprasart, M.C. Mukashyaka, R. Janknecht, et al., Multifunctional APJ pathway promotes ovarian Cancer progression and metastasis, Mol. Cancer Res. 17 (2019) 1378–1390.
[39] Z.S. Ozkan, H. Cilgin, M. Simsek, B. Cobanoglu, N. Ilhan, Investigation of apelin expression in endometriosis, J. Reprod .Infertil. 14 (2013) 50–55.
[40] M.C. Beattie, L. Adekola, V. Papadopoulos, H. Chen, B.R. Zirkin, Leydig cell aging and hypogonadism, Exp. Gerontol. 68 (2015) 87–91.
[41] E.G. Skurikhin, A.V. Pakhomova, O.V. Pershina, V.A. Krupin, N.N. Ermakova, E.S. Pan, et al., Role of Sertoli and Leydig cells in the regulation of Spermatogonial stem cell and development of reproductive disorders in male C57Bl/6 mice with type 1 diabetes mellitus, Bull. Exp. Biol. Med. 164 (2017) 127–131.
[42] S. Nef, L.F. Parada, Hormones in male sexual development, Genes Dev. 14 (2000) 3075–3086.
[43] Y. Kawamata, Y. Habata, S. Fukusumi, M. Hosoya, R. Fujii, S. Hinuma, et al., Molecular properties of apelin: tissue distribution and receptor binding, Biochim. Biophys. Acta 1538 (2001) 162–171.
[44] S. Sandal, S. Tekin, F.B. Seker, A. Beytur, N. Vardi, C. Colak, et al., The effects of intracerebroventricular infusion of apelin-13 on reproductive function in male rats, Neurosci. Lett. 602 (2015) 133–138.