Section A -Research paper

Role of Spexin in Health and Disease

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Abstract:

Spexin is a novel neuropeptide playing an emerging role in metabolic diseases such as obesity and diabetes via involvement in energy homeostasis and food intake.

Keywords: Spexin, diabetes, neuropeptide.

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Introduction:

Spexin (SPX) is an endogenous peptide discovered through the Hidden Markov Model in 2007 **(1)**. The C12orf39 gene encoding preprospexin (116 amino acid) was found on chromosome 12 in the human genome **(2)**. After a series of protein synthesis processes on preprospexin "mature SPX" is formed, that effective form on cellular physiological processes. The amino acid sequence of the SPX peptide has been evolutionarily conserved in all vertebrates and invertebrates **(3).**

SPX mRNA/protein is commonly present in the main systems of the body, such as cardiovascular, skeletal, digestive, urinary, reproductive, endocrine, and central nervous systems. The widespread synthesis of SPX has indicated that it regulates many physiological functions in the body as food intake glucose/fat metabolism gastrointestinal motility, reproductive **(4, 5)** and cardiovascular functions. Furthermore, important roles of SPX have been discovered in pathological conditions such as obesity, anorexia nervosa, diabetes, anxiety, and depression. SPX does the above-mentioned effects by binding to galanin-2 (GAL2) and galanin-3 (GAL3) receptors **(3)**.

Spexin peptide structure:

Ma et al. (6) Showed that preprospexin peptide contains both a hydrophobic signal peptide (SP) and two dibasic prohormone cleavage/amidation sites (RR/KR & GRR) (Fig. 1). A small amino acid region among dibasic cleavage sites forms the 14 amino acids of SPX, also named neuropeptide Q (NPQ). The C12orf39 gene consists of 6 exons and 5 introns in humans. The 1st and 2nd exons have encoded the signal peptide while the 3rd and 4th exons encode the active peptide **(1).** The 14 amino acids sequence of SPX is highly conserved with only minor changes in humans and other species. Like cats, dogs, and pandas as, serine amino acid is replaced with alanine in the 6th position (Fig. 2).

Wong et al. (7) introduced the three-dimensional structure of SPX in goldfish for the first time. In the amino acids sequence of SPX, while the first 4 amino acids (Asn1-Pro4) form a structure at the amino terminus (N-terminal), from the 5th to 14th amino acids (Gln5-Gln14) constitute an α-helix structure that extends to the carboxyl group [C-termius (COOH)]. This study also revealed that the Lys11 position of the SPX sequence is hydrophobic, and this region plays an important role in the activation of its receptor. **Kim et al, (8)** Firstly investigated the evolutionary mechanisms of SPX, and they revealed that SPX is phylogenetically a member of the GAL/KISS peptide family, but SPX is closer to the GAL family than the KISS family. Additionally, they discovered another form of SPX, called SPX2. Therefore, the first discovered SPX is now termed SPX1. Unlike SPX1, SPX2 is not found in mammals, but has been detected in many species such as chickens, fish, birds and frogs. SPX2 is encoded by a different gene and differs from SPX1 in terms of the prohormone cleavage/amidation sites, amino acid sequence, and species in which it is located (Fig. 2). Nonetheless, the amino acid sequences of SPX1/2 are highly conserved, suggesting that SPX has performed vital functions for survival **(6, 8).**

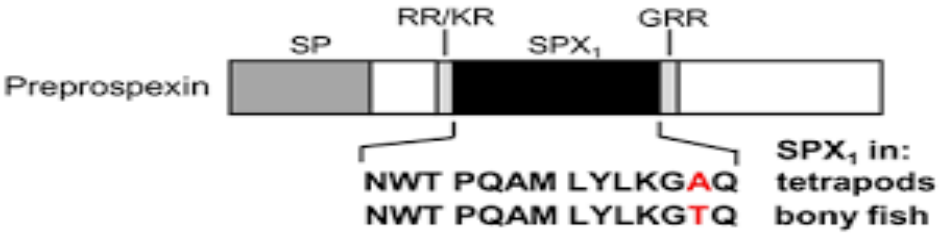

Figure (1): The amino acid sequence of SPX in various species. **(3).**

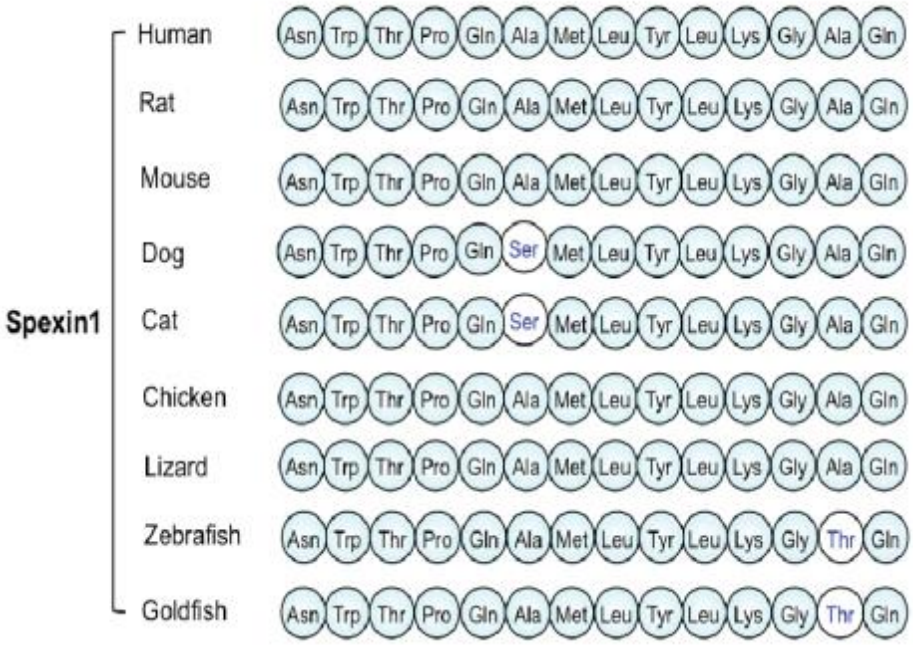

Figure (2): Organization of SPX1 (SPX) coding sequence in tetrapods and fish models, SP: signal peptide; N: asparagine; W: tryptophan; T: threonine; P: proline; Q: glutamine; A: alanine; M: methionine; L: leucine; Y: valine; K: lysine; G: glycine; SP: signal peptide; RR/KR and GRR: cleavage sites **(6).**

Receptor of spexins:

Molecular studies have found that the SPX peptide is somewhat similar to the GAL peptide. The amino acids at positions 2, 3, 9, 10, and 12 (Trp2, Thr3, Tyr9, Leu10, Gln12) in the amino acid sequence of SPX1 are the same as at the corresponding position in the GAL. Because amino acids at positions 2, 3 and 9 in the GAL sequence (corresponding to amino acid Trp2, Thr3, Tyr9) are the main criteria for binding to and activation of GAL receptors it has been suggested that SPX can also bind and activate galanin receptors **(8)**.

Three types of GAL receptors have been known in mammals: GAL1 (1a and 1b), GAL2 (2a and 2b), and GAL3 receptors **(9)**. GAL receptors are G-protein coupled; Gq/11 coupled receptors activate the intracellular signaling pathway by activating the phospholipase C/protein kinase C pathway. Moreover, Gi/o coupled receptors show inhibitory effects on target cells by suppressing the adenylate cyclase/protein kinase A pathway **(10)**. While GAL1 and GAL3 receptors have generally mediated inhibitory effects through Gi/o protein-coupled receptors; activation of GAL2 receptors causes inhibitory effects through Gi/o and excitatory effects via Gq/11. The ligand-receptor interaction study proved that the SPX (SPX1 and SPX2) can activate GAL2 and GAL3 receptors but not GAL1 receptors **(9, 11)**. SPX could be considered the

endogenous ligand for GAL2 and GAL3 receptors, and this is also consistent with the knowledge that SPX is from the galanin/kisspeptin gene family **(8)** (fig. 3).

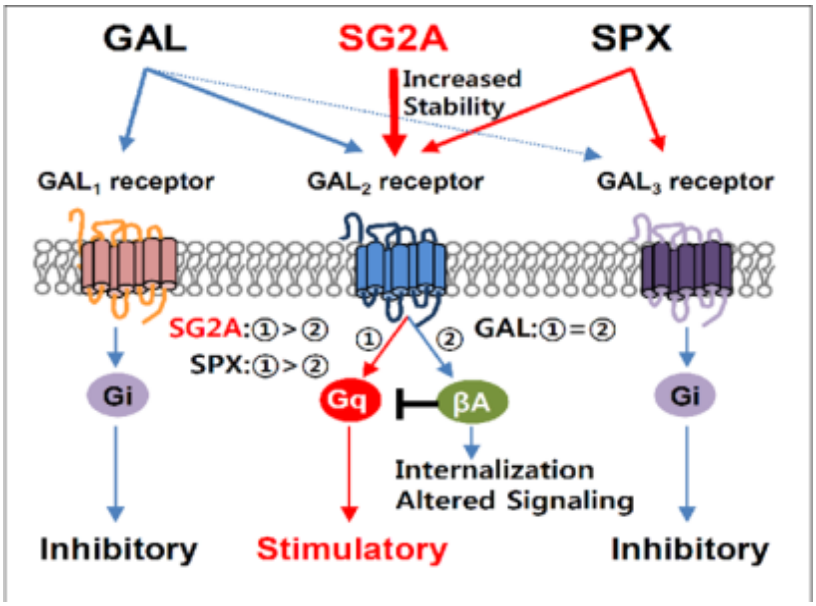

Figure (3): Complexity of GAL/SPX and receptor systems and pharmacological properties of Spexin-Based Galanin Receptor Type 2 Agonist (SG2A). The natural ligand GAL binds to the GAL_1 receptor and GAL_2 receptor with high affinity but shows relatively low affinity to the GAL_3 receptor. SPX has a high potency to activate the GAL_2 receptor and GAL_3 receptor but does not activate the GAL_1 receptor **(8).**

Effects of spexin:

The widespread distribution of SPX in both central and peripheral tissues in many living species suggests that it plays a critical role in many physiological and pathological functions. The studies show that SPX is involved in the physiology/pathophysiology of reproductive, gastrointestinal, cardiovascular, and endocrine systems including especially in food intake and, energy metabolism (carbohydrate/fat) **(7, 12).**

Food intake and energy metabolism:

The initial studies on SPX show a probable role in the regulation of obesity, energy homeostasis, appetite control, satiety, glucose and lipid metabolism, fatty acid uptake, cardiovascular/renal functions, endocrine homeostasis, reproduction and GI function **(6)**. SPX expression in adipose tissue and its levels are remarkably decreased in obese individuals **(13, 14)**, moreover the serum data collected from obese vs. non-obese subjects, a negative correlation has been detected between SPX and leptin **(15, 16)**, and a low serum level of SPX has been supposed to be a biomarker for childhood **(14)** and adult obesity **(13)**.

Furthermore, according to the results from animal studies, SPX might also act as an anorexigenic factor. In goldfish, injection of SPX to the brain, inhibited both basal and neuropeptide Y (NPY)-induced food consumption, and decreased the expression of orexigenic factors, while augmenting that of the anorexigenic factors, and increased brain levels of SPX mRNA in the post-prandial state **(7).**

On the other hand, Human pancreatic islets express SPX; this suggests that SPX might be released together with insulin **(17).** The limited studies shown in humans, have exposed that SPX has an inverse correlation with blood glucose, hemoglobin A1C (HbAlc), triglyceride, and low-density lipoprotein (LDL)-cholesterol **(18)**. lower SPX levels have been found in type 2 diabetes mellitus (T2DM) patients as compared to non-diabetic individuals, which suggests that SPX might affect glucose and lipid metabolism **(19).**

Since obesity contributes markedly to the prevalence of diabetes, which adversely affects public health **(20)**, investigating the role of SPX in glucose homeostasis might help discover novel functions for it.

In obese women, serum SPX had a negative correlation with serum levels of insulin and glucagon, this observation raises the possibility that SPX might be implicated in insulin resistance and glucose metabolism **(15)**.This idea is also in accord with the findings from a study in obese type 2 diabetic mouse model, in which SPX administration not only decrease the body weight but also improve glucose tolerance by decreasing insulin resistance as well as HbAlc **(21)**. However, there are no correlations between serum SPX and diabetes, serum levels of the insulin sensitivity-related parameters, or blood lipids were demonstrated in adolescents afflicted with type 2 diabetes **(22)**. On the other hand, there is a low SPX concentration in obese in comparison with lean adults and adolescents, and suggests a probable satiety-inducing role for the neuropeptide in humans **(14).**

Nociception:

Early immunohistochemical investigations indicated that SPX mRNA/protein is found in the brainstem, periaqueductal gray, brain cortex, and trigeminal ganglia, which is well known to be associated with nociceptive processes **(23, 24**). So, it has been thought that SPX plays a role in nociceptive pain transmission/modulation. Intracerebro-ventricular injection of SPX caused anti-nociception in mice tail withdrawal test **(25)**. Also, **Pirzeh and Taherianfard (26)** showed that pain sensitivity decreased in the formalin test in female rats administrated SPX into the intra-hippocampal CA3 region **(26)**. Similarly, hippocampal CA3 injection of SPX caused a reduction in pain sensitivity in the same pain test in ovariectomized rats. Additionally, **Lv et al. (3)** found that central SPX showed an antinociceptive effect in both tonic pain (formalin test) and visceral pain tests (writhing test) at the supraspinal level.

Endocrine:

The effect of SPX has been demonstrated in many endocrine tissues, including the hypothalamus, thyroid, adrenal gland, testicles, ovaries, pancreas, and adipose tissue. **Rucinski et al. (27)** found that incubation with SPX enhanced aldosterone secretion in zona glomerulosa cells and induced corticosterone secretion in adrenocortical cells in rats in vitro **(27)**.

Moreover, treatment with SPX indicated increases in the viability and proliferation of pancreatic islet cells in vitro **(28**). SPX decreases insulin gene expression as well as insulin promoter factor 1, a transcription factor, but not the expression of insulin receptors. Additionally, SPX reduces glucose-stimulated insulin secretion in isolated pancreatic islets **(29).** Also, SPX increases the serum level of T3 and glucagon levels **(30).**

Reproduction:

SPX has an inhibitory effect on LH hormone release **(31)**. It was revealed that administration with 17β-estradiol reduced the expression of the SPX in the hypothalamic nuclei of spotted scat **(32)**. While intraperitoneal treatment with SPX increases gonadotropin-inhibitory hormone (GnIH), and gonadotropin-releasing hormone (GnRH) expression in the hypothalamus, it suppresses GH, FSH **(4).** These findings suggest that SPX has an endocrine effect on reproduction **(31)**.

SPX has been shown to inhibit the reproductive axis in different fish species in vitro and in vivo **(31)**. Additionally, **Liu et al. (31)** firstly demonstrated that intraperitoneal injection of SPX suppresses LH release in goldfish. It was also shown that expression of SPX in the hypothalamic nuclei is modulated by gonadal hormones such as estrogens **(32).** Additionally, SPX injection induces the expression of gonadotropin-inhibitory hormone (GnIH) and gonadotropin-releasing hormone-3 (GnRH3). However, in a study with grouper fishes, SPX treatment does not affect the mRNA expression of LH and FSH in the pituitary **(33**).

In another study, the effect of SPX on gamete maturation and puberty onset was investigated, and it was reported that SPX knockout zebrafish has fertility without abnormality in the timing of pubertal onset or gamete maturation in the testes and ovary **(34).** This indicates that SPX is not essential for fish reproduction.

Cardiovascular and renal:

The central injections of SPX in rats lead to an enhancement in mean blood pressure and a reduction in renal excretion and heart rate **(25).** On the other hand, peripherally administered SPX causes a sharp pressor effect and bradycardia **(25)**, SPX does not alter renal urine output rate. The reason for the opposite result may be that peripherally administered SPX is rapidly metabolized.

Porzionato et al. (35) found that there was SPX expression in both human and rat carotid body, and SPX mRNA levels were increased by hypoxia exposure for 2 weeks in neonatal rats,

suggesting that SPX expression in the carotid body may be related to sensing O2/CO2 levels **(35)**. Moreover, it was shown that SPX improves mitochondrial dysfunction and the imbalance in energy homeostasis of cardiomyocytes due to exposure to hypoxia **(36).**

Role of SPX on diabetes and metabolic diseases:

SPX is expected to be involved in metabolic disorders such as obesity, diabetes, and metabolic syndromes because of its potential regulatory roles in energy intake **(13)** and inhibition of satiety **(7)**. SPX mRNA expression is affected in the forebrain region under different feeding status or metabolic states **(37).**

The regulation of SPX in diabetes is controversial. **Gu et al. (17).** Showed that there is no correlation between SPX levels and glycemic parameters in diabetes (type 1 and 2).

However, **Karaca et al. (19)** found low SPX levels in diabetic patients. In both type 1 and type 2 diabetes mellitus patients, SPX levels are reduced in serum and negatively correlate with blood glucose levels **(17, 19)**. Additionally, treatment with SPX to diet-induced obese mice with type-2 diabetes mellitus improves glucose tolerance and decreases insulin resistance **(21)**.

Zheng et al. (34). demonstrated that SPX knockout zebrafish exhibit high appetite and high levels of glucose, triacylglycerol and cholesterol in the serum, further proving the essential role of SPX in glucose tolerance and fat metabolism. Recent evidence has also shown that there is a decrease in insulin response to glucose following SPX treatment in pancreatic cells cultured from obese mice **(28)**. This decrease in insulin release is accompanied by an increase in cell viability and proliferation of the cultured pancreatic cells. A decrease in insulin release is associated with increased insulin sensitivity, this indicates a potential use of SPX treatment for diabetic patients **(28)**.

References:

1. Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, Birney E, Rosenthal N, Gross C (2007). Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res 2007;17:320–7.
2. Wan B, Wang XR, Zhou YB, Zhang X, Huo K, Han ZG (2009). C12ORF39, a novel secreted protein with a typical amidation processing signal. Biosci Rep. Sep 17; 30(1):1-10.
3. Lv SY, Zhou YC, Zhang XM, Chen WD, Wang YD (2019). Emerging roles of NPQ/spexin in physiology and pathology Front. Pharmacol., 10, p. 457.
4. Cohen Y, Hausken K, Bonfil Y, Gutnick M, Levavi-Sivan B (2020). Spexin and a Novel Cichlid-Specific Spexin Paralog Both Inhibit FSH and LH Through a Specific Galanin

Receptor (Galr2b) in Tilapia. Front Endocrinol (Lausanne). Feb 20;11:71. doi: 10.3389/fendo.2020.00071. PMID: 32153508; PMCID: PMC7044129.

5. Lomet D, Robert V, Poissenot K, Beltramo M, Dardente H (2020). No evidence that Spexin impacts LH release and seasonal breeding in the ewe. Theriogenology. Dec;158:1-7.
6. Ma A, Bai J, He M, Wong AOL (2018). Spexin as a neuroendocrine signal with emerging functions. Gen Comp Endocrinol. Sep 1;265:90-96. doi: 10.1016/j.ygcen.2018.01.015. Epub 2018 Jan 31. PMID: 29355530.
7. Wong MK, Sze KH, Chen T, Cho CK, Law HC, Chu IK et al. (2013). Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. Am J Physiol Endocrinol Metab. Aug 1;305(3):E348-66.
8. Kim DK, Yun S, Son GH, Hwang JI, Park CR, Kim JI, et al. (2014). Coevolution of the spexin/galanin/kisspeptin family: Spexin activates galanin receptor type II and III. Endocrinology; 155(5):1864–73.
9. Branchek TA, Smith KE, Gerald C, Walker MW (2000). Galanin receptor subtypes. Trends Pharmacol Sci. Mar;21(3):109- 17. doi: 10.1016/s0165-6147(00)01446-2.
10. Webling KE, Runesson J, Bartfai T, Langel U (2012). Galanin receptors and ligands. Front Endocrinol (Lausanne). Dec 7;3:146. doi: 10.3389/fendo.2012.00146. PMID: 23233848; PMCID: PMC3516677.
11. Smith KE, Walker MW, Artymyshyn R, Bard J, Borowsky B, Tamm JA et al. (1998). Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. J Biol Chem. Sep 4;273(36):23321-6. doi: 10.1074/jbc.273.36.23321. PMID: 9722565.
12. Jeong B, Kim KK, Lee TH, Kim HR, Park BS, Park JW et al. (2022). Spexin Regulates Hypothalamic Leptin Action on Feeding Behavior. Biomolecules. Jan 31; 12(2): 236.
13. Walewski JL, Ge F, Lobdell H 4th, Levin N, Schwartz GJ, Vasselli JR, Pomp A, Dakin G, Berk PD (2014). Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity; 22:1643–52.
14. Kumar S, Hossain J, Nader N, Aguirre R, Sriram S, Balagopal PB (2016). Decreased circulating levels of Spexin in obese children. J Clin Endocrinol Metab. ;101(7):2931–2936.

15. Kolodziejski PA, Pruszynska-Oszmalek E, Micker M, Skrzypski M, Wojciechowicz T, Szwarckopf P, et al. (2018). Spexin: a novel regulator of adipogenesis and fat tissue metabolism. Biochim Biophys Acta Mol Cell Biol Lipids.1863(10):1228–36
16. Kumar S, Hossain MJ, Javed A, Kullo IJ, Balagopal PB (2018). Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity. Pediatr Obes. Jun;13(6): 374-380.
17. Gu L, Ma Y, Gu M, Zhang Y, Yan S, Li N, Wang Y, Ding X, Yin J, Fan N, Peng Y (2015). Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides. ;71:232–239.
18. Kolodziejski PA, Leciejewska N, Chmurzynska A, Sassek M, Szczepankiewicz A, Szczepankiewicz D, Malek E, Strowski MZ, Checinska-Maciejewska Z, Nowak KW, Pruszynska-Oszmalek E. (2021). 30-Day spexin treatment of mice with diet-induced obesity (DIO) and type 2 diabetes (T2DM) increases insulin sensitivity, improves liver functions and metabolic status. Mol Cell Endocrinol;536:111420.
19. Karaca A, Bakar-Ates F, Ersoz Gulcelik N (2018). Decreased Spexin levels in patients with type 1 and type 2 diabetes. Med Princ Pract;27:549–54.
20. Agha M, Agha R (2017). The rising prevalence of obesity: part A: impact on public health. Int J Surg Oncol (N Y) 2017;2(7):e17.
21. Ge JF, Walewski JL, Anglade D, Berk PD (2016). Regulation of Hepatocellular Fatty Acid Uptake in mouse models of fatty liver disease with and without functional leptin signaling: roles of NfKB and SREBP-1C and the effects of spexin. Semin Liver Dis. 36:360–72. doi: 10.1055/s-0036-15 97248
22. Hodges SK, Teague AM, Dasari PS, Short KR (2018). Effect of obesity and type 2 diabetes, and glucose ingestion on circulating spexin concentration in adolescents. Pediatr Diabetes; 19(2):212–6.
23. Sonmez K, Zaveri NT, Kerman IA, Burke S, Neal CR, Xie X et al. (2009). Evolutionary sequence modeling for discovery of peptide hormones. PLoS Comput Biol. Jan;5(1):e1000258. doi: 10.1371/journal.pcbi.1000258. Epub 2009 Jan 9. PMID: 19132080; PMCID: PMC2603333. Darakcı Saltık and Bozkurt / J Exp Clin Med 898
24. Porzionato A, Rucinski M, Macchi V, Stecco C, Malendowicz LK, De Caro R (2010). Spexin expression in normal rat tissues. J Histochem Cytochem; 58:825–37.

25. Toll L, Khroyan TV, Sonmez K, Ozawa A, Lindberg I, McLaughlin JP et al. (2012). Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception. FASEB J. Feb;26(2):947-54.
26. Pirzeh L and Taherianfard M (2014). Effect of intra hippocampal CA1 injection of spexin on pain sensitivity in female rat. Bull Env. Pharmacol. Life Sci. 3, 71–74.
27. Rucinski M, Porzionato A, Ziolkowska A, Szyszka M, Macchi V, De Caro R et al. (2010). Expression of the spexin gene in the rat adrenal gland and evidences suggesting that spexin inhibits adrenocortical cell proliferation. Peptides. Apr;31(4):676- 82.
28. Sassek M, Kolodziejski PA, Strowski MZ, Nogowski L, Nowak KW, Mackowiak P (2018). Spexin Modulates Functions of Rat Endocrine Pancreatic Cells. Pancreas. Aug;47(7):904-909 .
29. Sassek M, Kolodziejski PA, Szczepankiewicz D, Pruszynska Oszmalek E (2019). Spexin in the physiology of pancreatic islets-mutual interactions with insulin. Endocrine. 2019 Mar;63(3):513-519.
30. Pruszynska-Oszmalek E, Sassek M, Szczepankiewicz D, Nowak KW, Kolodziejski PA (2020). Short-term administration of spexin in rats reduces obesity by affecting lipolysis and lipogenesis: An in vivo and in vitro study. Gen Comp Endocrinol. Dec 1;299:113615. doi: 10.1016/j.ygcen.2020.113615. Epub 2020 Sep 17. PMID: 32950584.
31. Liu Y, Li S, Qi X, Zhou W, Liu X, Lin H et al. (2013). A novel neuropeptide in suppressing luteinizing hormone release in goldfish, Carassius auratus. Mol Cell Endocrinol. Jul 15;374(1-2):65-72. doi: 10.1016/j.mce.2013.04.008. Epub 2013 Apr 25. PMID: 23623870.
32. Deng SP, Chen HP, Zhai Y, Jia LY, Liu JY, Wang M et al. (2018). Molecular cloning, characterization and expression analysis of spexin in spotted scat (Scatophagus argus). Gen Comp Endocrinol. Sep 15;266:60-66.
33. Li S, Liu Q, Xiao L, Chen H, Li G, Zhang Y et al. (2016). Molecular cloning and functional characterization of spexin in orangespotted grouper (Epinephelus coioides). Comp Biochem Physiol B Biochem Mol Biol. Jun-Jul;196-197:85-91. doi: 10.1016/j.cbpb.2016.02.009. Epub 2016 Mar 2. PMID: 26944307.
34. Zheng B, Li S, Liu Y, Li Y, Chen H, Tang H et al. (2017). Spexin Suppress Food Intake in Zebrafish: Evidence from Gene Knockout Study. Sci Rep. Nov 7; 7(1):14643.

35. Porzionato A, Rucinski M, Macchi V, Stecco C, Sarasin G, Sfriso MM et al. (2012). Spexin is expressed in the carotid body and is upregulated by postnatal hyperoxia exposure. Adv Exp Med Biol. ;758:207-13. doi: 10.1007/978-94-007-4584-1_29. PMID: 23080164
36. Liu Y, Sun L, Zheng L, Su M, Liu H, Wei Y, Li D, Wang Y, Dai C, Gong Y, Zhao C, Li Y.(2020). Spexin protects cardiomyocytes from hypoxia-induced metabolic and mitochondrial dysfunction. Naunyn Schmiedebergs Arch Pharmacol; 393:25–33.
37. Wu H, Lin F, Chen H, Liu J, Gao Y, Zhang X et al. (2016). Ya-fish (Schizothorax prenanti) spexin: identification, tissue distribution and mRNA expression responses to periprandial and fasting. Fish Physiol Biochem. Feb; 42(1):39-49.