Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Review Article

J Exp Clin Med
2022; 39(3): 893-900
doi: 10.52142/omujecm.39.3.56

Structure and functions of spexin as a new neuroendocrine signal

Özge DARAKCI SALTIK*, Ayhan BOZKURT

Department of Physiology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey

Received: 26.05.2022• **Accepted/Published Online:** • **Final Version:** 30.08.2022

Abstract

Spexin (SPX) is a recently discovered endogenous peptide consisting of 14 amino acids. It was found that SPX, kisspeptin (KISS), and galanin (GAL) peptides belong to the same gene family and are also endogenous ligands of GAL2 and GAL3 receptors. The amino acid sequence of the SPX peptide is relatively conserved in vertebrates and invertebrates. The mRNA and protein of SPX are highly expressed both in peripheral organs and in the peripheral/central nervous system of mammals, birds, and fishes. Many biological roles of SPX has been found in non-mammal/mammals, including food intake, energy metabolism, reproduction, nociception, gastrointestinal motility, stress, and endocrine functions. This review collectively mentions the peptide structure of SPX, its receptors and distribution in tissues, and the biological activities of SPX on various organs.

Keywords: galanin-2 receptor, galanin-3 receptor, Neuropeptide, NPQ, spexin

1. Introduction

Spexin (SPX) is an endogenous peptide discovered through the Hidden Markov Model in 2007 (1). The C12orf39 gene, located on chromosome 12 in the human genome, encodes preprospexin (2). After preprospexin has undergone through a series of protein synthesis processes “mature SPX” is formed, which is an effective form for cellular physiological processes. The amino acid sequence of the SPX peptide has been evolutionarily conserved in all vertebrates and invertebrates (1, 3-5). Therefore, it is claimed that SPX is essential for survival and may exist in various tissues/organs as it carries out many body functions. In fact, SPX mRNA/protein commonly presents 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 pointed out that it regulates many physiological functions in the body. For instance, SPX affects food intake (6-10, 25) glucose/fat metabolism (11-15) gastrointestinal motility (1, 16), pain perception (5, 17, 18), endocrine (10, 12, 19-21), reproductive (7, 10, 20, 22-24) and cardiovascular functions (17). Additionally, the important roles of SPX have been revealed in pathological conditions such as obesity, anorexia nervosa, diabetes, anxiety, and depression (26-31). SPX exerts the above-mentioned effects by binding to galanin-2 (GAL2) and galanin-3 (GAL3) receptors (5, 14, 16, 32). Studies on SPX have increased significantly in recent years. This review discusses the molecular structure and roles of SPX in physiological and pathological conditions in both mammalian and non-mammalian species.

2. Spexin peptide structure

Spexin gene (called C12orf39 gene) located in chromosome 12 in the human genome encodes a preprospexin peptide of 116 amino acid residues (2). The preprospexin peptide contains both a hydrophobic signal peptide (SP) and two dibasic prohormone cleavage/amidation sites (RR/KR & GRR) (Fig. 1) (4). A small amino acid region among dibasic cleavage sites forms the 14 amino acids of spexin, also called neuropeptide Q (NPQ). 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. In cats, dogs, and pandas, for example, serine amino acid is replaced with alanine in the 6th position (Fig. 2).

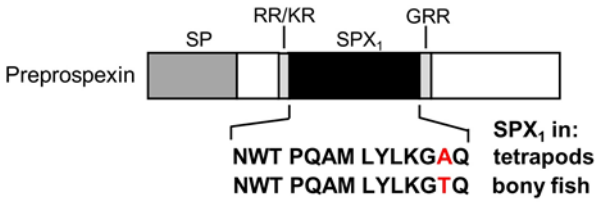

Fig. 1. Organization of SPX1 (SPX) coding sequence in tetrapods and fish models, SP: signal peptide; N: asparagine; W: tryptophan; T: threonine; P: proline; Q: glutamine; A: alanine; M: methionine; L: leucine; Y: valine; K: lysine; G: glycine; SP: signal peptide; RR/KR and GRR: cleavage sites. Figure is taken from Ma et al. (4).

*Correspondence: ozge.darakci@omu.edu.tr

Wong et al. introduced the three-dimensional structure of SPX in goldfish for the first time. In 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 a key role in the activation of its receptor (6).

In 2014, Kim et al. 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. Moreover, they discovered another form of SPX, called SPX2 (33). Accordingly, 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 (Fig. 3). 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 (Figs. 2 and 3). Nonetheless, the amino acid sequences of SPX1/2 are highly conserved, suggesting that SPX has performed essential functions for survival (4, 33).

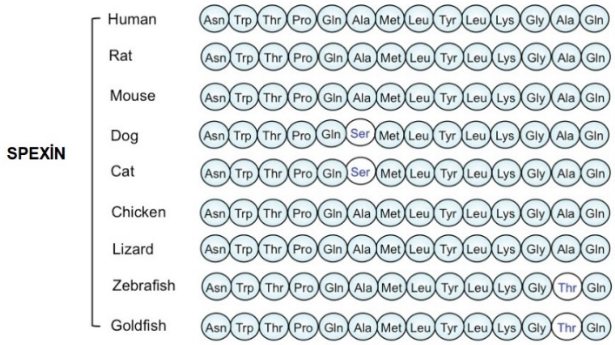

Fig. 2. The amino acid sequence of SPX in various species. The figure modified from Lv et al. (32).

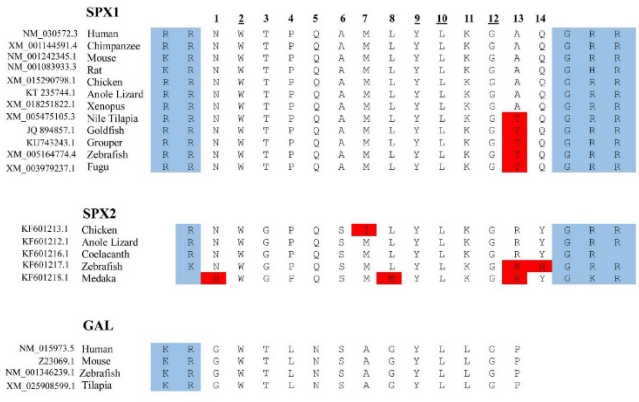

Fig. 3. Comparison of the protein sequence of SPX and GAL in diverse species. The prohormone cleavage/amidation sites is in the red box and the amino acid replacementis is in the red box. Figure taken from Reference 38.

3. Receptor of spexins

Molecular studies have shown that SPX peptide is somewhat similar to 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 (Fig. 3). 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 (33). Three types of GAL receptors have been identified in mammals: GAL1 (1a and 1b), GAL2 (2a and 2b), and GAL3 receptors. GAL receptors are G-protein coupled; Gq/11 coupled receptors activate the intracellular signaling pathway by activating the phospholipase C/protein kinase C pathway. On the other hand, Gi/o coupled receptors show inhibitory effects on target cells by suppressing the adenylate cyclase/protein kinase A pathway (34). While GAL1 and GAL3 receptors have generally mediated inhibitory effects through Gi/o protein coupled receptors; activation of GAL2 receptors causes both inhibitory effects through Gi/o and excitatory effects via Gq/11. The ligand-receptor interaction study demonstrated that the SPX (SPX1 and SPX2) can activate GAL2 and GAL3 receptors but not GAL1 receptors (33, 35, 36, 37). It is concluded that SPX is the endogenous ligand for GAL2 and GAL3 receptors, and this conclusion is also consistent with the knowledge that SPX is from the galanin/kisspeptin gene family.

4. Distribution of spexin in tissues/organs

After discovering SPX, a comprehensive analysis of its localization in various species (such as the rat, mouse, human, and fish) has been carried out. While SPX1 was found in both mammalian and non-mammalian vertebrates, SPX2 was only described in non-mammalian vertebrates.

4.1. Distribution in mammalian vertebrates

SPX was first discovered in the submucosal layer of the rat esophagus and stomach (1). Sonmez et al. have shown that SPX is found in neurons secreting the CRH in mesopontine tegmentum including neurons secreting tyrosine hydroxylase and tryptophan hydroxylase in PAG and the Barrington nucleus in rats (3). Additionally, it was demonstrated in the locus cerelous and laterodorsal tegmental nucleus. Studies using immunohistochemical staining methods revealed that SPX is present in ependymal cells of the choroid plexus, superior cervical neurons, trigeminal ganglia, retinal photoreceptors, cerebellar purkinje cells, paraventricular and supraoptic nuclei in the hypothalamus (39). Moreover, SPX immunoreactivity was found in rat skin (epidermis and adipocyte), gastrointestinal system (esophagus, stomach, small/large intestine, liver, exocrine part of pancreas), endocrine tissues (adrenal cortex and medulla, thyroid and parathyroid glands, endocrine part of pancreas), the respiratory system (alveolar epithelium and bronchi), muscle tissue (heart, skeleton and smooth muscle), the genital system

(ovary and testis) and the placenta in rat, fish, human (19, 39-42). Besides, it has been demonstrated in trophoblastic cells of the human placenta (2) and type I glomic cells in carotid body (43).

4.2. Distribution in non-mammalian vertebrates

Both SPX1 and SPX2 have been shown in many living non-mammalian genomes such as chicken, lizard, frog, and fish. However, there are very few studies on the distribution and functions of SPX2 (33, 44). Tian et al. (2020) have demonstrated that spexin mRNA is widely expressed in the gastrointestinal tract, liver, and hypothalamus in Siberian sturgeon (Acipenser baeri) which is a type of experimental fish (40). Also, it was indicated that SPX1 is found in the hypothalamus, telencephalon, optic tectum, pituitary gland, cerebellum, brainstem, ventromedial thalamic nuclei and medial longitudinal fasciculi in goldfish. In a study conducted on zebrafish through the in-situ hybridization method, the localization of SPX1 and SPX2 in the brain was determined in detail (44). According to this study, while SPX1 positive neurons are highly found in the middle and hindbrain, SPX2 has been found densely in the preoptic area of hypothalamus. SPX1-positive neurons in the hindbrain project to the spinal cord and these neurons are GABAergic inhibitory neurons, and it has also been demonstrated that these neurons connect with neurons in the spinal cord where GAL2b receptors are located. Additionally, SPX1 neurons have been detected in the dorsal habenula in which GAL2a and GAL2b receptors are located, and these neurons have projected to the interpeduncular nucleus from the dorsal habenula (44).

5. Effects of spexin

The widespread distribution of SPX in both central/peripheral tissues in many living species suggests that it plays a very important role in many physiological and pathological functions. In studies conducted consistent with the suggestion, SPX has been found to be involved in the physiology/pathophysiology of reproductive, gastrointestinal, cardiovascular and endocrine systems including especially in food intake and, energy metabolism (carbohydrate/fat). Below, these effects are comprehensively discussed under the main topic.

5.1. Food intake

Studies have shown that SPX suppresses food intake and nutritional behaviors (6, 7, 8, 9, 10, 25). As it is known, the level of circulating insulin increases following food intake. A study revealed that increased insulin following food intake mediates rises of SPX expression in both brain and the liver of fish (45). Therefore, it is thought that there is a link between food intake and SPX expression through insulin. Furthermore, in studies conducted in fish, it was observed that SPX expression increases in telencephelon, hypothalamus, and optic tectum after food intake (6, 8). However, there are also studies claiming the opposite of these findings (7, 9, 10).

Ma et al. (2017) highlighted the mechanism of SPX's inhibitory effect on food intake in their study on fish. Following food intake, the level of glucose in circulation rises and glucose increases insulin secretion in the liver (concurrently in the pancreas); then, insulin causes SPX upregulation at the hepatic level through insulin receptors (small amounts of IGF1R), which is coupled to MKK3/6/P38 MAPK and PI3K/Akt pathway, acting in an autocrine and paracrine manner. With the hepatic exit of SPX, SPX levels in the blood increase and the peripheral SPX reaches the central nervous system by passing through the blood-brain barrier. Meanwhile, insulin released by the liver (together with secretion from the pancreas) can increase insulin level in circulation and has a central effect of upregulating SPX in brain areas involved in control of food intake via InsR. Later, the combined effect of both central expression and peripheral secretion of SPX can regulate the central orexigenic and anorexigenic signals pathway. Eventually, SPX suppresses food intake either by suppressing orexigenic factors [neuropeptide Y (NPY), agouti-Related Peptide (AgRP), and apelin] or by increasing anorexigenic factors [proopiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), kholecystokinin (CCK), nucleobindin-2 (NUCB-2), and peptide YY (PYY)] in the nuclei in the hypothalamus (Fig. 4) (4, 6, 8). Besides, in a recent study in mice, it was revealed that spexin suppresses food intake via GAL3 receptors (40).

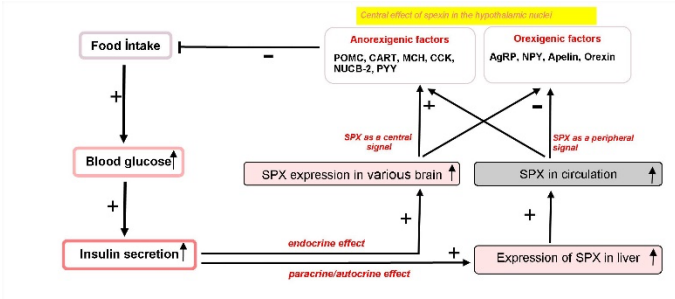

Fig. 4. Functional role of SPX on food intake in fish models (e.g., goldfish, ya fish, grouper, and zebrafish) (refer to the text for details). SPX: Spexin; POMC: Proopiomelanocortin; CART: cocaine-and amphetamine-regulated transcript; MCH: Melanin-concentrating hormone; CCK: Cholecystokinin; NUCB-2: Nucleobindin-2; PYY: Peptide YY; AgRP: Agouti-Related Peptide; NPY: Neuropeptide Y. Figure is rearranged from Ma et a.l (4).

5.2. Energy metabolism

SPX plays an important role in lipid metabolism associated with body weight control. It is well known that the long-chain fatty acid uptake and storage into adipocytes are significant factors in the control of body weight (46). Waleswski et al. (2014) found that SPX treatment decreases the uptake of long-chain fatty acids into adipocytes in mice (47). Similary, SPX inhibits the uptake of fatty acids into hepatocytes, resulting in a reduction in hepatic lipid content (48). Kolodziejski et al. demonstrated that SPX decreases adipogenesis and lipogenesis but increases lipolysis in murine and human (49). In the same study, they also found that it

suppresses glucose uptake into visceral adipose tissue. Additionally, it has been shown that SPX leads to lipid oxidation and increases locomotor activity (47). Moreover, SPX levels were also compared in obese and non-obese adults with clinical human studies. It has been shown that the C12orf39 gene was 14.9-fold lower in omental and subcutaneous adipose tissue of obese individuals compared to individuals with normal body weight (47). Also, circulating SPX levels and SPX expression in adipocytes are low in obese patients (50). As it is known, obese patients have high leptin levels. A negative correlation was revealed between leptin and SPX levels in obese individuals (13, 51). These two peptides may have an antagonist role in the regulation of body weight, hunger/satiety, and energy metabolism. Additionally, Kumar et al. (2018) showed that circulating SPX levels dropped after Roux-en-Y gastric bypass surgery in youth with severe obesity, and the low SPX levels negatively correlated with insulin resistance (HOMA-IR) and body mass index (52). Therefore, it is suggested that SPX can be a biomarker especially in adult and childhood obesity (47, 50). SPX can also be an important marker in diseases associated with energy metabolism. It has been reported that SPX may potentially have beneficial effects in various metabolic diseases. Bitarafan et al. found that SPX decreases hepatic lipids, serum alanine aminotransferase (ALT) and aspartate aminotransferase in mice with hepatic steatosis/nonalcoholic fatty liver disease (HS/NAFLD), which are markers of HS/NAFLD (53). Another clinical study results showed that serum SPX levels were significantly lower in the metabolic syndrome (MetS) group than the healthy individuals in women (54).

5.3. Reproduction

Spexin has been shown to inhibit the reproductive axis in different fish species in vitro and in vivo (20). Liu et al. (2013) firstly demonstrated that intraperitoneal injection of SPX suppresses LH release in goldfish. Also, it was found that the expression of SPX is lower during the breeding season than during non-breeding seasons (20) and in the breeding season; SPX injection has not affected LH expression in pituitary in the orange spotted grouper (7). In this study, it was also shown that expression of spexin in the hypothalamic nuclei is modulated by gonadal hormones such as estrogens (10, 20). For example, estrogen treatment causes downregulation of SPX expression (20). Additionally, while SPX injection induces expression of gonadotropin-inhibitory hormone (GnIH) and gonadotropin-releasing hormone-3 (GnRH3) (9). SPX suppresses the growth hormone expression in orange-spotted grouper and half-smooth tongue sole (7, 9). However, in a study with grouper fishes, SPX treatment does not affect mRNA expression of LH and FSH in the pituitary (7). Contrary to this data, there are studies showing that SPX can inhibit LH and FSH secretion (9, 20, 22). 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 (55). This data demonstrates that SPX is not essential for fish reproduction. Finally, it has recently been revealed that there was no in vivo evidence of the role of SPX in modulation of the ovine gonadotropic axis. These data show that the effect of SPX on the reproductive axis may be type-specific, and further investigations are needed to find out whether it is effective in various types of reproduction.

5.4. Nociception

Early immunohistochemical investigations indicated that spexin mRNA/protein is found in the brainstem, periaqueductal gray, brain cortex, and trigeminal ganglia, which is well known to be associated with nociceptive processes (3, 39). So, it has been thought SPX can play a role in nociceptive pain transmission/modulation. Indeed, intracerebroventricular injection of SPX caused antinociception in mice tail withdrawal test (17). Also, Pirzeh et al. (2014) show that pain sensitivity was decreased in the formalin test in female rats administrated SPX into intrahippocampal CA3 region (56). Similarly, hippocampal CA3 injection of SPX caused a reduction in pain sensitivity in the same pain test in ovariectomized rats. Furthermore, co-injection of SPX and progesterone caused a higher antinociceptive effect than progesterone administered alone (18). In another study to elucidate its possible mechanism in SPX antinociception, Lv et al. (2019) found that central spexin showed an antinociceptive effect in both tonic pain (formalin test) and visceral pain tests (writhing test) at supraspinal level. Additionally, findings of molecular analysis indicated that after central SPX is injected in mice formalin pain test, GAL3 receptors are activated and then GAL3 receptors cause to up-regulate dynorphin and k-opioid receptor gene/protein. As for the writhing test, SPX administrated in the lateral ventricle stimulates GAL2 receptors and then activates POMC/mu-opioid receptor gene/protein (5). All results have shown the antinociceptive effect of the central SPX, but the effect of spexin is still unclear at the peripheral level.

5.5. Digestive system

Mirabeau et al. (2007) firstly showed that SPX mRNA is located in the submucosal layer of the esophagus and the stomach fundus in mice by using in situ hybridization (1). Additionally, they demonstrated SPX induces contraction in rat stomach fundus smooth muscle in a dose-dependent manner in vitro, providing evidence of the first known physiological effect for SPX (1). According to another in vitro study, spexin also stimulates intestinal and colonic contraction via GAL-2 receptors in mice. Besides, intraperitoneal injection of SPX enhances all of the bowel transit via GAL2 receptor by activating L-type voltage-gated calcium channels in fed mice, but not GAL3 receptors (16). Nevertheless, it is not known whether centrally administered SPX has an effect on gastrointestinal motility and therefore,

further studies are needed to show the effect of spexin on intestine motility mediated by central nervous system. The effect of SPX on bile acid synthesis has also been investigated. It was found that both acute and chronic peripheral SPX treatment reduce the levels of total bile acid in circulating and liver, and also decrease hepatic cholesterol 7α-hydroxylase 1 (CYP7A1) mRNA level through GAL2 and GAL3 receptors (14). Furthermore, it was revealed that SPX plasma levels are reduced in patients with constipation. Finally, the mRNA levels of SPX were reported to be reduced in rat jejunum and ileum after hunger stress in rat (16).

5.6. Stress

The presence of SPX was shown in the Barrington nuclei by using in-situ hybridization, indicating that it can play a role in stress response. In fact, Sonmez et al. (2009) showed that SPX collocates with CRF (corticotropin-releasing factor) in the Barrington nuclei (3). Moreover, Zhuang et al. (2020) showed in their detailed study in rats exposed to chronic stress that SPX mRNA level was decreased, while CRF mRNA level was raised in hippocampus (57). Additionally, it was indicated that hippocampal CRF administration reduces expression of SPX mRNA in hippocampus, hypothalamus, pituitary in mice, and this inhibitory effect is mediated by the CRF receptors.

5.7. Endocrine effects

SPX has been demonstrated in many endocrine tissues, including the hypothalamus, thyroid, adrenal gland, testicles, ovaries, pancreas, and adipose tissue. Rucinski et al. (2010) found that incubation with SPX enhanced aldosterone secretion in zona glomerulosa cells and induced corticosterone secretion in adrenocortical cells in rat in vitro (19) showing that SPX plays a role in the control of adrenocortical endocrine functions. Additionally, SPX has an inhibitory effect on LH hormone release (20). It was revealed that administration with 17β-estradiol reduced expression of the SPX in the hypothalamic nuclei of spotted scat (10). While intraperitoneal treatment with SPX increases GniH, GNrH expression in the hypothalamus, it suppresses GH, FSH expression. These findings suggest that SPX has an endocrine effect on reproduction; however, all studies have been conducted on fish models (9). Furthermore, treatment with spexin indicated increases in the viability and proliferation of pancreatic islets cells in vitro (12). Spexin decreases insulin gene expression as well as insulin promoter factor 1, a transcription factor, but not expression of insulin receptor. The same study revealed that SPX reduces glucose-stimulated insulin secretion in isolated pancreatic islets (12). In another study in obese rats, it was shown that while SPX treatment decreases levels of serum ghrelin, leptin, corticosteron, it increases serum T3 and glucagon levels (21). These studies suggest that SPX may be an endocrine factor.

5.8. Other effects

It has been suggested SPX has cardiovascular and renal roles. In rats, the central injections of SPX lead to an enhancement in mean blood pressure and a reduction in renal excretion and heart rate (17). However, whereas peripherally administered SPX causes a sharp pressor and bradycardia, SPX does not alter renal urine output rate. The reason for the opposite result may be that peripherally administered SPX is rapidly metabolized. Additionally, Porzionato et al. (2012) 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 (43). Moreover, it was shown that SPX improves mitochondrial dysfunction and the imbalance in energy homeostasis of cardiomyocytes due to exposure to hypoxia (58).

In conclusion, SPX is a 14-amino-acid endogenous peptide that is well-conserved. To date, SPX1 and SPX2 are the two forms of SPX discovered. SPX1 was identified in both mammalian and non-mammalian cells, but SPX2 was exclusively discovered in non-mammalian cells. Studies have shown that SPX performs its functions through GAL2 and GAL3 receptors. The biological function of SPX includes feeding behavior, reproduction, nociception, glucose/lipid metabolism, gastrointestinal motility, and stress. Also, SPX plays a role in pathological processes such as obesity, anorexia nervosa, diabetes, anxiety, and depression. Therefore, its possible potential role in therapeutic targets will be beneficial for the development of new technology for the cure of various disorders. Furthermore, the functional relevance of SPX2, which is found in non-mammalian vertebrates, should be investigated further.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ö.D.S., A.B., Design: Ö.D.S., A.B., Data Collection or Processing: Ö.D.S., Analysis or Interpretation: Ö.D.S., A.B., Literature Search: Ö.D.S., Writing: Ö.D.S., A.B.

References

1. Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R et al. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res. 2007 Mar;17(3):320-7. doi: 10.1101/gr.5755407. Epub 2007 Feb 6. PMID: 17284679; PMCID: PMC1800923.
2. Wan B, Wang XR, Zhou YB, Zhang X, Huo K, Han ZG. C12ORF39, a novel secreted protein with a typical amidation processing signal. Biosci Rep. 2009 Sep 17;30(1):1-10. doi: 10.1042/BSR20080156. PMID: 19193193.
3. Sonmez K, Zaveri NT, Kerman IA, Burke S, Neal CR, Xie X et al. Evolutionary sequence modeling for discovery of peptide hormones. PLoS Comput Biol. 2009 Jan;5(1):e1000258. doi: 10.1371/journal.pcbi.1000258. Epub 2009 Jan 9. PMID: 19132080; PMCID: PMC2603333.

4. Ma A, Bai J, He M, Wong AOL. Spexin as a neuroendocrine signal with emerging functions. Gen Comp Endocrinol. 2018 Sep 1;265:90-96. doi: 10.1016/j.ygcen.2018.01.015. Epub 2018 Jan 31. PMID: 29355530.

5. Lv SY, Cui B, Yang Y, Du H, Zhang X, Zhou Y et al. Spexin/NPQ Induces FBJ Osteosarcoma Oncogene (Fos) and Produces Antinociceptive Effect against Inflammatory Pain in the Mouse Model. Am J Pathol. 2019 Apr;189(4):886-899. doi: 10.1016/j.ajpath.2018.12.009. Epub 2019 Jan 19. PMID: 30664863.

6. Wong MK, Sze KH, Chen T, Cho CK, Law HC, Chu IK, et al. Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. Am J Physiol Endocrinol Metab. 2013 Aug 1;305(3):E348-66. doi: 10.1152/ajpendo.00141.2013. Epub 2013 May 28. PMID: 23715729.

7. Li S, Liu Q, Xiao L, Chen H, Li G, Zhang Y et al. Molecular cloning and functional characterization of spexin in orange-spotted grouper (Epinephelus coioides). Comp Biochem Physiol B Biochem Mol Biol. 2016 Jun-Jul;196-197:85-91. doi: 10.1016/j.cbpb.2016.02.009. Epub 2016 Mar 2. PMID: 26944307.

8. Wu H, Lin F, Chen H, Liu J, Gao Y, Zhang X et al. Ya-fish (Schizothorax prenanti) spexin: identification, tissue distribution and mRNA expression responses to periprandial and fasting. Fish Physiol Biochem. 2016 Feb;42(1):39-49. doi: 10.1007/s10695-015-0115-0. Epub 2015 Aug 27. PMID: 26311351.

9. Wang S, Wang B, Chen S. Spexin in the half-smooth tongue sole (Cynoglossus semilaevis): molecular cloning, expression profiles, and physiological effects. Fish Physiol Biochem. 2018 Jun;44(3):829-839. doi: 10.1007/s10695-018-0472-6. Epub 2018 Feb 5. PMID: 29404821.

10. Deng SP, Chen HP, Zhai Y, Jia LY, Liu JY, Wang M et al. Molecular cloning, characterization and expression analysis of spexin in spotted scat (Scatophagus argus). Gen Comp Endocrinol. 2018 Sep 15;266:60-66. doi: 10.1016/j.ygcen.2018.04.018. Epub 2018 May 10. PMID: 29753927

11. Sassek M, Kolodziejski PA, Szczepankiewicz D, Pruszynska-Oszmalek E. Spexin in the physiology of pancreatic islets-mutual interactions with insulin. Endocrine. 2019 Mar;63(3):513-519. doi: 10.1007/s12020-018-1766-2. Epub 2018 Sep 28. PMID: 30267353.

12. Sassek M, Kolodziejski PA, Strowski MZ, Nogowski L, Nowak KW, Mackowiak P. Spexin Modulates Functions of Rat Endocrine Pancreatic Cells. Pancreas. 2018 Aug;47(7):904-909. doi: 10.1097/MPA.0000000000001083. PMID: 29912854.

13. Kołodziejski PA, Pruszyńska-Oszmałek E, Korek E, Sassek M, Szczepankiewicz D, Kaczmarek P et al. Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women. Physiol Res. 2018 Mar 16;67(1):45-56. doi: 10.33549/physiolres.933467. Epub 2017 Nov 10. PMID: 29137471.

14. Lin CY, Zhao L, Huang T, Lu L, Khan M, Liu J et al. Spexin Acts as Novel Regulator for Bile Acid Synthesis. Front Physiol. 2018 Apr 10;9:378. doi: 10.3389/fphys.2018.00378. PMID: 29692737; PMCID: PMC5902714.

15. Pruszynska-Oszmalek E, Sassek M, Szczepankiewicz D, Nowak KW, Kolodziejski PA. Short-term administration of spexin in rats reduces obesity by affecting lipolysis and lipogenesis: An in vivo and in vitro study. Gen Comp Endocrinol. 2020 Dec 1;299:113615. doi: 10.1016/j.ygcen.2020.113615. Epub 2020 Sep 17. PMID: 32950584.

16. Lin CY, Zhang M, Huang T, Yang LL, Fu HB, Zhao L et al. Spexin Enhances Bowel Movement through Activating L-type Voltage-dependent Calcium Channel via Galanin Receptor 2 in Mice. Sci Rep. 2015 Jul 10;5:12095. doi: 10.1038/srep12095. PMID: 26160593; PMCID: PMC4498193.

17. Toll L, Khroyan TV, Sonmez K, Ozawa A, Lindberg I, McLaughlin JP et al. Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception. FASEB J. 2012 Feb;26(2):947-54. doi: 10.1096/fj.11-192831. Epub 2011 Oct 28. PMID: 22038051; PMCID: PMC3290442.

18. Moazen P, Taherianfard M, Ahmadi Soleimani M, Norozpor M. Synergistic effect of spexin and progesterone on pain sensitivity attenuation in ovariectomized rats. Clin Exp Pharmacol Physiol. 2018 Apr;45(4):349-354. doi: 10.1111/1440-1681.12862. Epub 2017 Dec 11. PMID: 28949407.

19. Rucinski M, Porzionato A, Ziolkowska A, Szyszka M, Macchi V, De Caro R et al. Expression of the spexin gene in the rat adrenal gland and evidences suggesting that spexin inhibits adrenocortical cell proliferation. Peptides. 2010 Apr;31(4):676-82. doi: 10.1016/j.peptides.2009.12.025. Epub 2010 Jan 4. PMID: 20045034.

20. Liu Y, Li S, Qi X, Zhou W, Liu X, Lin H et al. A novel neuropeptide in suppressing luteinizing hormone release in goldfish, Carassius auratus. Mol Cell Endocrinol. 2013 Jul 15;374(1-2):65-72. doi: 10.1016/j.mce.2013.04.008. Epub 2013 Apr 25. PMID: 23623870.

21. Pruszynska-Oszmalek E, Sassek M, Szczepankiewicz D, Nowak KW, Kolodziejski PA. Short-term administration of spexin in rats reduces obesity by affecting lipolysis and lipogenesis: An in vivo and in vitro study. Gen Comp Endocrinol. 2020 Dec 1;299:113615. doi: 10.1016/j.ygcen.2020.113615. Epub 2020 Sep 17. PMID: 32950584.

22. Cohen Y, Hausken K, Bonfil Y, Gutnick M, Levavi-Sivan B. Spexin and a Novel Cichlid-Specific Spexin Paralog Both Inhibit FSH and LH Through a Specific Galanin Receptor (Galr2b) in Tilapia. Front Endocrinol (Lausanne). 2020 Feb 20;11:71. doi: 10.3389/fendo.2020.00071. PMID: 32153508; PMCID: PMC7044129.

23. Wang L, Tran A, Lee J, Belsham DD. Palmitate differentially regulates Spexin, and its receptors Galr2 and Galr3, in GnRH neurons through mechanisms involving PKC, MAPKs, and TLR4. Mol Cell Endocrinol. 2020 Dec 1;518:110991. doi: 10.1016/j.mce.2020.110991. Epub 2020 Aug 22. PMID: 32841709.

24. Lomet D, Robert V, Poissenot K, Beltramo M, Dardente H. No evidence that Spexin impacts LH release and seasonal breeding in the ewe. Theriogenology. 2020 Dec;158:1-7. doi: 10.1016/j.theriogenology.2020.08.031. Epub 2020 Sep 1. PMID: 32916519.

25. Jeong B, Kim KK, Lee TH, Kim HR, Park BS, Park JW et al. Spexin Regulates Hypothalamic Leptin Action on Feeding Behavior. Biomolecules. 2022 Jan 31;12(2):236. doi: 10.3390/biom12020236. PMID: 35204737; PMCID: PMC8961618.

26. Gu L, Ma Y, Gu M, Zhang Y, Yan S, Li N et al. Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides. 2015 Sep;71:232-9. doi: 10.1016/j.peptides.2015.07.018. Epub 2015 Jul 23. PMID: 26211893.

27. Hodges SK, Teague AM, Dasari PS, Short KR. Effect of obesity and type 2 diabetes, and glucose ingestion on circulating spexin concentration in adolescents. Pediatr Diabetes. 2018 Mar;19(2):212-216. doi: 10.1111/pedi.12549. Epub 2017 Jun 19. PMID: 28626942.

28. Kumar S, Hossain MJ, Javed A, Kullo IJ, Balagopal PB. Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity. Pediatr Obes. 2018 Jun;13(6):374-380. doi: 10.1111/ijpo.12249. Epub 2017 Oct 10. PMID: 29045048; PMCID: PMC5906205.

29. Pałasz A, Janas-Kozik M, Borrow A, Arias-Carrión O, Worthington JJ. The potential role of the novel hypothalamic neuropeptides nesfatin-1, phoenixin, spexin and kisspeptin in the pathogenesis of anxiety and anorexia nervosa. Neurochem Int. 2018 Feb;113:120-136. doi: 10.1016/j.neuint.2017.12.006. Epub 2017 Dec 15. PMID: 29253527.

30. Jeong I, Kim E, Seong JY, Park HC. Overexpression of Spexin 1 in the Dorsal Habenula Reduces Anxiety in Zebrafish. Front Neural Circuits. 2019 Aug 14;13:53. doi: 10.3389/fncir.2019.00053. PMID: 31474838; PMCID: PMC6702259.

31. Kolodziejski PA, Leciejewska N, Chmurzynska A, Sassek M, Szczepankiewicz A, Szczepankiewicz D et al. 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. 2021 Oct 1;536:111420. doi: 10.1016/j.mce.2021.111420. Epub 2021 Aug 9. PMID: 34384849.

32. Lv SY, Zhou YC, Zhang XM, Chen WD, Wang YD. Emerging Roles of NPQ/Spexin in Physiology and Pathology. Front Pharmacol. 2019 May 7;10:457. doi: 10.3389/fphar.2019.00457. PMID: 31133851; PMCID: PMC6514225.

33. Kim DK, Yun S, Son GH, Hwang JI, Park CR, Kim JI et al. Coevolution of the spexin/galanin/kisspeptin family: Spexin activates galanin receptor type II and III. Endocrinology. 2014 May;155(5):1864-73. doi: 10.1210/en.2013-2106. Epub 2014 Feb 11. PMID: 24517231.

34. Webling KE, Runesson J, Bartfai T, Langel U. Galanin receptors and ligands. Front Endocrinol (Lausanne). 2012 Dec 7;3:146. doi: 10.3389/fendo.2012.00146. PMID: 23233848; PMCID: PMC3516677.

35. Wang S, Hashemi T, Fried S, Clemmons AL, Hawes BE. Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. Biochemistry. 1998 May 12;37(19):6711-7. doi: 10.1021/bi9728405. PMID: 9578554.

36. Branchek TA, Smith KE, Gerald C, Walker MW. Galanin receptor subtypes. Trends Pharmacol Sci. 2000 Mar;21(3):109-17. doi: 10.1016/s0165-6147(00)01446-2. PMID: 10689365.

37. Smith KE, Walker MW, Artymyshyn R, Bard J, Borowsky B, Tamm JA et al. Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. J Biol Chem. 1998 Sep 4;273(36):23321-6. doi: 10.1074/jbc.273.36.23321. PMID: 9722565.

38. Lim CH, Lee MYM, Soga T, Parhar I. Evolution of Structural and Functional Diversity of Spexin in Mammalian and Non-mammalian Vertebrate Species. Front Endocrinol (Lausanne). 2019 Jun 19;10:379. doi: 10.3389/fendo.2019.00379. PMID: 31275244; PMCID: PMC6593056.

39. Porzionato A, Rucinski M, Macchi V, Stecco C, Malendowicz LK, De Caro R. Spexin expression in normal rat tissues. J Histochem Cytochem. 2010 Sep;58(9):825-37. doi: 10.1369/jhc.2010.956300. Epub 2010 Jun 7. PMID: 20530460; PMCID: PMC2924798.

40. Tian Z, Xu S, Wang M, Li Y, Chen H, Tang N, et al. Identification, tissue distribution, periprandial expression, and anorexigenic effect of spexin in Siberian sturgeon, Acipenser baeri. Fish Physiol Biochem. 2020 Dec;46(6):2073-2084. doi: 10.1007/s10695-020-00856-y. Epub 2020 Aug 14. PMID: 32794103.

41. Lim CH, Soga T, Levavi-Sivan B, Parhar IS. Chronic Social Defeat Stress Up-Regulates Spexin in the Brain of Nile Tilapia (Oreochromis niloticus). Sci Rep. 2020 May 6;10(1):7666. doi: 10.1038/s41598-020-64639-4. PMID: 32376994; PMCID: PMC7203209.

42. Pałasz A, Suszka-Świtek A, Kaśkosz A, Plewka D, Bogus K, Filipczyk Łet al. Spexin-expressing neurons in the magnocellular nuclei of the human hypothalamus. J Chem Neuroanat. 2021 Jan;111:101883. doi: 10.1016/j.jchemneu.2020.101883. Epub 2020 Nov 5. PMID: 33161073.

43. Porzionato A, Rucinski M, Macchi V, Stecco C, Sarasin G, Sfriso MM et al. Spexin is expressed in the carotid body and is upregulated by postnatal hyperoxia exposure. Adv Exp Med Biol. 2012;758:207-13. doi: 10.1007/978-94-007-4584-1_29. PMID: 23080164.

44. Kim E, Jeong I, Chung AY, Kim S, Kwon SH, Seong JY et al. Distribution and neuronal circuit of spexin 1/2 neurons in the zebrafish CNS. Sci Rep. 2019 Mar 22;9(1):5025. doi: 10.1038/s41598-019-41431-7. PMID: 30903017; PMCID: PMC6430828.

45. Ma A, He M, Bai J, Wong MK, Ko WK, Wong AO. Dual Role of Insulin in Spexin Regulation: Functional Link Between Food Intake and Spexin Expression in a Fish Model. Endocrinology. 2017 Mar 1;158(3):560-577. doi: 10.1210/en.2016-1534. PMID: 28359089.

46. Petrescu O, Fan X, Gentileschi P, Hossain S, Bradbury M, Gagner M et al. Long-chain fatty acid uptake is upregulated in omental adipocytes from patients undergoing bariatric surgery for obesity. Int J Obes (Lond). 2005 Feb;29(2):196-203. doi: 10.1038/sj.ijo.0802868. PMID: 15570311.

47. Walewski JL, Ge F, Lobdell H 4th, Levin N, Schwartz GJ, Vasselli JR et al. 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 (Silver Spring). 2014 Jul;22(7):1643-52. doi: 10.1002/oby.20725. Epub 2014 Mar 8. PMID: 24550067; PMCID: PMC4077920.

48. Ge JF, Walewski JL, Anglade D, Berk PD. 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. 2016 Sep;36(4):360-372. doi: 10.1055/s-0036-1597248. Epub 2016 Dec 20. PMID: 27997977.

49. Kolodziejski PA, Pruszynska-Oszmalek E, Micker M, Skrzypski M, Wojciechowicz T, Szwarckopf P et al.Spexin: A novel regulator of adipogenesis and fat tissue metabolism. Biochim Biophys Acta Mol Cell Biol Lipids. 2018 Oct;1863(10):1228-1236. doi: 10.1016/j.bbalip.2018.08.001. Epub 2018 Aug 4. PMID: 30305242.

50. Kumar S, Hossain J, Nader N, Aguirre R, Sriram S, Balagopal PB. Decreased Circulating Levels of Spexin in Obese Children. J Clin Endocrinol Metab. 2016 Jul;101(7):2931-6. doi: 10.1210/jc.2016-1177. Epub 2016 May 24. PMID: 27218269; PMCID: PMC4929838.

51. Yun S, Reyes-Alcaraz A, Lee YN, Yong HJ, Choi J, Ham BJ et al. Spexin-Based Galanin Receptor Type 2 Agonist for Comorbid Mood Disorders and Abnormal Body Weight. Front Neurosci. 2019 Apr 18;13:391. doi: 10.3389/fnins.2019.00391. PMID: 31057364; PMCID: PMC6482256.

52. Kumar S, Hossain MJ, Inge T, Balagopal PB. Roux-en-Y gastric bypass surgery in youth with severe obesity: 1-year longitudinal changes in spexin. Surg Obes Relat Dis. 2018 Oct;14(10):1537-1543. doi: 10.1016/j.soard.2018.07.007. Epub 2018 Jul 20.

PMID: 30131311.

53. Bitarafan V, Esteghamati A, Azam K, Yosaee S, Djafarian K. Comparing serum concentration of spexin among patients with metabolic syndrome, healthy overweight/obese, and normal-weight individuals. Med J Islam Repub Iran. 2019 Sep 6;33:93. doi: 10.34171/mjiri.33.93. PMID: 31696087; PMCID: PMC6825375.

54. Al-Daghri NM, Alenad A, Al-Hazmi H, Amer OE, Hussain SD, Alokail MS. Spexin Levels Are Associated with Metabolic Syndrome Components. Dis Markers. 2018 Sep 4;2018:1679690. doi: 10.1155/2018/1679690. PMID: 30254709; PMCID: PMC6142736.

55. Zheng B, Li S, Liu Y, Li Y, Chen H, Tang H et al. Spexin Suppress Food Intake in Zebrafish: Evidence from Gene Knockout Study. Sci Rep. 2017 Nov 7;7(1):14643. doi: 10.1038/s41598-017-15138-6. PMID: 29116147; PMCID: PMC5677112.

56. Pirzeh L, and Taherianfard, M. Effect of intra hippocampal CA1 injection of spexin on pain sensitivity in female rat. Bull Env. Pharmacol. 2014.Life Sci. 3, 71–74.

57. Zhuang M, Lai Q, Yang C, Ma Y, Fan B, Bian Z et al. Spexin as an anxiety regulator in mouse hippocampus: Mechanisms for transcriptional regulation of spexin gene expression by corticotropin releasing factor. Biochem Biophys Res Commun. 2020 Apr 30;525(2):326-333. doi: 10.1016/j.bbrc.2020.02.023. Epub 2020 Feb 21. PMID: 32093887.

58. Liu Y, Sun L, Zheng L, Su M, Liu H, Wei Y et al. Spexin protects cardiomyocytes from hypoxia-induced metabolic and mitochondrial dysfunction. Naunyn Schmiedebergs Arch Pharmacol. 2020 Jan;393(1):25-33. doi: 10.1007/s00210-019-01708-0. Epub 2019 Aug 8. PMID: 31396649.