An update on galanin and spexin and their potential for the treatment of type 2 diabetes and related metabolic disorders

Published in:
Peptides

Publication Status:
Published online: 14/09/2023

DOI:
10.1016/j.peptides.2023.171096

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Peptides xxx (xxxx) xxx

Contents lists available at ScienceDirect

Peptides

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

An update on galanin and spexin and their potential for the treatment of type 2 diabetes and related metabolic disorders

Daniel M. Gallagher, Finbarr P.M. O'Harte, Nigel Irwin *

Diabetes Research Centre, Biomedical Sciences Research Institute, Ulster University, Coleraine, Northern Ireland, BT52 1SA, UK

ARTICLE INFO

Keywords:
Spexin
Galanin
Appetite
Metabolism
Type 2 diabetes
Obesity

ABSTRACT

Spexin (SPX) and galanin (GAL) are two neuropeptides widely expressed in the central nervous system as well as within peripheral tissues in humans and other species. SPX and GAL mediate their biological actions through binding and activation of galanin receptors (GALR), namely GALR1, GALR2 and GLAR3. GAL appears to trigger all three galanin receptors, whereas SPX interacts more specifically with GALR2 and GLAR3. Whilst the biological effects of GAL have been well-described over the years, in-depth knowledge of physiological action profile of SPX is still in its preliminary stages. However, it is recognised that both peptides play a significant role in modulating overall energy homeostasis, suggesting possible therapeutically exploitable benefits in diseases such as obesity and type 2 diabetes mellitus. Accordingly, although both peptides activate GALR's, it appears GAL may be more useful for the treatment of eating disorders such as anorexia and bulimia, whereas SPX may find therapeutic application for obesity and obesity-driven forms of diabetes. This short narrative review aims to provide an up-to-date account of SPX and GAL biology together with putative approaches on exploiting these peptides for the treatment of metabolic disorders.

1. Introduction

Diabetes Mellitus (DM) is a major global epidemic, with around 10% of the adult population currently presenting with the disease equating to just over half a billion people globally [72]. The underlying pathophysiology of the most prevalent form of the disease, namely type 2 diabetes mellitus (T2DM), is often a consequence of modern-day lifestyles and consumption of highly calorific meals combined with a physical inactivity [91], leading to issues with obesity and associated dysfunctional metabolic control. As such, T2DM is characterised by the impaired insulin secretion from pancreatic beta-cells and reduced insulin sensitivity of peripheral tissues, that ultimately leads to overt hyperglycaemia [23]. In a vicious circle type scenario, hyperglycaemia then results in excessive pancreatic beta-cell stimulation, beta-cell exhaustion and ultimately a reduction in total beta-cell mass [23]. If left untreated, this can lead to hyperglycaemic-induced oxidative stress and inflammation, and subsequent longer-term damage to the micro- and macro-vasculature that culminates in characteristic T2DM disease complications [31]. Although there is a proverbial arsenal of therapeutics to manage T2DM, many patients still fail to achieve adequate blood glucose control [61], largely as a result of treatment failure over time linked to the degenerative nature of this disease.

Obesity is recognised as an important independent risk factor for T2DM. Obesity refers to abnormal or excessive fat accumulation in the body, both within adipocytes as well as metabolically active tissues [2]. Like T2DM, the prevalence of obesity has increased significantly during the last few decades, and it is now considered an epidemic of the 21st Century [84]. In that respect, it has been established that worldwide over 500 million adults currently present with obesity [84]. Beyond T2DM, obesity is strongly linked to the increased prevalence of cardiovascular disease, stroke and cancer [10]. Although there have been a number of recently approved drugs for obesity, with some of these being linked to augmentation of the biological activity profile of the incretin peptide hormone glucagon-like peptide-1 (GLP-1) that is also employed for T2DM treatment [10], there is still a scarcity of effective pharmacological interventions to effectively manage the disease. Indeed, the most successful approach for obesity is often lifestyle intervention including increased physical exercise and consumption of a healthy diet [10], but long-term compliance and motivation can be a barrier to success. Thus, there is a real need for development of new safe and effective therapies for both obesity and related T2DM. Galanin (GAL) and spexin (SPX), neuropeptides with postulated benefits on energy

* Correspondence to: Ulster University, Diabetes Research Centre, Coleraine, Northern Ireland BT52 1SA, UK.
E-mail address: n.irwin@ulster.ac.uk (N. Irwin).

https://doi.org/10.1016/j.peptides.2023.171096
Received 11 August 2023; Received in revised form 4 September 2023; Accepted 11 September 2023
Available online 14 September 2023

homeostasis and the cardiovascular system, may have potential important therapeutic application for both obesity and T2DM, with this narrative review considering the use of both peptides in this regard.

2. Galanin (GAL)

2.1. GAL discovery

GAL is a biologically active neuropeptide first isolated from the porcine intestine in 1983 by Tatemoto and co-workers, who initially discovered that the peptide induced mild hyperglycaemia in mice [75]. The GAL family of peptides is now known to consist of GAL, galanin message-associated protein (GMAP), galanin-like peptide (GALP) and alarin [36]. GAL has a 29 amino acid primary sequence (30 amino acids in humans) and is distributed widely throughout the peripheral and central nervous systems (Table 1; [54]). GAL is highly conserved across species (Table 1) and appears to be involved in a variety of physiological functions in humans including effects on the cardiovascular, respiratory, gastrointestinal, endocrine, reproductive and renal systems, as well as a role in inflammation and various other centrally mediated actions [18, 38,51].

2.2. GAL synthesis, secretion and distribution

GAL is encoded by a gene on the 11Q13.3 human chromosome and is processed from a 123 amino acid precursor pro-peptide known as prepro galanin (Fig. 1A; [14]). GAL expression, as demonstrated in bovine chromaffin cells, is regulated by three distinct signal transduction systems that includes depolarisation-stimulated Ca^{2+} influx, activation of protein kinase C (PKC) as well as protein kinase A (PKA), which together differentially upregulate and downregulate the expression of several other neuropeptides, resulting in different patterns of expression between GAL and other neuropeptides [63]. GAL is distributed widely throughout the brain in mammals but is most abundant in the hypothalamus and pituitary [44]. As such, GAL mRNA is detectable in various regions of the brain in non-mammalian vertebrates that are known to impact energy regulation [54], being co-expressed alongside various neurotransmitters such as acetylcholine, serotonin, glutamate, GABA, noradrenaline, dopamine, enkephalin, neuropeptide Y (NPY), substance P, vasopressin, calcitonin gene-regulated peptide (CGRP) and gonadotropin-releasing hormone (GRH) [83]. As well as been located centrally, GAL is also evidenced within the periphery including the gastrointestinal tract and endocrine system [24], where the full bioactivity profile is now beginning to be resolved, as described below. Studies to date reveal that the secretion of GAL is modulated by several factors within the bloodstream including oestrogens, thyroid hormones, corticosteroids, growth hormone-releasing hormone, dopamine, somatostatin and thyrotropin-releasing hormone (TRH) [35].

2.3. GAL receptors and signalling

GAL receptors (GALRs) are responsible for mediating the functions of GAL, as well as the more recently discovered phylogenetically related peptide SPX [39]. GALRs are G-protein coupled receptors (GPCRs) with three different GLARs identified to date, namely GALR1, GALR2 and GLAR3 [54]. All three receptors are expressed in the periphery and centrally. For example, GALR1 and GALR2 mRNA are present in the similar brain regions including the hippocampus, hypothalamus and cortex, as well as within the gastrointestinal tract [36]. Accordingly, GALR3 has been evidenced in peripheral tissue and the central nervous system [88]. Interestingly, Barreto et al. conducted in situ hybridisation studies with an aim of localising pancreatic GALRs, with GALR1 and GALR2 detected in endocrine pancreatic islets only, and GALR3 located in both endocrine and exocrine pancreatic tissue [5]. In terms of cell signalling, GALR1 and GALR3 interact with the G_i-class of GPCR to induce inhibitory effects at the cellular level [3]. Specifically, GALR1

Table 1
Amino acid sequences of SPX, and GAL including species variations. Amino acids are abbreviated using standard single letter amino acid codes.

Peptide name	Amino acid sequence																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Human Spexin (1–14)	N	W	T	P	Q	A	M	L	Y	L	K	G	A	Q																
Mouse Spexin (1–14)	N	W	T	P	Q	A	M	L	Y	L	K	G	A	Q																
Rat Spexin (1–14)	N	W	T	P	Q	A	M	L	Y	L	K	G	A	Q																
Human Galanin (1–30)	G	W	T	L	N	S	A	G	Y	L	L	G	P	H	A	V	G	N	H	R	S	F	S	D	K	N	G	L	T	S
Rat Galanin (1–29)	G	W	T	L	N	S	A	G	Y	L	L	G	P	H	A	I	D	N	H	R	S	F	S	D	K	H	G	L	T	
Porcine Galanin (1–29)	G	W	T	L	N	S	A	G	Y	L	L	G	P	H	A	V	G	N	H	R	S	F	H	D	K	Y	G	L	A	
Human Galanin (1–16)	G	W	T	L	N	S	A	G	Y	L	L	G	P	H	A	V														
Rat/Porcine Galanin (1–16)	G	W	T	L	N	S	A	G	Y	L	L	G	P	H	A	I														

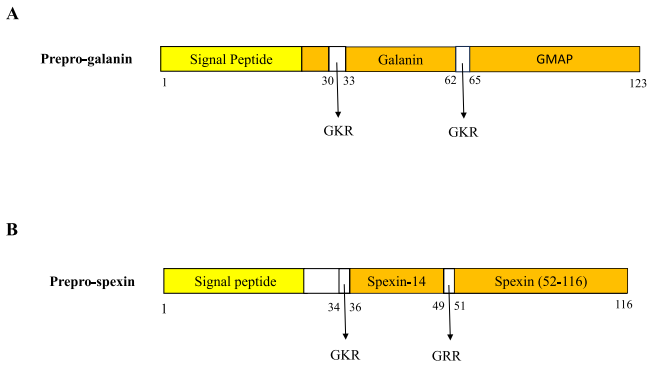

Fig. 1. Processing of (A) GAL and (B) SPX peptides from respective precursor pro-peptides. The enzymes involved on the cleavage and liberation of GAL include endopeptidase, chymotrypsin, pepsin and trypsin. Whereas for SPX peptide-glycine α- amidatingmonoxygenase yield the native peptide from the precursor. Single letter amino acid code notation is used to highlight the cleavage sites for both GAL (GKR at residues 30–33 and GKR at residues 62–65) and SPX (GKR at residues 34–36 and GRR at residues 49–51).

modulates the G protein-coupled inwardly-rectifying potassium channel (GIRK) and stimulates mitogen-activated protein kinase (MAPK) activity [43]. In terms of GALR3, signalling interactions are more poorly defined. However, because GALR3 acts via $G_{i/o}$-type GPCR, resulting in activation of G protein–regulated inwardly rectifying potassium channels (GIRK), it seems likely that GALR3 signalling pathways are at least partially similar to that of GALR1 and GALR2 [69]. Fascinatingly however, GALR2 can couple with different G-protein classes, including G_s, $G_{q/11}$ and $G_{i/o}$ to mediate both stimulatory and inhibitory downstream effects [11]. The best characterised GALR2 signalling pathway involves phospholipase C activation to induce Ca^{2+} release from intracellular stores [44]. Of interest, GALR2 signalling has also been linked to promotion of cell survival, associated with activation of phosphoinositide 3-kinases (PI3Ks) leading to inhibition of caspase activity [9].

2.4. GAL structure

The N-terminal 16 amino acid sequence of GAL, GAL(1−16), is considered important for receptor binding and activation [22,70]. As such, this N-terminal region of GAL is highly conserved among species (Table 1), with peptides that have a similar structure to GAL and bind to GALRs, such as in GALP and SPX, displaying similarly prominent levels of N-terminal amino acid sequence conservation [36]. Interestingly, the C-terminal region of GAL is less well conserved and is thought to be more important for protection against proteolytic attack rather than receptor binding or recognition (Table 1; [83]). It is predicted that GAL forms a horseshoe-like conformational shape, where the N-terminal region is organized in an alpha-helix form, followed by a beta-bend around the Pro^{13} residue, with a more uncertain configuration of the C-terminal region [67]. Studies with GAL and GAL(1−16) suggest that phosphoramidon sensitive zinc-metalloprotease are primarily responsible for the degradation of GAL within cerebrospinal fluid [8], whereas endopeptidases seem to be responsible for GAL degradation within the circulation [42]. However, the exact degradation profile of GAL in plasma has yet to be confirmed.

2.5. GAL biological actions

The most well described physiological role of GAL relates to its effects on appetite control and energy regulation in rodents ([20]; Fig. 2). Direct administration of GAL into the paraventricular nucleus (PVN) of rats elicits a preferential increase in fat and carbohydrate consumption, with no effect on protein ingestion [76]. As such, lipid intake increases GAL levels in the paraventricular hypothalamic nucleus (PVH) in a dose-dependent fashion, suggesting GAL signalling could play a role in the onset of obesity [51]. These GAL mediated effects are thought to be related specifically to activation of GALR1 [20,51]. In terms of direct effects of GAL on the endocrine system, at the level of the pancreatic islet GAL appears to function in a comparable manner as noradrenergic modulation of islet hormone secretion in both rodents and humans [14]. In keeping with this, GAL reduces glucose-induced insulin secretion in isolated rat and pig islets [30] as well as augmenting glucagon secretion ([24,26]; Fig. 2). These findings have been confirmed in the in vivo environment in obese hyperglycaemic mice [1], although there is still debate as to whether this effect fully translates to humans. Conversely, GAL exerts beneficial effects on insulin sensitivity (Fig. 2) with GAL levels thought to decline during the onset of T2DM [19]. Thus, intracerebroventricular (ICV) administration of GAL in animals with obesity, insulin resistance and T2DM, leads to increased GLUT4 expression with related beneficial effects on insulin sensitivity [29]. To reinforce this concept, both M35 and M617, recognised are GALR1 agonists [47], also augment GLUT4 expression in muscle and adipose tissues in T2DM rats [30]. In addition, a GALR2 agonist, M1145 [64], was reported to

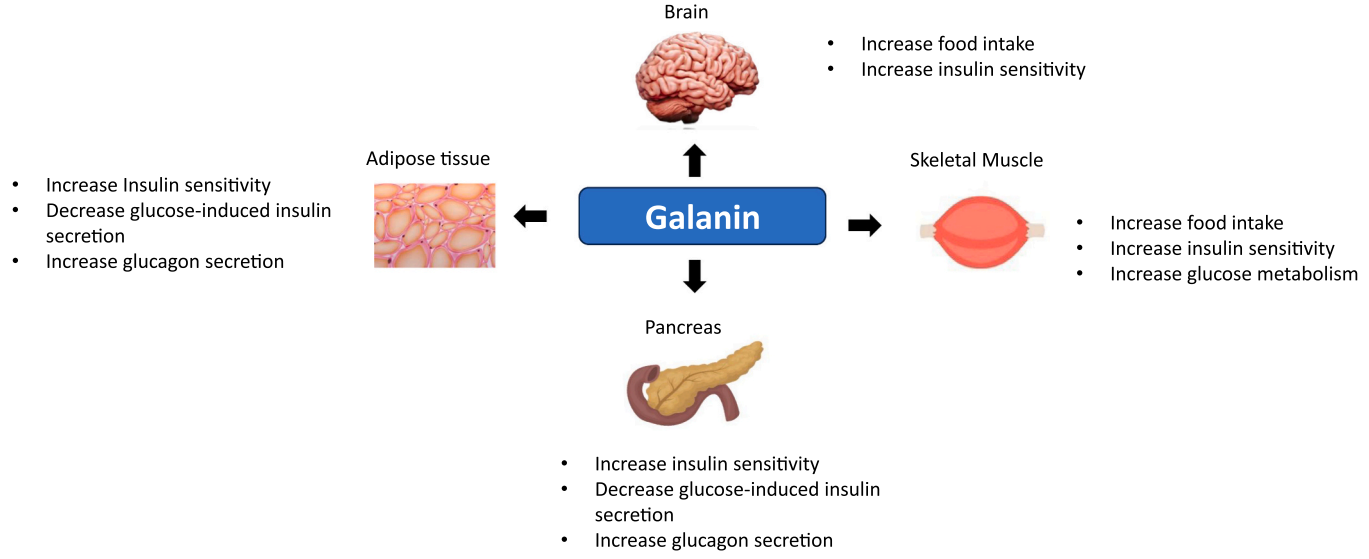

Fig. 2. Biological actions of GAL brain, muscle, pancreas and adipose tissue. Chief physiological effects documented within the text are depicted. Distinction between effects observed in humans and other animals is provided within the text.

improve glycaemic control via restoration of insulin and leptin sensitivity in diet-induced obese (DIO) mice [17]. Overall, studies would indicate that GAL can alleviate insulin resistance in myocytes, hepatocytes and adipocytes [20], suggesting potential therapeutically exploitable actions (Fig. 2).

3. Other GALR ligands

Several peptide-type chimeric ligands that function as GALR antagonists, such as galantide (M15), M35 and M40, have been synthesised and characterised [55]. Interestingly, although M35 is characterised as a GALR1 agonist [47], in the presence of GAL is appears to inhibit GAL-mediated biological actions, suggesting that it may be a GALR1 partial agonist. Despite this, M35 has been demonstrated to inhibit glucose utilisation and oxidation in mice [34]. The appetite suppressive actions of GAL are blocked by galantide in goldfish [79]. In addition, M40 and galantide decrease fat intake when injected directly into the hypothalamus in rodents, in contrast to native GAL [92]. Interestingly, M40 fails to antagonise GAL-induced inhibition of glucose-induced insulin release from mouse pancreatic islets [14] but is thought to displace GAL from receptor binding sites in the hippocampus, hypothalamus and spinal cord [6], suggesting possible tissue-specific effects of this antagonist. Non-peptide agonists of GALR1 and GALR2, such as galmic and galnon, have been described and intriguingly shown to stimulate insulin secretion from isolated rat pancreatic islets [7,58].

3.1. GMAP and GALP

GMAP is a 59 amino acid residue peptide (60 amino acids in humans) that serves as a GAL precursor [62]. GMAP has been reported to possess biological effects distinct from that of GAL, which include antimicrobial actions as part of the innate immune system [83]. GMAP also effects nociception via the spinal cord in rats, and it is likely that GMAP exerts this and other actions independent of GALRs [87], but to date no further actions of GMAP have been acknowledged. There is an overall lack of mechanistic knowledge in relation to these ascribed actions of GMAP [68]. GALP is a 60 amino acid peptide, with residues 9–21 of sharing 100% sequence homology with GAL(1−13) [60]. GALP was isolated from the porcine hypothalamus in 1999 and found to bind to GALR1 and GALR2, with greater affinity than GAL itself [56]. However, there is an indication that GALP may interact with other receptors, and that its putative role in the regulation of metabolism and energy homeostasis may not involve GALRs [45]. GALP has been shown to reduce body weight and food intake in both obese-diabetic *ob/ob* and DIO mice following intranasal administration [37], but the underlying mechanism (s) remain unclear.

4. Spexin (SPX)

4.1. SPX discovery

SPX, also known as neuropeptide Q (NPQ), is a 14 amino acid peptide hormone that signals through GAL membrane bound receptors to exert its physiological effects (Table 1; [77]). SPX was first recognised in 2007 through a bioinformatic approach, using evolutionary probabilistic models based on the genome database in different vertebrates [53]. Subsequently, in situ hybridization (ISH) studies confirmed detection of SPX mRNA within the submucosal layer of the oesophagus and stomach [71]. Elevated levels of SPX have also been detected in the liver, brain, ovaries, hypothalamus and thyroid in both mammalian and non-mammalian species [71,77]. In 2014, SPX was confirmed as a member of GAL/kisspeptin (KISS) peptide family of peptides, where a second form of SPX, namely SPX2, was discovered in non-mammalian species [39]. SPX2 represents a mature peptide with a somewhat similar amino acid sequence to SPX and a further amidation at the C terminus [48]. However, to date most focus on SPX biology relates to the actions of SPX, rather than SPX2, given that SPX2 is not detected in humans.

4.2. SPX synthesis and secretion

As noted above, SPX belongs to the GAL/KISS family of peptides and is encoded by the C12ORF39 gene located on chromosome 12 [53]. Fig. 1B highlights how SPX is initially synthesised from a 116 amino acid precursor peptide [48], with the highly conserved 14 amino acid form of SPX noted in both mammalian and non-mammalian species [77]. Strong evolutionary conservation would imply that SPX exerts important biological actions (Table 1; [13]). As such, SPX has a range of pleiotropic metabolic effects including an increase of glucose and lipid metabolism as well as modulation of insulin secretion and energy homeostasis (Fig. 3; [89]). SPX secretion appears to be restricted to metabolically active tissues and organs such as adipose tissue, liver, stomach and the pancreas in mammals [48]. In this regard, although SPX is defined as and adipokine, SPX immunoreactivity has been observed across various tissues in humans [48], with expression and secretion actually revealed to be down-regulated within omental and subcutaneous fat in obese individuals [80]. Exercise is the primary physiological secretory stimulus for SPX, as evidence in both rodents and humans [78]. In addition, hormones such as adrenocorticotropic hormone, insulin, glucagon and oestradiol are known to modulate SPX secretion in rodents, supporting the idea that SPX is responsive to input from the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal circuits [81]. Notably, previous studies document that SPX is co-localised with insulin in pancreatic beta-cell secretory vesicles of mice, implying exocytosis as the primary method of secretion, similar to many other peptide hormones [25]. In the porcine pancreas, SPX secretion is increased after a short-term 90 min incubation with glucose but decreased after 24 h glucose exposure [65], implying that glucose augments SPX secretion from pancreatic islets but that insulin may inhibit this. Accordingly, glucose ingestion has been demonstrated to increase the expression and secretion of SPX from the stomach of fish, mediated by GLUT4, whereas subsequent glucose-induced insulin secretion then inhibits SPX release [50].

4.3. SPX structure

The three-dimensional structure of SPX was first confirmed in goldfish. While the first 4 N-terminal amino acids (Asn-Trp-Thr-Pro) form a random coil-like structure at the N-terminus, the amino acids from position 5–14 create an alpha-helical motif that is common in many peptides [86]. The same study also revealed that Lys^{11} of SPX plays a critical role in receptor activation [86]. Using a combination of molecular dynamic simulations and nuclear magnetic resonance (NMR) analysis, a beta-turn-helix-beta-turn (βαβ) conformity for human SPX has been demonstrated, relating to interactions between amino acid residues Asn^{1} and Ala^{13} [13]. Furthermore, cyclic analogues of SPX with a disulphide bond between Asn^{1} and Ala^{13} are still capable of activating GALR2 and GALR3, and N-terminal acetylation can increase peptide potency [33].

4.4. SPX biological actions

SPX is known to inhibit feeding with an elevation of SPX expression observed following nutrient ingestion Fig. 3; [13]. The inhibitory effect of SPX on food intake is regulated centrally by either suppressing orexigenic factors such as neuropeptide Y (NPY), agouti-related peptide (AgRP) and apelin or increasing anorexigenic factors such as proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), cholecystokinin (CCK), nucleobindin-2 (NUCB-2) or peptide YY (PYY) in the nuclei of the hypothalamus, as evidenced in piscine models [49,86]. In addition, nutrient intake augments SPX secretion from the stomach, which may contribute this satiety signal through either activation of vagal afferents

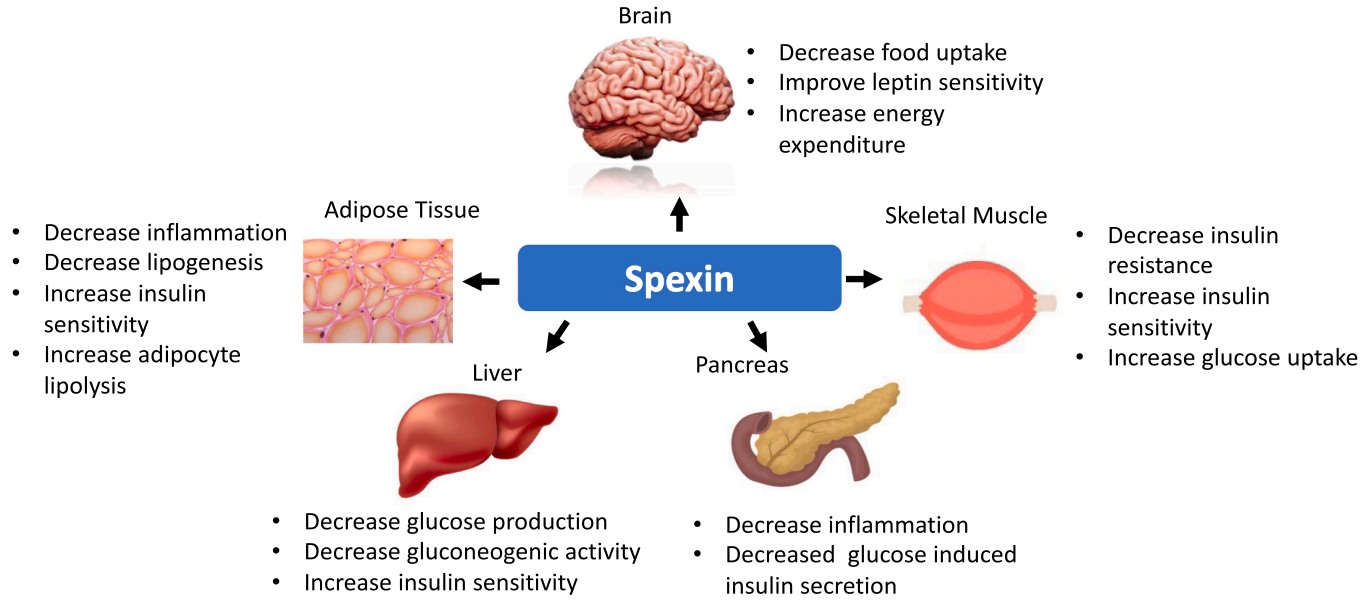

Fig. 3. Biological actions of SPX within brain, muscle, pancreas, liver and adipose tissue. Chief physiological effects documented within the text are depicted. Distinction between effects observed in humans and other animals is provided within the text.

or direct passage of SPX through the blood brain barrier [89].

SPX appears to be involved in the up-regulation of exercise related insulin action in liver, adipose tissue and skeletal muscle (Fig. 3; [89,78,16]). In agreement, SPX was previously shown to induce weight loss in DIO mice, with related improvements in HbA1c, glucose tolerance and insulin resistance observed [41]. This would suggest that SPX can improve metabolic status in T2DM through direct improvements on insulin action (Fig. 3). SPX also increases lipolysis while suppressing lipogenesis and hepatic fat accumulation in adipose tissue through activating GALR2 and GALR3 in DIO mice (Fig. 3; [89]). It follows that SPX may be able to exert metabolic benefits in diseases such as non-alcoholic fatty liver (NAFLD), that are often linked with obesity and insulin resistance [32]. In keeping with this, SPX has been revealed to reverse hepatic and hepatocellular steatosis in DIO mice [82]. In addition, treatment with 50 μg/kg/day SPX for 21 days in DIO mice improved liver function through positive effects on the regulation of carbohydrate and lipid metabolism leading to reduced levels of pro-inflammatory cytokines such as IL-6 and TNF-α (Fig. 3; [41]). At the level of the pancreatic islet, akin to the actions of GAL [73] SPX has been shown to inhibit glucose-induced insulin secretion both in vitro and in vivo (Fig. 3; [66]). Thus, benefits of GAL and SPX in T2DM could be partly linked to induction of beta-cell rest that is known to improve enduring glycaemic control under situations of metabolic stress [74], as well as improvements of insulin action. Furthermore, in obese-diabetic patients, improvements of metabolic indicators such as waist circumference and BMI lead to increased serum SPX concentrations [28]. In keeping with this, circulating SPX levels appear to be directly correlated with a decline in pancreatic beta-cell function in a Chinese population of T2DM subjects [12]. Other reported, but less well characterised, functions of SPX in rodents include inhibition of adrenocortical cell proliferation, stimulation of bowel movements and up-regulation of hyperoxia [57,80].

4.5. SPX receptor and signalling

SPX binds specifically to GALR2 and GALR3, but not to GALR1, corresponding to primary effects on modulation of insulin action and feeding behaviour [21,46]. SPX is believed to regulate energy homeostasis through interaction with hypothalamic circuits linked to augmentation of leptin and melanocortin 4 (MC4) receptor signalling, whilst diminishing neuropeptide Y5 (NPYR5) and ghrelin receptor signalling [85]. Thus, intranasal administration of a SPX-based GALR2 agonist effectively reduced food intake and body weight in obese mice [90]. SPX has also been shown to inhibit lipogenesis and glucose absorption in murine 3T3-L1 as well as human adipocytes, through activating both GALR2 and GALR3 [40]. Specifically, SPX activation of GALR2 and GALR3 results in the triggering of the PI3K/PKB and cyclic adenosine 3,5-monophosphate (cAMP)/PKA cell signalling pathways in most tissues [89]. Interestingly, SPX also regulates the phosphorylation and activation of forkhead box O1 (FoxO1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathways, including extracellular signal-regulated kinase1/2 (ERK1/2), phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase), which are involved in gluconeogenesis and glycogenolysis metabolism pathways, respectively [27,46].

4.6. SPX metabolism

Studies reveal that modifications of the N-terminal amino acid of SPX, Asn^1, via substitutions of L-Asn^1 to D-Asn^1, addition of fluorenyl methoxycarbonyl (Fmoc), acetylation or polyethylene glycosylation, increase the enzymatic stability of the peptide when compared to native SPX [59]. In addition, substitution of L-Gln^{14} for D-Gln^{14} also enhanced stability of the peptide in plasma [46]. Thus, although the exact degradation profile of SPX is unknown, cleavage of N- and C-terminal residues by serum proteases seems the most likely process in this regard, but further confirmation is required. Furthermore, L-Ala^4 for D-Ala^4 substitution improved enzymatic stability of the peptide, indicating other potentially important enzyme cleavage sites beyond the N- and C-termini [59]. Together it is clear that further investigation of SPX mediated degradation within the bloodstream is warranted.

5. GAL and SPX: opportunities for the treatment of T2DM and obesity

It seems apparent that both GAL and SPX have untapped therapeutic promise for obesity and obesity-driven forms of diabetes such as T2DM [52]. The peptides induce direct effects on to appetite and overall energy homeostasis, making them desirable targets for clinical application. Interestingly, from the current literature it seems that SPX may have

more potential in this regard as a treatment for obesity, possibly owing to the GALR selectivity of this peptide [54]. On the other hand, GAL stimulates increased fat intake and may be better suited to the treatment of eating disorders such as anorexia and bulimia [4]. However, compounds that antagonise the actions of GAL could yet find therapeutic applicability for diseases such as obesity. In contrast to GAL, SPX suppresses appetite and can reduce body weight in obese rodents [52]. Indeed, circulating fatty acids may act as a trigger for SPX secretion to induce satiety [54]. Such effects of GAL and SPX are predominantly mediated centrally through direct modulation of hypothalamic circuits [51]. Notably, SPX is documented to inhibit adipogenesis and stimulate lipolysis in both human and murine adipocytes [40], suggesting direct benefits in obesity. In good agreement with this, once daily SPX administration (25 μg/kg body weight) for 30 days in DIO mice lead to reductions of body weight, with subsequent improvements in insulin action and overall metabolism [41], similar to previous observations [80]. In keeping with this, GAL and SPX have previously been shown to improve peripheral insulin action and up-regulate GLUT4 membrane expression, resulting in positive effects on glucose tolerance [27]. Interestingly, at the level of the pancreatic beta-cell, SPX inhibits glucose-stimulated insulin secretion (GSIS) [65]. This inhibitory effect of GAL and SPX on GSIS may at first appear counterintuitive for T2DM treatment. However, there are well documented benefits of inducing beta-cell rest in diabetes [74], with such agents shown to promote beta-cell health and prevent beta-cell apoptosis that is characteristic of all forms of diabetes. Indeed, SPX-induced enhancement of pancreatic islet cell viability and proliferation would fully support this notion [65], with the presence of SPX in islet beta-cells suggesting possible important autocrine and paracrine interactions within and between endocrine islet cells [66]. Therefore, the impact of GAL and SPX in this setting requires further detailed study.

6. Conclusion and future developments

Recent literature clearly documents that GAL and SPX and exert numerous biological actions both within the CNS and periphery (Fig. 2 and 3). However, the relative importance of each of these peptide mediated effects in terms of overall physiology and control of metabolism is still largely unknown, but their impact on the modulation of energy homeostasis appears to be particularly significant. Of the two peptides, GAL is the most extensively investigated to date given its earlier discovery, although potential anti-obesity actions of SPX may see interest in this molecule increase exponentially over the coming years. Development of pharmacological and/or genetic methods to determine the precise actions of GAL and SPX at individual GALRs, such as characterisation and use of specific GALR antagonists or CRISPR-Cas9 mediated knockdown of GALRs [15], may help to uncover the underlying mechanisms of such benefits. Thus, there are still unanswered questions regarding the pharmacology and cellular mechanisms that coordinate GAL and SPX biological actions. Nevertheless, owing to direct positive effects on energy homeostasis and insulin sensitivity, GAL and SPX related peptides have exciting potential for the future treatment of obesity and associated metabolic disorders such as T2DM.

Ethical statement

There are no ethical issues related to this review article.

Declaration of Competing Interest

Finbarr P.M. O'Harte and Nigel Irwin are named on patents filed by the University of Ulster for exploitation of incretin-based drugs and other peptide therapeutics.

Data availability

No data was used for the research described in the article.

Acknowledgements

The research was supported by the Northern Ireland (UK), Department for the Economy and Ulster University (UK), Strategic Funding.

References

[1] B. Ahrén, P. Arkhammar, P.O. Berggren, T. Nilsson, Galanin inhibits glucose-stimulated insulin release by a mechanism involving hyperpolarization and lowering of cytoplasmic free Ca2+ concentration, Biochem. Biophys. Res. Commun. 140 (1986) 1059–1063.
[2] A.M. Angelidi, M.J. Belanger, A. Kokkinos, C.C. Koliaki, C.S. Mantzoros, Novel noninvasive approaches to the treatment of obesity: from pharmacotherapy to gene therapy, Endocr. Rev. 43 (2022) 507–557.
[3] Y.F. Bai, H.T. Ma, L.N. Liu, H. Li, X.X. Li, Y.T. Yang, B. Xue, D. Wang, Z.Q.D. Xu, Activation of galanin receptor 1 inhibits locus coeruleus neurons via GIRK channels, Biochem. Biophys. Res. Commun. 503 (2018) 79–85.
[4] B. Baranowska, E. Wolinska-Witort, E. Wasilewska-Dziubinska, K. Roguski, M. Chmielowska, Plasma leptin, neuropeptide Y (NPY) and galanin concentrations in bulimia nervosa and in anorexia nervosa, Neuroendocrinol. Lett. 22 (2001) 356–358.
[5] S.G. Barreto, M. Bazargan, M. Zotti, D.J. Hussey, O.A. Sukocheva, H. Peiris, M. Leong, D.J. Keating, A.C. Schloithe, C.J. Carati, C. Smith, Galanin receptor 3–a potential target for acute pancreatitis therapy, Neurogastroenterol. Motil. 23 (2011) 141–151.
[6] T. Bartfai, U. Langel, K. Bedecs, S. Andell, T. Land, S. Gregersen, B. Ahren, P. Girotti, S. Consolo, R. Corwin, Galanin-receptor ligand M40 peptide distinguishes between putative galanin-receptor subtypes, Proc. Natl. Acad. Sci. 90 (1993) 11287–11291.
[7] T. Bartfai, X. Lu, H. Badie-Mahdavi, A.M. Barr, A. Mazarati, X.Y. Hua, T. Yaksh, G. Haberhauer, S.C. Ceide, L. Trembleau, L. Somogyi, Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests, Proc. Natl. Acad. Sci. 101 (2004) 10470–10475.
[8] K. Bedecs, Ü. Langel, T. Bartfai, Metabolism of galanin and galanin (1–16) in isolated cerebrospinal fluid and spinal cord membranes from rat, Neuropeptides 29 (1995) 137–143.
[9] M. Brzozowska, J. Całka, Occurrence and distribution of galanin in the physiological and inflammatory states in the mammalian gastrointestinal tract, Front. Immunol. 11 (2021), 602070.
[10] V.P. Chavda, J. Ajabiya, D. Teli, J. Bojarska, V. Apostolopoulos, Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review, Molecules 27 (2022) 4315.
[11] Y. Cohen, K. Hausken, Y. Bonfil, M. Gutnick, B. Levavi-Sivan, Spexin and a novel cichlid-specific spexin paralog both inhibit FSH and LH through a specific galanin receptor (Galr2b) in tilapia, Front. Endocrinol. 11 (2020) 71.
[12] J. Dai, Y. Ni, D. Wu, Y. Jiang, S. Jin, S. Zhang, X. Yu, R. Liu, Circulating spexin levels are influenced by the glycemic status and correlated with pancreatic β-cell function in Chinese subjects, Acta Diabetol. 60 (2023) 305–313.
[13] Ö. Darakci, A. Bozkurt, Structure and functions of spexin as a new neuroendocrine signal, J. Exp. Clin. Med. 39 (2022) 893–900.
[14] B.E. Dunning, G.J. Taborsky Jr, Galanin—sympathetic neurotransmitter in endocrine pancreas, Diabetes 37 (1988) 1157–1162.
[15] A. Eskova, H.G. Frohnhöfer, C. Nüsslein-Volhard, U. Irion, Galanin signaling in the brain regulates color pattern formation in zebrafish, Curr. Biol. 30 (2020) 298–303.
[16] P. Fang, W. Guo, M. Ju, Y. Huang, H. Zeng, Y. Wang, M. Yu, Z. Zhang, Exercise training rescues adipose tissue spexin expression and secretion in diet-induced obese mice, Physiol. Behav. 256 (2022), 113958.
[17] P. Fang, B. He, M. Yu, M. Shi, Y. Zhu, Z. Zhang, P. Bo, Central galanin receptor 2 mediates galanin action to promote systemic glucose metabolism of type 2 diabetic rats, Biochem. Pharmacol. 156 (2018) 241–247.
[18] P. Fang, Y. She, J. Zhao, J. Yan, X. Yu, Y. Jin, Q. Wei, Z. Zhang, W. Shang, Emerging roles of kisspeptin/galanin in age-related metabolic disease, Mech. Ageing Dev. 199 (2021), 111571.
[19] P. Fang, M. Yu, M. Shi, P. Bo, Z. Zhang, Galanin peptide family regulation of glucose metabolism, Front. Neuroendocrinol. 56 (2020), 100801.
[20] P. Fang, M. Yu, M. Shi, Z. Zhang, Y. Sui, L. Guo, P. Bo, Galanin peptide family as a modulating target for contribution to metabolic syndrome, Gen. Comp. Endocrinol. 179 (2012) 115–120.
[21] P. Fang, L. Zhang, M. Yu, Z. Sheng, M. Shi, Y. Zhu, Z. Zhang, P. Bo, Activated galanin receptor 2 attenuates insulin resistance in skeletal muscle of obese mice, Peptides 99 (2018) 92–98.
[22] Z. Fathi, A.M. Cunningham, L.G. Iben, P.B. Battaglino, S.A. Ward, K.A. Nichol, K. A. Pine, J. Wang, M.E. Goldstein, T.P. Iismaa, I.A. Zimanyi, Cloning, pharmacological characterization and distribution of a novel galanin receptor, Mol. Brain Res. 51 (1997) 49–59.
[23] U. Galicia-Garcia, A. Benito-Vicente, S. Jebari, A. Larrea-Sebal, H. Siddiqi, K. B. Uribe, H. Ostolaza, C. Martín, Pathophysiology of type 2 diabetes mellitus, Int. J. Mol. Sci. 21 (2020) 6275–6309.

[24] I. Gesmundo, T. Villanova, D. Banfi, G. Gamba, R. Granata, Role of melatonin, Galanin, and RFamide neuropeptides QRFP26 and QRFP43 in the neuroendocrine control of pancreatic β-cell function, Front. Endocrinol. 8 (2017) 143.
[25] T. Gowdu, C.D. Dayanand, Spexin in metabolic syndrome-an overview, SAS J. Med. 7 (2021) 15–25.
[26] S. Gregersen, K. Hermansen, Ü. Langel, G. Fisone, T. Bartfai, B. Ahren, Galanin-induced inhibition of insulin secretion from rat islets: effects of rat and pig galanin and galanin fragments and analogues, Eur. J. Pharmacol. 203 (1991) 111–114.
[27] L. Gu, X. Ding, Y. Wang, M. Gu, J. Zhang, S. Yan, N. Li, Z. Song, J. Yin, L. Lu, Y. Peng, Spexin alleviates insulin resistance and inhibits hepatic gluconeogenesis via the FoxO1/PGC-1α pathway in high-fat-diet-induced rats and insulin resistant cells, Int. J. Biol. Sci. 15 (2019) 2815.
[28] L. Gu, S. Yan, Y. Huang, J. Yang, Y. Peng, Y. Wang, Serum spexin differed in newly diagnosed type 2 diabetes patients according to body mass index and increased with the improvement of metabolic status, Front. Endocrinol. 13 (2022) 1086497.
[29] L. Guo, B. He, P. Fang, P. Bo, Z. Zhang, Activation of central galanin receptor 2 mitigated insulin resistance in adipocytes of diabetic rats, J. Endocrinol. Investig. 44 (2021) 515–522.
[30] L. Guo, M. Shi, L. Zhang, G. Li, L. Zhang, H. Shao, P. Fang, Y. Ma, J. Li, Q. Shi, Y. Sui, Galanin antagonist increases insulin resistance by reducing glucose transporter 4 effect in adipocytes of rats, Gen. Comp. Endocrinol. 173 (2011) 159–163.
[31] J.L. Harding, M.E. Pavkov, D.J. Magliano, J.E. Shaw, E.W. Gregg, Global trends in diabetes complications: a review of current evidence, Diabetologia 62 (2019) 3–16.
[32] J.M. Hazlehurst, C. Woods, T. Marjot, J.F. Cobbold, J.W. Tomlinson, Non-alcoholic fatty liver disease and diabetes, Metabolism 65 (2016) 1096–1108.
[33] T. Huang, Toward novel therapeutics for functional constipation: from traditional Chinese medicine herbal formula MaZiRenWan to cyclic spexin analogues. *Doctoral dissertation*, Hong Kong Baptist University,, 2017.
[34] A. Idelevich, K. Sato, K. Nagano, G. Rowe, F. Gori, R. Baron, Neuronal hypothalamic regulation of body metabolism and bone density is galanin dependent, J. Clin. Investig. 128 (2018) 2626–2641.
[35] C. Invitti, F.P. Giraldi, A. Dubini, P. Moroni, M. Losa, R. Piccoletti, F. Cavagnini, Galanin is released by adrenocorticotropin-secreting pituitary adenomas in vivo and in vitro. J. Clin. Endocrinol. Metab. 84 (1999) 1351–1356.
[36] W. Jiang, S. Zheng, Structural insights into galanin receptor signaling, Proc. Natl. Acad. Sci. 119 (2022) 2121465119.
[37] H. Kageyama, K. Shiba, S. Hirako, N. Wada, S. Yamanaka, Y. Nogi, F. Takenoya, N. Nonaka, T. Hirano, S. Inoue, S. Shioda, Anti-obesity effect of intranasal administration of galanin-like peptide (GALP) in obese mice, Sci. Rep. 6 (2016) 1–11.
[38] A. Kastin (Ed.), Handbook of Biologically Active Peptides, Academic press, 2013.
[39] D.K. Kim, S. Yun, G.H. Son, J.I. Hwang, C.R. Park, J.I. Kim, K. Kim, H. Vaudry, J. Y. Seong, Coevolution of the spexin/galanin/kisspeptin family: spexin activates galanin receptor type II and III, Endocrinology 155 (2014) 1864–1873.
[40] P.A. Kolodziejski, E. Pruszynska-Oszmalek, M. Micker, M. Skrzypski, T. Wojciechowicz, P. Szwarckopf, K. Skieresz-Szewczyk, K.W. Nowak, M. Z. Strowski, Spexin: a novel regulator of adipogenesis and fat tissue metabolism, Biochim. Et. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids 1863 (2018) 1228–1236.
[41] P.A. Kolodziejski, N. Leciejewska, A. Chmurzynska, M. Sassek, A. Szczepankiewicz, D. Szczepankiewicz, E. Malek, M.Z. Strowski, Z. Checinska-Maciejewska, K. W. Nowak, E. Pruszynska-Oszmalek, 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 (2021 30), 111420.
[42] T. Land, U. Langel, T. Bartfai, Hypothalamic degradation of galanin (1–29) and galanin (1–16): identification and characterization of the peptidolytic products, Brain Res. 558 (1991) 245–250.
[43] R. Lang, A.L. Gundlach, F.E. Holmes, S.A. Hobson, D. Wynick, T. Hoekfelt, B. Kofler, Physiology, signaling, and pharmacology of galanin peptides and receptors: three decades of emerging diversity, Pharmacol. Rev. 67 (2015) 118–175.
[44] R. Lang, A.L. Gundlach, B. Kofler, The galanin peptide family: receptor pharmacology, pleiotropic biological actions, and implications in health and disease, Pharmacol. Ther. 115 (2007) 177–207.
[45] C. Lawrence, G.S. Fraley, Galanin-like peptide (GALP) is a hypothalamic regulator of energy homeostasis and reproduction, Front. Neuroendocrinol. 32 (2011) 1–9.
[46] Y.N. Lee, A. Reyes-Alcaraz, S. Yun, C.S. Lee, J.I. Hwang, J.Y. Seong, Exploring the molecular structures that confer ligand selectivity for galanin type II and III receptors, PloS One 15 (2020), e0230872.
[47] L. Lundström, U. Sollenberg, A. Brewer, P.F. Kouya, K. Zheng, X.J. Xu, X. Sheng, J. K. Robinson, Z. Wiesenfeld-Hallin, Z.Q. Xu, T. Hökfelt, A galanin receptor subtype 1 specific agonist, Int. J. Pept. Res. Ther. 11 (2005) 17–27.
[48] S.Y. Lv, Y.C. Zhou, X.M. Zhang, W.D. Chen, Y.D. Wang, Emerging roles of NPQ/spexin in physiology and pathology, Front. Pharmacol. 10 (2019) 457.
[49] A. Ma, J. Bai, M. He, A.O. Wong, Spexin as a neuroendocrine signal with emerging functions, Gen. Comp. Endocrinol. 265 (2018) 90–96.
[50] A. Ma, M. He, J. Bai, M.K. Wong, W.K. Ko, A.O. Wong, Dual role of insulin in spexin regulation: functional link between food intake and spexin expression in a fish model, Endocrinology 158 (2017) 560–577.
[51] P. Marcos, R. Coveñas, Neuropeptidergic control of feeding: focus on the galanin family of peptides, Int. J. Mol. Sci. 22 (2021) 2544.
[52] E.G. Mills, C. Izzi-Engbeaya, A. Abbara, A.N. Comninos, W.S. Dhillo, Functions of galanin, spexin and kisspeptin in metabolism, mood and behaviour, Nat. Rev. Endocrinol. 17 (2021) 97–113.
[53] O. Mirabeau, E. Perlas, C. Severini, E. Audero, O. Gascuel, R. Possenti, E. Birney, N. Rosenthal, C. Gross, Identification of novel peptide hormones in the human proteome by hidden Markov model screening, Genome Res. 17 (2007) 320–327.
[54] I.A. Mohd Zahir, S. Ogawa, N.A. Dominic, T. Soga, I. Parhar, Spexin and galanin in metabolic functions and social behaviors with a focus on non-mammalian vertebrates, Front. Endocrinol. 13 (2022), 882772.
[55] S.O. Ögren, E. Kuteeva, T. Hökfelt, J. Kehr, Galanin receptor antagonists, CNS Drugs 20 (2006) 633–654.
[56] T. Ohtaki, S. Kumano, Y. Ishibashi, K. Ogi, H. Matsui, M. Harada, C. Kitada, T. Kurokawa, H. Onda, M. Fujino, Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus, J. Biol. Chem. 274 (1999) 37041–37045.
[57] E. Pruszynska-Oszmalek, M. Sassek, D. Szczepankiewicz, K.W. Nowak, P. A. Kolodziejski, Short-term administration of spexin in rats reduces obesity by affecting lipolysis and lipogenesis: an in vivo and in vitro study, Gen. Comp. Endocrinol. 299 (2020), 113615.
[58] N.T.T. Quynh, S.M. Islam, A. Florén, T. Bartfai, Ü. Langel, C.G. Östenson, Effects of galnon, a non-peptide galanin-receptor agonist, on insulin release from rat pancreatic islets, Biochem. Biophys. Res. Commun. 328 (2005) 213–220.
[59] A. Reyes-Alcaraz, Y.N. Lee, G.H. Son, N.H. Kim, D.K. Kim, S. Yun, D.H. Kim, J. I. Hwang, J.Y. Seong, Development of spexin-based human galanin receptor type II-specific agonists with increased stability in serum and anxiolytic effect in mice, Sci. Rep. 6 (2016) 21453.
[60] J.K. Robinson, T. Bartfai, U. Langel, Galanin/GALP receptors and CNS homeostatic processes, CNS Neurol. Disord. Drug Targets Former. Curr. Drug Targets-CNS Neurol. Disord. 5 (2006) 327–334.
[61] T.V. Rohm, D.T. Meier, J.M. Olefsky, M.Y. Donath, Inflammation in obesity, diabetes, and related disorders, Immunity 55 (2022) 31–55.
[62] Å. Rökaeus, M.J. Brownstein, Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor, Proc. Natl. Acad. Sci. 83 (1986) 6287–6291.
[63] A. Rokaeus, R.M. Pruss, L.E. Eiden, Galanin gene expression in chromaffin cells is controlled by calcium and protein kinase signaling pathways, Endocrinology 127 (1990) 3096–3102.
[64] J. Runesson, I. Saar, L. Lundström, J. Järv, Ü. Langel, A novel GalR2-specific peptide agonist, Neuropeptides 43 (2009) 187–192.
[65] M. Sassek, P.A. Kolodziejski, M.Z. Strowski, L. Nogowski, K.W. Nowak, P. Mackowiak, Spexin modulates functions of rat endocrine pancreatic cells, Pancreas 47 (2018) 904–909.
[66] M. Sassek, P.A. Kolodziejski, D. Szczepankiewicz, E. Pruszynska-Oszmalek, Spexin in the physiology of pancreatic islets-mutual interactions with insulin, Endocrine 63 (2018) 513–519.
[67] E. Schrank, G.E. Wagner, K. Zangger, Solution NMR studies on the orientation of membrane-bound peptides and proteins by paramagnetic probes, Molecules 18 (2013) 7407–7435.
[68] J. Šipkova, I. Kramarikova, S. Hynie, V. Klenerova, The galanin and galanin receptor subtypes, its regulatory role in the biological and pathological functions, Physiol. Res. 66 (2017) 729–740.
[69] K.E. Smith, M.W. Walker, R. Artymyshyn, J. Bard, B. Borowsky, J.A. Tamm, W. J. Yao, P.J.J. Vaysse, T.A. Branchek, C. Gerald, K.A. Jones, Cloned human and rat galanin GALR3 receptors: pharmacology and activation of G-protein inwardly rectifying K+ channels, J. Biol. Chem. 273 (1998) 23321–23326.
[70] D. Sobrido-Cameán, L.A. Yáñez-Guerra, F. Lamanna, C. Conde-Fernández, H. Kaessmann, M.R. Elphick, R. Anadón, M.C. Rodicio, A. Barreiro-Iglesias, Galanin in an agnathan: precursor identification and localisation of expression in the brain of the sea lamprey Petromyzon marinus, Front. Neuroanat. 13 (2019) 83.
[71] K. Sonmez, N.T. Zaveri, I.A. Kerman, S. Burke, C.R. Neal, X. Xie, S.J. Watson, L. Toll, Evolutionary sequence modeling for discovery of peptide hormones, PLoS Comput. Biol. 5 (2009) 1000258.
[72] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova, B.B. Duncan, C. Stein, A. Basit, J.C. Chan, J.C. Mbanya, M.E. Pavkov, IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, Diabetes Res. Clin. Pract. 183 (2022), 109119.
[73] X. Sun, Z. Yu, Y. Xu, S. Pu, X. Gao, The role of spexin in energy metabolism, Peptides 164 (2023), 170991.
[74] N. Tanday, R.A. Lafferty, P.R. Flatt, N. Irwin, Beneficial metabolic effects of recurrent periods of beta-cell rest and stimulation using stable neuropeptide Y1 and glucagon-like peptide-1 receptor agonists, Diabetes Obes. Metab. 24 (2022) 2353–2363.
[75] K. Tatemoto, Å. Rökaeus, H. Jörnvall, T.J. McDonald, V. Mutt, Galanin—a novel biologically active peptide from porcine intestine, FEBS Lett. 164 (1983) 124–128.
[76] D.L. Tempel, K.J. Leibowitz, S.F. Leibowitz, Effects of PVN galanin on macronutrient selection, Peptides 9 (1988) 309–314.
[77] İ. Türkel, G. Memi, B. Yazgan, Impact of spexin on metabolic diseases and inflammation: an updated minireview, Exp. Biol. Med. 247 (2022) 567–573.
[78] İ. Türkel, B. Özerkliğ, M.M. Atakan, S. Aktitiz, Ş.N. Koşar, B. Yazgan, Exercise and metabolic health: the emerging roles of novel exerkines, Curr. Protein Pept. Sci. 33 (2022) 437–455.
[79] S. Unniappan, J.M. Cerdá-Reverter, R.E. Peter, In situ localization of preprogalanin mRNA in the goldfish brain and changes in its expression during feeding and starvation, Gen. Comp. Endocrinol. 136 (2004) 200–207.
[80] J.L. Walewski, F. Ge, H. Lobdell IV, N. Levin, G.J. Schwartz, J.R. Vasselli, A. Pomp, G. Dakin, P.D. Berk, 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 (2014) 1643–1652.

[81] L. Wang, A. Tran, J. Lee, D.D. Belsham, Palmitate differentially regulates Spexin, and its receptors Galr2 and Galr3, in GnRH neurons through mechanisms involving PKC, MAPKs, and TLR4, Mol. Cell. Endocrinol. 518 (2020), 110991.
[82] M. Wang, Z. Zhu, Y. Kan, M. Yu, W. Guo, M. Ju, J. Wang, S. Yi, S. Han, W. Shang, Z. Zhang, Treatment with spexin mitigates diet-induced hepatic steatosis in vivo and in vitro through activation of galanin receptor 2, Mol. Cell. Endocrinol. 552 (2022), 111688.
[83] K.E. Webling, J. Runesson, T. Bartfai, Ü. Langel, Galanin receptors and ligands, Front. Endocrinol. 3 (2012) 146.
[84] M. Wolosowicz, S. Prokopiuk, T.W. Kaminski, Recent advances in the treatment of insulin resistance targeting molecular and metabolic pathways: fighting a losin battle, Medicina 58 (2022) 472.
[85] M.K. Wong, Y. Chen, M. He, C. Lin, Z. Bian, A.O. Wong, Mouse spexin:(II) functional role as a satiety factor inhibiting food intake by regulatory actions within the hypothalamus, Front. Endocrinol. 12 (2021), 681647.
[86] M.K. Wong, K.H. Sze, T. Chen, C.K. Cho, H.C. Law, L.K. Chu, A.O. Wong, Goldfish spexin: solution structure and novel function as a satiety factor in feeding control, Am. J. Physiol. Endocrinol. Metab. 305 (2013) 348–366.
[87] X.J. Xu, S. Andell, J.X. Hao, Z. Wiesenfeld-Hallin, T. Bartfai, The effects of intrathecal galanin message-associated peptide (GMAP) on the flexor reflex in rats, Regul. Pept. 58 (1995) 19–24.
[88] M. Yu, P. Fang, H. Wang, G. Shen, Z. Zhang, Z. Tang, Beneficial effects of galanin system on diabetic peripheral neuropathic pain and its complications, Peptides 134 (2020), 170404.
[89] M. Yu, M. Ju, P. Fang, Z. Zhang, Emerging central and peripheral actions of spexin in feeding behaviour, leptin resistance and obesity, Biochem. Pharmacol. 202 (2022), 115121.
[90] S. Yun, A. Reyes-Alcaraz, Y.N. Lee, H.J. Yong, J. Choi, B.J. Ham, J.W. Sohn, D. H. Kim, G.H. Son, H. Kim, S.G. Kwon, Spexin-based galanin receptor type 2 agonist for comorbid mood disorders and abnormal body weight, Front. Neurosci. 13 (2019) 391.
[91] Y. Zheng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat. Rev. Endocrinology 14 (2018) 88–98.
[92] E.P. Zorrilla, M. Brennan, V. Sabino, X. Lu, T. Bartfai, Galanin type 1 receptor knockout mice show altered responses to high-fat diet and glucose challenge, Physiol. Behav. 91 (2007) 479–485.