Molecules and Cells

Minireview

Sensory nerve and neuropeptide diversity in adipose tissues

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ABSTRACT

Both brown and white adipose tissues (BAT/WAT) are innervated by the peripheral nervous system, including efferent sympathetic nerves that communicate from the brain/central nervous system out to the tissue, and afferent sensory nerves that communicate from the tissue back to the brain and locally release neuropeptides to the tissue upon stimulation. This bidirectional neural communication is important for energy balance and metabolic control, as well as maintaining adipose tissue health through processes like browning (development of metabolically healthy brown adipocytes in WAT), thermogenesis, lipolysis, and adipogenesis. Decades of sensory nerve denervation studies have demonstrated the particular importance of adipose sensory nerves for brown adipose tissue and WAT functions, but far less is known about the tissue's sensory innervation compared to the better-studied sympathetic nerves and their neurotransmitter norepinephrine. In this review, we cover what is known and not yet known about sensory nerve activities in adipose, focusing on their effector neuropeptide actions in the tissue.

Keywords: Metabolism, Brown adipose tissue (BAT), Sensory nerves, Sensory neuropeptides, White adipose tissue (WAT)

INTRODUCTION

The homeostatic regulation of whole-body metabolism and energy balance is modulated by the bidirectional neural communication between adipose tissues and the central nervous system (CNS), and facilitated by the peripheral nervous system (PNS) that innervates tissues and organs. The PNS, in general, is comprised of somatosensory, motor, sympathetic, and parasympathetic nerves. Among these, the sensory nerves innervate the majority of the body's organs and tissues, transmit internal and external cues to the CNS via afferent signaling, while simultaneously releasing neuropeptides like calcitonin gene-related peptide (CGRP) and Substance P (SP) to the innervated peripheral tissue. Conversely, motor, sympathetic, and parasympathetic nerves partake in efferent signaling from the CNS to the periphery. Motor neurons elicit both voluntary and involuntary responses in the effector muscle or gland and can be either sympathetic and parasympathetic in nature. Sympathetic nerves mediate the "fight or flight" response by releasing neurotransmitters like norepinephrine (NE), whereas parasympathetic nerves generally counteract this by promoting the "rest and digest" response in peripheral tissues, like in the gastrointestinal system via efferent vagal nerve activity (Altschuler et al., 1993; Berthoud et al., 1990; Kalia and Sullivan, 1982; Shapiro and Miselis, 1985; Uvnas-Moberg, 1994) and the release of neurotransmitters like acetylcholine. However, as evidenced by multiple viral tracing studies (Fishman and Dark, 1987; Ryu et al., 2015; Song et al., 2009; Vaughan and Bartness, 2012; Wang et al., 2022), adipose tissues only receive sensory and sympathetic innervation (Ryu and Bartness, 2014; Ryu et al., 2017) and lack parasympathetic or vagal innervation (Bartness et al., 2014) (*see below "A note: Parasympathetic innervation of adipose tissues*"). As such, whether sensory nerves and their secreted neuropeptides exert their effects in part to counteract sympathetic signaling in adipose tissues remains an active area of research and one that is currently fraught with conflicting data (reviewed in Mishra and Townsend [2023]).

Adipose tissue nerves exert important functional roles in adipogenesis, browning (development of brown adipocytes in white adipose tissue [WAT]), thermogenesis, lipolysis, and more (Mishra and Townsend, 2023). Obesity, a consequence of the imbalance between energy storage and expenditure, presents with adipose neuropathy, or the loss of adipose innervation (neurite density, expression of neurotrophic factors, axon outgrowth markers, demyelination, etc), in both mice and human subjects and is also evident in aged adipose tissue (Blaszkiewicz et al., 2019). Multiple surgical (Shi et al., 2005; Vaughan et al., 2014), chemical (Shi et al., 2005), and genetic (Makwana et al., 2021; Wang et al., 2022) denervation studies have emphasized the need for intact adipose innervation for preventing metabolic dysregulation. For instance, Siberian hamsters that had received surgical resection of inguinal subcutaneous WAT (ing-scWAT)-innervating nerves (unilateral) presented with increased NE levels and larger depot mass in the denervated depot compared to the contralateral control depot (Youngstrom and Bartness, 1998). Chemical ablation of sensory afferents in ing-scWAT by high-dose capsaicin injections in Siberian hamsters resulted in adipocyte hypertrophy (Shi et al., 2005). Lastly, adeno-

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associted virus (AAV)-mediated sensory denervation of unilateral ing-scWAT in mice upregulated both thermogenic and lipogenic gene expression in the denervated depot, but not the contralateral control depot (Wang et al., 2022). Taken together, these studies emphasize the importance of adipose innervation, particularly sensory innervation, for adipose metabolic health.

While the specific contributions of sympathetic nerves and the neurotransmitter NE to adipose metabolism have been well documented, the role of sensory nerves and neuropeptides in the context of adipose metabolism has only recently garnered attention (Makwana et al., 2021; Wang et al., 2022). For instance, viral ablation of sensory nerves innervating ing-scWAT enhanced both lipogenesis and thermogenesis in mice (Wang et al., 2022), and systemic, genetic ablation of CGRP protected mice against high-fat diet-induced obesity by enhancing whole-body energy expenditure (Makwana et al., 2021). Interestingly, why there is adipose neuropathy with obesity, humans with obesity also have elevated circulating CGRP (Zelissen et al., 1991). Together, these studies using viral or genetic ablation of sensory nerves or neuropeptide expression in adipose suggest a negative feedback loop between sensory and sympathetic nerves in adipose tissues. These findings, however, challenge the outcomes observed following surgical and chemical denervation of scWAT in rodents, which support a positive feedback loop between sensory and sympathetic nerves in adipose tissues. For instance, high-dose capsaicin-induced sensory denervation of epididymal WAT in Siberian hamsters enhanced both retroperitoneal and ing-scWAT mass, suggesting a potential decrease in lipolysis or increase in lipogenesis (this was not explored by the authors) (Shi and Bartness, 2005) (reviewed in Mishra and Townsend [2023]). Together, here we provide an overview of the latest findings on sensory nerve and neuropeptide contributions to adipose tissue functions and metabolic health (Table 1).

NEUROANATOMY OF THE SENSORY NERVOUS SYSTEM

Sensory neurons are pseudo-unipolar and located within the dorsal root ganglia (DRG) of the spinal cord. Axons originating from the sensory neurons extend bidirectionally, toward the peripheral tissues and centrally towards the brain via the spinal cord. The unique ability of sensory nerves to detect a vast set of incoming sensory stimuli can be attributed, in part, to their specialized structural and functional adaptations at the tissue terminal ends. For instance, sensory nerves express receptors such as mechanoreceptors, thermoreceptors, chemoreceptors, nociceptors, and photoreceptors (Genovese et al., 2021; Iheanacho and Vellipuram, 2023; Messlinger, 1997; O'Regan

Table 1. Summary of sensory neuropeptide effects on energy balance

Animal or cell model	Treatment	Outcome	Citation
3T3-L1	CGRP	↑cAMP production	Chatzipanteli et al. (1996)
3T3-L1	CGRP	↑ Extracellular free-fatty acid, no change in cAMP production	Walker et al. (2014)
Male Wistar rats, exercised	CGRP (intravenous)	↑ Lipolysis via PIPLC/IP_3 pathway in epididymal WAT	Aveseh et al. (2018)
Obese human subjects with type II diabetes	Substance P	↑ Substance P in circulation	Fu et al. (2011)
Obese children	Substance P	↑ Substance P in circulation	Baroncelli et al. (1989)
3T3-L1	Substance P	↓ Lipid deposition (FABP4 and DGAT-1 mRNA), reduced free fatty acid uptake, increased lipolysis, increased CD36 expression	Miegueu et al. (2013)
Mice	CJ 12,255 (ip) (Substance P receptor Neurokinin-1R agonist)	Protected mice against high-fat diet-induced obesity, ↓ fat mass, ↓ both food intake and circulating leptin levels	Karagiannides et al. (2008)
Mice	Cold-exposure (3.5 weeks)	↓ PACAP receptor (PAC-1R) expression in BAT, ↑ PAC-1R expression in WAT	Cline et al. (2019)
Mice	Thermoneutrality and cold-exposure (3.5 weeks)	↑ VIP receptor (VPAC-1R) expression in both BAT and WAT	Cline et al. (2019)
Orexin-null mice	N/A	↓ Energy expenditure	Hara et al. (2001)
Orexin-null mice	N/A	↓ Lipid content, ↓mitochondrial content, and ↓ UCP1 mRNA expression in BAT. ↓ Ppar- 1, Pgc-1 , and Pgc-1 mRNA levels in BAT preadipocytes—all phenotypes rescued by orexin administration	Sellayah et al. (2011)
Mice	[Ala11, D-Leu15]-OxB (OB-Ala)	Microinjection into BAT = ↓ thermogenic gene expression (UCP1, Ppar- 1, and Cidea) in BAT. Chronic Ob-Ala treatment ↓ TH immunopositivity and -3 adrenoceptor expression in BAT. OX2R expression only seen in BAT DRG neurons.	Jia et al. (2022)

cAMP, cyclic adenosine monophosphate; DGAT-1, diacylglycerol O-acyltransferase 1; FABP4, fatty-acid binding protein 4; IP_3, inositol-1,4,5-triphosphate; mRNA, messenger ribonucleic acid; PIPLC, phosphatidylinositol-specific phospholipase C; UCP1, uncoupling protein 1.

and Majcherczyk, 1982; Pierau and Wurster, 1981). Our understanding of sensory nerve structure and function, however, largely relies on studies assessing sensory innervation of the skin (Hsieh et al., 1997) and various end-terminal structures and sensory nerve subtypes have been mapped to their stimuli and responses in this tissue. In the skin, sensory innervation patterns and sensory nerve functions have been well characterized. For other tissues, particularly metabolic tissues like adipose, sensory nerve knowledge remains nascent. Likely, each tissue and organ in the body has unique structures to allow for specialized interoceptive stimuli, but the field is far from understanding these tissue and organ-specific specializations. Recent work deciphering the identity and diversity of nerve fiber subtypes in adipose tissues by us (Willows et al., 2021, 2022) and others (Cao et al., 2018; Zhang et al., 2018) has provided new evidence for the existence of nerves with myelination patterns and neuropeptide expression unique from those observed in the skin, as well as peptidergic sensory nerve subtype in adipose (Willows et al., 2021). We have also identified the first nerve terminal structure in adipose, the neuro-adipose nexus (NAN) where nerve terminals containing synaptic markers and synaptic vesicles wrap around individual adipocytes on the surface of adipose depots (Willows et al., 2021). NANs may represent a specialized sensory nerve ending prone to form around a subset of tissue surface adipocytes, but further validation is needed to determine how these structures are formed and function within the tissue.

Peptidergic vs Nonpeptidergic Sensory Innervation in Adipose Tissues

In recent years, advancements in whole-mount immunofluorescent (IF) labeling and confocal imaging techniques have enabled the mapping of innervation patterns in adipose depots (Fig. 1). These techniques take advantage of nerve-specific proteins, neuropeptides, and receptors to identify and distinguish sensory nerve subtypes. Firstly, sensory neurons can be divided into peptidergic (ie, releasing neuropeptides) vs nonpeptidergic subpopulations. In mouse DRG, peptidergic neurons synthesize neuropeptides like CGRP and SP and persistently express the nerve growth factor receptor neurotrophic receptor tyrosine kinase 1 (NTRK1) (Bennett et al., 1996; Rosenfeld et al., 1983). Conversely, nonpeptidergic neurons are marked by glial-derived neurotrophic factor receptor Ret (Bennett et al., 1998) and purinergic receptor purinergic 2X3 (P2X3) (Vulchanova et al., 1998) expression, and bind isolectin B4 (Silverman and Kruger, 1988). These findings were in other tissues and have not yet been extended to adipose. Anatomically, peptidergic nerves mostly innervate skin and deeper tissues (Yang et al., 2013; Zylka et al., 2005), whereas nonpeptidergic nerves typically innervate the epidermis (Zylka et al., 2005).

Sensory Innervation Patterns in Adipose Tissues

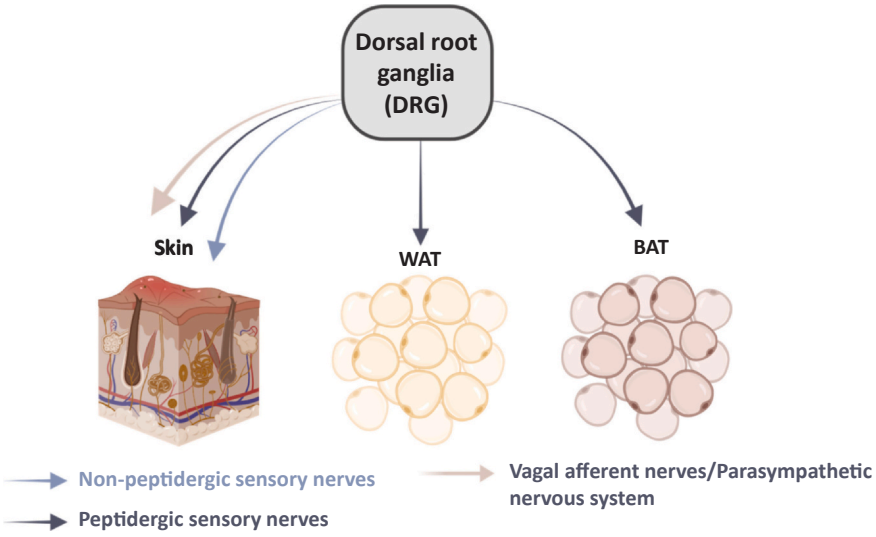
Sensory nerves are further distinguished by nerve-specific protein expression. While some markers like Nav1.8 (a voltage-gated sodium channel) and Advillin (Avil) are considered pan-sensory (ie, expressed in all sensory nerves), CGRP/SP and P2X3 mark peptidergic and nonpeptidergic sensory nerves, respectively (Akopian et al., 1996; Bird et al., 2013; Hunter et al., 2018). However, both Nav1.8 and Avil have limitations in terms of their pan-sensory expression. Avil expression was recently reported in isolectin-binding 4 (IB4)-binding nonpeptidergic axons originating from DRG in mice, with low to no expression within the DRG itself, by immunoreactivity (Hunter et al., 2018), suggesting preferential localization of Avil in sensory processes. In contrast, Nav1.8 expression is well documented in both the DRG (Hunter et al., 2018; Ye et al., 2016) and sensory projections (Ramachandra et al., 2013) in rodents. Moreover, Avil expression was inversely co-related with cell size in the DRG (Chuang et al., 2018), as measured by Avil immunofluorescence, challenging the assumption that all sensory neurons uniformly express Avil. Nav1.8 expression, however, was observed in both large and small sensory afferent neurons of rats by immunocytochemistry (Ramachandra et al., 2013), suggesting Nav1.8 expression is more pan-sensory than that of Advillin.

Whole-mount confocal IF imaging was used to demonstrate that sensory nerves in mouse WAT travel either as part of a large nerve bundle with mixed nerve types along the vasculature or as smaller parenchymal axons that likely branch from these larger bundles (Wang et al., 2022; Willows et al., 2021). Whole-mount immunostaining of ing-scWAT from sensory nerve reporter mice (*Nav1.8-Cre x TdTomato*) validated the presence of small, distinct sensory axons innervating adipocytes in the parenchyma, and also supported earlier findings of sensory fibers within larger nerve bundles in ing-scWAT. Large nerve bundles in ing-scWAT of mice contained a small subset of Avil + fibers, but no Avil + IF was seen in small fiber axons (only tyrosine hydroxylase [TH]+ fibers were observed) traversing the depots (Willows et al., 2021). Until recently, TH (a classical marker for sympathetic axons and the rate-limiting step for catecholamine production, including NE) expression was considered strictly restricted to sympathetic nerves in adipose (Wang et al., 2022). Recently, however, Wang et al. (2022) reported a 40% overlap in thin fiber sensory afferents with TH immunopositivity in mouse DRG innervating ing-scWAT, thus challenging the belief that all TH+ fibers are purely sympathetic in nature (Wang et al., 2022). This finding draws into question the conclusions of many prior studies that have relied on TH to mark sympathetic nerves, without confirming changes to sympathetic nerve products like NE or co-staining with sensory nerve markers like CGRP. Lastly, it is also important to note that Willows et al. (2021) reported Avil + vascular proteins, thereby disqualifying Advilin as a distinct sensory nerve marker (Willows et al., 2021) in scWAT.

Myelination Patterns of Vascular and Parenchymal Sensory Innervation in Adipose Tissues

Myelination patterns further distinguish sensory nerve subtypes and may provide insight into their adipose-specific functions. In spite of the historical view that sensory neuropeptides mainly control vasomodulation, the notion that distinct, parenchymal sensory nerves exist and innervate adipocytes was not uncovered until the 1990s (De Matteis et al., 1998; Giordano et al., 1996). Thick, mixed nerve bundles containing both myelinated and unmyelinated fibers were also immunohistologically labeled

A)



B)

Peptidergic sensory nerves

Markers	Expression in PNS	Tissue innervated (Peripheral)	Fiber type	Citation
Calcitonin Gene-Related Peptide (CGRP)	DRG	Skin, WAT and BAT	C/Aδ	PMID: 8377941 PMID: 2352644 PMID: 17348016 PMID: 7551174 PMID: 15550613 PMID: 33412345 PMID: 26010480 PMID: 2459317
Substance P (SP)	DRG	Skin, WAT and BAT	C/Aδ	PMID: 17986814 PMID: 2459317 PMID: 9157365 PMID: 2469036 PMID: 10025074
Pituitary adenylate cylase polypeptide (PACAP)	DRG; Receptor PAC1 expressed in BAT and WAT	Skin, WAT and BAT – expression not reported	C/Aδ	PMID: 29982965 PMID: 20889562 PMID: 7508577
Orexin	Receptor OX2R expressed in BAT	BAT	Not reported	PMID: 21982708 PMID: 35660653

Non-Peptidergic sensory nerves

Markers	Binds	Tissue innervated	Fiber type	Citation
Ret (Glial-derived neurotrophic factor receptor)	Isolectin B4	**Skin** (predominantly epidermis)	C	PMID: 28728214 PMID: 17952660
P2X purinoceptor 3 (ATP-gated ion channel)				

(caption on next page)

Fig. 1. Comparison of sensory innervation in skin vs white and brown adipose tissues. (A) Skin, white adipose tissue (WAT), and brown adipose tissue (BAT) receive sensory innervation from the dorsal root ganglia (DRG). Skin receives both peptidergic and non-peptidergic innervation, whereas WAT and BAT receive peptidergic innervation. (B) Summary of peptidergic and non-peptidergic sensory innervation of skin and adipose tissues. ATP, adenosine triphosphate.

with CGRP and SP in brown adipose tissue (BAT) of rats (De Matteis et al., 1998). The observation of CGRP+ parenchymal nerves in the same study first revealed a sensory nerve population in BAT that was distinct from CGRP+ nerves innervating the vasculature, suggesting possible roles for CGRP beyond vasodilation in adipose tissues (De Matteis et al., 1998). Conversely, in rat BAT only unmyelinated nerves contained TH (De Matteis et al., 1998), however, whether these were truly sympathetic or sensory in nature remains unclear.

Although direct evidence for myelinated sensory nerves in WAT was lacking, studies examining sympathetic nerve myelination patterns in WAT have provided useful insights. Myelin protein zero (MPZ) and myelin basic protein (MBP) both mark the peripheral myelin sheath (D'urso et al., 1990; Willows et al., 2023). MPZ-IF was restricted to smaller, TH+ axons in ing-scWAT of mice, which suggested that all parenchymal nerves were solely sympathetic and myelinated (Willows et al., 2021). In a follow-up study, Willows et al. (2023) observed co-labeled TH and MBP fibers in ing-scWAT of a fluorescently-tagged-PGP9.5 mouse and confirmed myelinated axons by electron microscopy (Willows et al., 2023). Moreover, MPZ+ nerves in mouse scWAT ran in parallel and in close proximity to IB4-marked vasculature, as evidenced by whole-depot confocal imaging (Willows et al., 2021). Sensory neuropeptides exhibit vasomodulatory properties (Brain et al., 1985; Newby et al., 1999), as do sympathetic nerve products (Nette et al., 2006; Vatner et al., 1985), and thus likely both nerve types innervate adipose vasculature (Frei et al., 2022). Additional CGRP (or another sensory marker) and MPZ/MBP costaining will be required to confirm whether myelinated sensory nerves innervate scWAT, and along which anatomical structures (such as vasculature) or cell types.

A Note: Parasympathetic Innervation of Adipose Tissues

The parasympathetic nervous system (PSNS) is classically understood to act in opposition to the sympathetic response in peripheral tissues (Tindle and Tadi, 2023). The consensus on PSNS in WAT, however, suggests that these nerves are low or absent, as measured by cholinergic markers that are specific to parasympathetic nerves (Giordano et al., 2006; Kreier et al., 2002; Vaughan and Bartness, 2012). These include the excitatory neurotransmitter acetylcholine, the enzymes responsible for its transport (vesicular acetylcholine transporter [VAChT]) or degradation (acetylcholinesterase), and vasoactive intestinal peptide (VIP), a neuropeptide normally synthesized in the parasympathetic ganglia, that is also expressed in some sensory ganglia (Zhang et al., 1995). scWAT, epididymal WAT, and retroperitoneal pads of rats or mouse strain (C57BL/6) and black tan brown (mouse strain) (BTBR) *ob/ob* mice lacked any cholinergic PSNS marker expression and VIP immunoreactivity. Together, these data strongly point to the lack of PSNS innervation in WAT. It is currently unknown if this translates to human adipose tissues. Interestingly, recent work in the adipo-neuroimmunology field has identified a subset of acetylcholine-producing macrophages in murine subcutaneous fat that promote thermogenesis in beige adipocytes (Jun et al., 2018; Knights et al., 2021). Mice expressing green fluorescent protein (GFP) under transcriptional regulatory element for choline acetyltransferase (ChAT), the rate-limiting enzyme mediating acetylcholine biosynthesis, had GFP+ cells in stromal vascular fraction (SVF) isolated from inguinal scWAT. Additionally, cold exposure augmented both acetylcholine and ChAT levels in SVF from inguinal scWAT, as measured by the median fluorescence intensity (Jun et al., 2018). Similarly, differentiated pre-adipocytes treated with freshly isolated SVF had enhanced thermogenic gene expression (uncoupling protein 1 (UCP1), Iodothyronine deiodenase 2 (Dio2)), both of which remained upregulated after acetylcholinesterase antagonist treatment, suggesting that SVF-derived acetylcholine was potentially, in part, responsible for the enhanced thermogenic profile observed. In a following study, macrophages (termed cholinergic adipose macrophages [ChAMs]) were identified as the source of SVF-derived acetylcholine using fluorescence imaging and fluorescence-activated cell sorting (FACS) sorted cells from ChAT-enhanced green flurorescent protein (eGFP+) reporter mice (Knights et al., 2021). Mice with macrophage-specific ChAT ablation (*ChAT* $^{fl/fl}$; *LysM-Cre*) had impaired cold-induced thermogenesis, suggesting macrophage-derived acetylcholine may be required for cold-induced thermogenesis in beige adipocytes. Therefore, the lack of parasympathetic innervation in scWAT may be accounted for by ChAMs. However, how sensory neuropeptide activity impacts ChAM activation, and where crosstalk potentially occurs, remain unknown. The significance of immune-cell-derived neurotransmitters in adipose remains unclear, and it is also uncertain if most of the neurotransmitter content of phagocytic immune cells may be due to the uptake and clearance of neuronal products.

The data on PSNS in BAT tells a slightly different story. Histochemical analysis revealed that acetylcholine and active acetyl-cholinesterase were absent in interscapular BAT (Bryant et al., 1983). However, 2 smaller BAT depots, the mediastinal (Giordano et al., 2004) and pericardial (Schafer et al., 1998), contained VAChT+ perivascular and parenchymal innervation. However, the absence of VIP-immunopositivity in these fibers suggested that while these smaller BAT depots contained putative PSNS innervation, these nerves may not necessarily be cholinergic (Giordano et al., 2004). Based on a study that noted elevated lipolysis in human scWAT adipocytes after VIP treatment in vitro (Richter et al., 1989), VIP simply may not be needed in tissues with relatively lower levels of lipolysis that may need to be turned on and off as regularly, such as BAT. Therefore, a subset of PSNS nerves seems unique to smaller BAT depots, and whether the lack of VIP is compensated for by other sensory, sympathetic, or parasympathetic neurotransmitters and neuropeptides requires additional data.

SENSORY NEUROPEPTIDES

Sensory neuropeptides are synthesized, stored, and released by sensory neurons, and can act in an autocrine, paracrine, or endocrine fashion (Van Den Pol, 2012). Neuropeptides are initially synthesized within the cell soma and stored in large dense core vesicles in axon terminals until their release. Centrally synthesized sensory neuropeptides often act as neuromodulators, enhancing or dampening the activity of co-stored neuropeptides (Merighi, 2002) or co-released neurotransmitters (Gibbins et al., 1985; Russo, 2017) to modulate synaptic signaling, in addition to acting as signaling molecules themselves (reviewed in Mendel et al. [2020]). In contrast, peripherally synthesized neuropeptides behave as signaling molecules (Schlereth et al., 2016) and bind to surface receptors on target tissues, other peripheral nerves, and cells in the ganglia. Additionally, centrally synthesized sensory neuropeptides may act on peripheral tissues (and vice versa) through the endocrine circulation, as neuropeptides can cross the blood-brain barrier (Zhang et al., 2002). However, how changes in central levels of sensory neuropeptides impact sensory neuropeptide signaling in the periphery, and vice versa, remains poorly explored.

Sensory Neuropeptides and Their Metabolic Functions in Adipose Tissues

Our current understanding of sensory nerves and their neuropeptides is largely derived from studies in the skin (Fig. 1). Based on these studies, neuropeptides are primarily synthesized in Aδ-type and C-type sensory neurons in the DRG (Brain and Cox, 2006), and they are released from tissue-innervating afferent terminals upon sensory nerve stimulation (Chen et al., 2010; Lawson et al., 2002; Mccarthy and Lawson, 1990). Sensory nerves, depending on the subtype and modality, can be stimulated by chemical, mechanical, or other signals. In adipose, these stimuli are just starting to be understood (Mishra and Townsend, 2023). Sensory neuropeptides are primarily known for their vasomodulatory, pro-inflammatory, and neuromodulatory effects (Brain, 1997; Russo, 2017; Smillie and Brain, 2011; Vollbracht and Rapoport, 2014).

The prominence of CGRP+ and SP+ fibers in BAT and WAT (Liu et al., 2019; Makwana et al., 2021; Shi and Bartness, 2005; Song, 2007) makes adipose tissues a highly relevant site for neuropeptide action. Beyond sensory neuropeptide ligands, receptors for CGRP, SP, and pituitary adenylate cyclase-activating polypeptide (PACAP) and those for sympathetic (NE) and parasympathetic (VIP, VAChT) neurotransmitters have also been identified in mouse WAT and BAT (Cline et al., 2019). Similarly, orexin (a sympathetic neuropeptide in the CNS) receptors were observed on vagal afferent neurons in the nodose ganglia in humans (Burdyga et al., 2003) and sensory afferents in BAT of cold-exposed mice (Jia et al., 2022) (Fig. 2).

Whether or not other neuropeptides are released in adipose remains unclear. Historically, sensory nerves and their neuropeptides have been understudied in adipose due to multiple technical reasons. First, these small-sized neuropeptides are transcribed in the DRG of sensory nerves innervating adipose tissues (and thus, little to no messenger ribonucleic acid (mRNA) transcripts exist in the axons innervating adipose depots itself), which requires protein-level assessments that are less often employed by adipose researchers. Additionally, low-level mRNA neuropeptide transcripts are notoriously undersampled in single cell ribonucelic acid sequencing (scRNAseq) and single nuclei ribonucleic acid sequencing (snRNAseq) studies, making sensory neuropeptides difficult to detect in unbiased omics studies. Secondly, due to their small size, sensory neuropeptides remain obscured in typical proteomics and western blot studies as well, instead requiring peptidomics or enzyme linked immunosorbent assay (ELISA) assays. Finally, neuropeptides have a short half-life in the tissue, lasting less than an hour (and only seconds for some that are more quickly cleared or degraded, like SP) (Kraenzlin et al., 1985; McGregor and Bloom, 1983), making their transient nature challenging to capture experimentally. Taken together, technical and methodological limitations have likely hampered the study of sensory innervation in adipose depots previously. Thus, despite their known presence in the tissue, sensory neuropeptide mechanisms in adipose remain poorly understood (De Matteis et al., 1998; Giordano et al., 1996; Himms-Hagen et al., 1990). Recent observations of metabolic dysregulation from targeted sensory denervation of adipose with subsequent sensory neuropeptide loss have revived interest in clarifying their roles in adipose tissues, and determining what sensory neuropeptide functions are distinct from those mediated by sympathetic nerves (Bartness et al., 2010a, 2010b; Bartness and Kay Song, 2005; Harris, 2018, 2012; Makwana et al., 2021; Nguyen et al., 2018; Wang et al., 2022; Youngstrom and Bartness, 1998).

Calcitonin Gene-Related Peptide

CGRP was first identified in 1982 as a 37-amino acid vasodilatory neuropeptide synthesized by the calcitonin gene (Amara et al., 1982). However, 2 distinct CGRP isoforms—*α*CGRP and *β*CGRP—exist, which are synthesized by either calcitonin A (*CALCA*) or calcitonin B (*CALCB*) genes, respectively. Although these isoforms share > 90% sequence homology, their distribution pattern suggests unique, tissue-specific functions that have not been fully deciphered to date (Russell et al., 2014). Sensory fibers in most central and peripheral tissues, including WAT and BAT, predominantly contain CGRP (Mulderry et al., 1985). Those in the gut, however, are largely CGRP + (Mulderry et al., 1985). Most studies assessing CGRP function in the context of energy balance regulation have focused on CGRP, but the possibility of additional CGRP isoforms existing in adipose and other peripheral tissues remains valid. Whether these isoforms bind and activate their receptor, the calcitonin-receptor-like receptor-receptor activity modifying protein 1 complex, on adipocytes is also not yet clear, although this receptor is present on adipocytes (Mishra and Willows, 2022). CGRP has a short half-life of 7 to 18 min on average in circulation (Kraenzlin et al., 1985) before it undergoes endosomal degradation by endothelin-converting enzyme-1 (Padilla et al., 2007). Tissue-level turnover may vary.

Some studies have linked CGRP to the pathophysiological progression of metabolic diseases like obesity. Women with obesity had significantly elevated serum CGRP and were more sensitive to serum CGRP spikes after isocaloric high-fat meals (Zelissen et al., 1991). Thus, the potential therapeutic capacity

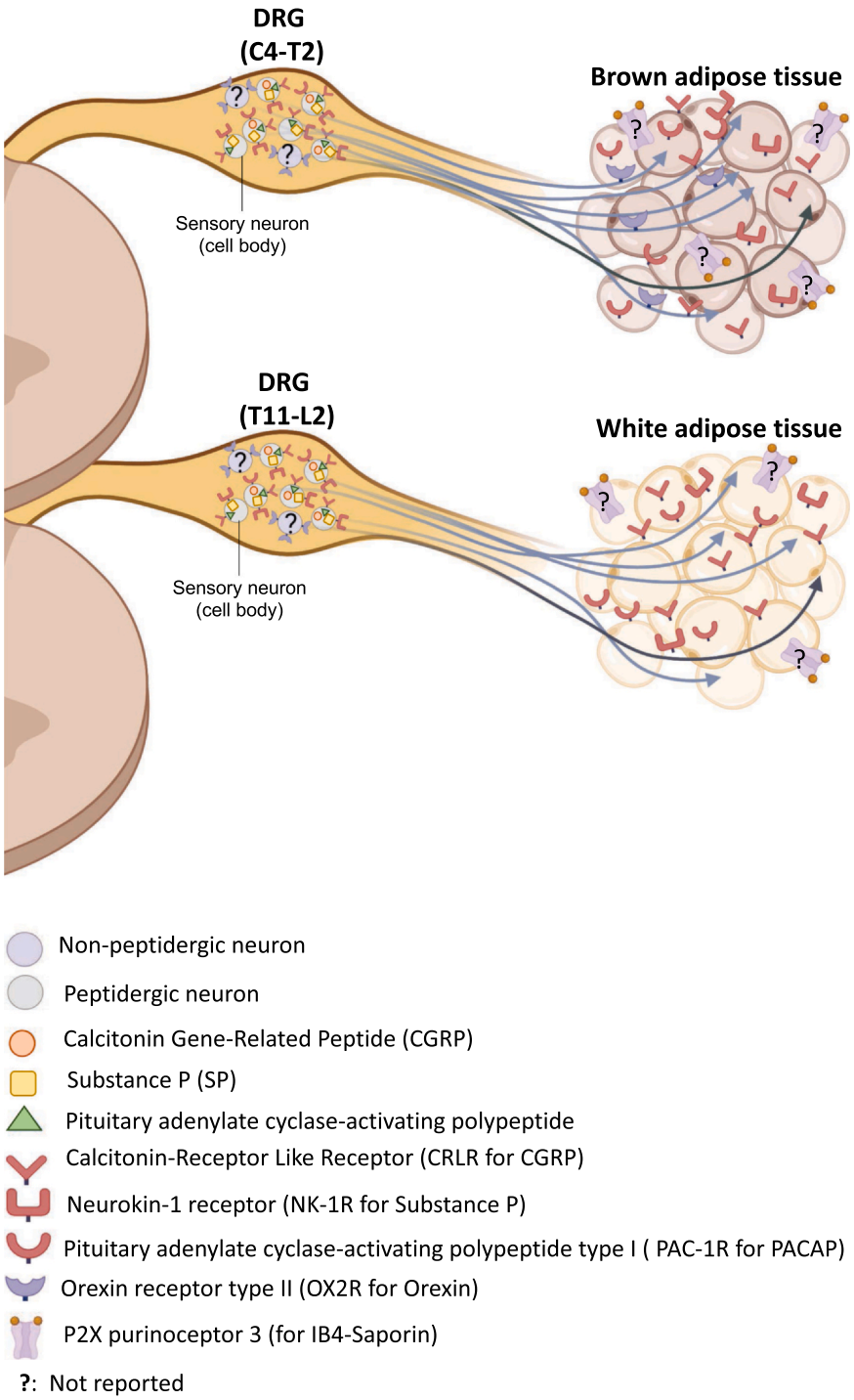


Fig. 2. Sensory neuropeptides and receptor distribution across dorsal root ganglia (DRG) and adipose tissues. Sensory neurons innervate brown adipose tissue (BAT) from C4-T2 DRG (in Siberian hamsters) and white adipose tissue (WAT) from T11-L2 (in mouse; T13-L1 in rats, T13-L3 in Siberian hamsters). (*Top*) Sensory neurons in the DRG innervating BAT express the neuropeptides CGRP, SP, PACAP, as well as their receptors CRLR, NK-1R, and PAC-1R. OX2R is also expressed on sensory neurons innervating BAT. Whether P2X3+ nonpeptidergic sensory nerves innervate BAT is not reported. CRLR, NK-1R, and PAC-1R are expressed in BAT. (*Bottom*) Sensory neurons in the DRG innervating WAT express the neuropeptides CGRP, SP, PACAP, as well as their receptors CRLR, NK-1R, and PAC-1R. Whether P2X3+ nonpeptidergic sensory nerves innervate WAT is not reported. CRLR, calcitonin-receptor-like receptor.

of anti-CGRP monoclonal antibodies has been explored in rodent models of obesity and diabetes (Halloran et al., 2020). Anti-CGRP monoclonal antibodies treatment ameliorated diabetes pathophysiology by improving glucose tolerance and insulin resistance, and reducing total adiposity, serum free fatty acid (FFA), and liver triglyceride content in BL6-db/db diabetic

mice (Halloran et al., 2020). In the same study, High-fat diet-induced obese mice receiving the same treatment had higher energy expenditure and lower total adiposity (Halloran et al., 2020). Collectively, CGRP is involved in adipose and whole-body metabolic regulation. Yet, any noted effects of CGRP in these studies could be attributed to plasma CGRP rather than secreted from sensory nerves innervating adipose depots. Thus, specific investigations on adipose actions of CGRP would clarify how this neuropeptide impacts this metabolic tissue directly.

Both in vivo and in vitro studies have examined CGRP's impacts on energy balance. In vitro studies support CGRP's lipolytic action in adipose-tissue-derived cell lines (Mishra and Willows, 2022) whereas the data on CGRP-mediated adipose regulation in vivo remains inconclusive. CGRP stimulated cyclic adenosine monophosphate (cAMP) production (a measure of lipolysis) at 10-fold greater half-maximal effective concentration (EC_{50}) than NE in 3T3-L1 adipocytes (Chatzipanteli et al., 1996), suggesting CGRP-induced lipolysis may occur independently of NE in vitro. Recently, enhanced extracellular FFA release without changes in cAMP production was measured in CGRP-treated 3T3-L1 adipocytes, suggesting CGRP may promote lipolysis through distinct pathways (Walker et al., 2014). An in vivo study using exercise-trained rats intravenously infused with CGRP supported this finding (Aveseh et al., 2018). CGRP upregulated the lipolytic pathway phosphatidylinositol-specific phospholipase C (PIPLC)/inositol-1,4,5-triphosphate (IP_3) in epididymal white adipose tissue (eWAT) of exercise-trained, but not sedentary or CGRP8-37 (CGRP receptor antagonist) treated rats (Aveseh et al., 2018), confirming CGRP-induced lipolysis through an cAMP-independent pathway. However, whether these outcomes occur in scWAT depots was not explored. Taken together, these findings collectively suggest that CGRP may be important for adipose tissue lipolysis, albeit by unique pathways distinct from NE.

CGRP+ neurites are expressed in adipose depots, including at the neuro-adipose nexus (NAN), and contribute to adipose functions such as lipolysis (Mishra and Willows, 2022), and may contribute to vasodilation in adipose to facilitate FFA clearance, although this has not been directly reported.

Substance P

Von Euler and Gaddum first identified SP in the horse brain and gastrointestinal tract in 1931 (Us and Gaddum, 1931). This 11 amino acid compound was later isolated in powder form (thus named SP) from the bovine hypothalamus in 1971 (Chang and Leeman, 1970). SP belongs to the tachykinin peptide hormone family and is 1 of the 4 neuropeptides encoded by the TAC1 gene (the rest being neurokinin A, neuropeptide K, and neuropeptide ɣ). Nociceptive neurons with SP-immunoreactivity were first observed in the spinal cord and the hind-paw skin of cats in 1975, and since then SP has been widely used as an immunohistological marker for C-fiber sensory nerves (Hokfelt et al., 1975). SP is expressed in both neuronal (Barker and Larner, 1992; Holzer, 1988; Pickel et al., 1977) and non-neuronal cells (Milner et al., 2004; Watanabe et al., 2002), with significant expression in multiple immune cells (Lai et al., 1998; Lambrecht et al., 1999; Weinstock et al., 1988). SP modulates neuroimmune crosstalk and aids in immune cell recruitment (Leonard et al., 2014; Mashaghi et al., 2016; Sideri et al., 2015), nociception (Li et al., 2012), angiogenesis, and vasodilation (Fan et al., 1993). Interestingly, SP may modulate CGRP function by being co-released from sensory nerve terminals in both peripheral and central tissues (Greco et al., 2008; Ohlen et al., 1987; Schlereth et al., 2016), although this has not been shown in all SP- and CGRP-expressing neurons, including those innervating adipose tissues. In adipose, SP binds to its high-affinity receptor neurokinin-1 receptor (NK-1R) and retains the ability to bind both neurokinin-2 and -3 in other neurons and immune cells (Ho et al., 1997; Karagiannides et al., 2006).

SP first became relevant in the context of energy balance and metabolism in the late 1980s. Obese children (Baroncelli et al., 1989) and adults (Fu et al., 2011) with type II diabetes had elevated SP levels in circulation. Mechanistic insights into SP function in adipose tissues, however, remain sparse, with only one in vitro and in vivo study to date. After SP treatment in culture, 3T3-L1 adipocytes had lower lipid deposition, which was associated with lower fatty-acid binding protein-4 and diacylglycerol *O-acetyltransferase-1* mRNA expression, reduced FFA uptake, and increased lipolysis and lipid export gene CD36 expression (Miegueu et al., 2013). Taken together, these indicate SP promotes a negative energy balance state in adipocytes. However, pharmacological antagonism of NK-1R by CJ 12,255 (ip) prevented weight gain in lean mice fed a high-fat diet, mainly by stimulating loss in fat mass, lowering food intake, and depressing circulating leptin levels (Karagiannides et al., 2008). In stark contrast to in vitro findings discussed earlier, this in vivo study deemed SP as an orexigenic neuropeptide that would increase fuel storage. However, given that NK-1R antagonism was systemic rather than adipose-tissue specific, and NK-1R is expressed across numerous peripheral and central tissues, these outcomes cannot be solely attributed to SP signaling in adipose tissues.

VIP and PACAP

VIP, a 28-amino acid peptide, was first isolated in 1970 from porcine duodenum by Said and Mutt (1970) and was soon characterized as a potent vasodilator due to its ability to enhance peripheral blood flow in vascular beds while simultaneously decreasing arterial blood pressure (John et al., 1972; Said and Mutt, 1972). VIP is expressed in the CNS (Fahrenkrug, 1993) and the PNS (Baldwin et al., 1991; Sasek et al., 1991). In the CNS, VIP modulates circadian rhythms, and based on rodent studies, may serve as a neuroprotective agent in CNS pathologies such as stroke (Yang et al., 2015) and Parkinson's disease (Delgado and Ganea, 2003). Within the PNS, VIP expression is largely restricted to cholinergic parasympathetic nerves (Lundberg et al., 1980) and is most prominently recognized for inducing pancreatic insulin, glucagon, and somatostatin secretion (Szecowka et al., 1980), in addition to its vasodilatory properties.

PACAP was first isolated from the bovine hypothalamus in 1989 (Miyata et al., 1989). The ADCYAP1 gene encodes PACAP (Hosoya et al., 1992), and post-translational processing of the peptide can result in either a 27- or 38-amino acid-long peptide (Cardoso et al., 2020). Mammals predominantly

express PACAP38 (Arimura, 1992), but the core PACAP sequence is estimated to be evolutionarily conserved across avian, rodent, and mammalian species for ~700 million years (Hirabayashi et al., 2018). PACAP is widely expressed across the CNS, where it stimulates sympatho-adrenal activity (Farnham et al., 2019). In the CNS, PACAP expression is seen in both parasympathetic and sensory nerves (Tanida et al., 2010). Historically, PACAP was understood to mediate fat metabolism solely through its hypothalamic actions (Bozadjieva-Kramer et al., 2021). However, Cline et al. (2019) showed direct evidence of PACAP receptor (PAC-1R) expression in BAT and WAT by RNA-Seq and quantitative polymerase chain reaction (qPCR) (Cline et al., 2019). Cold-exposure (3.5 weeks) decreased PAC-1R expression in BAT but increased PAC-1R expression in ing-scWAT of mice (Cline et al., 2019). However, whether PACAP acting in BAT and WAT was centrally or peripherally synthesized was not assessed. Moreover, qPCR confirmed VIP receptor (VPAC-1) expression in both BAT and ing-scWAT, both at thermoneutrality and post-cold exposure, albeit the expression levels remained low. However, VPAC1 was significantly higher in differentiated adipocytes compared to preadipocytes from BAT, suggesting the involvement of this neuropeptide in the maturation of thermogenic adipocytes (Cline et al., 2019). The lack of knowledge of these 2 neuropeptides in adipose could again be a direct consequence of the experimental and technical hurdles, as outlined above. Taken together, although VIP and PACAP receptors have been identified in preadipocytes and adipocytes, respectively, no evidence for VIP/PACAP expression in sensory afferents innervating adipose tissues exists till date.

Orexin

Orexins (or hypocretins) were first identified in 1998 in rat hypothalamus (De Lecea et al., 1998; Sakurai et al., 1998), specifically in regions associated with feeding behavior like the lateral hypothalamic area, paraventricular nucleus, and the arcuate nucleus. Orexin A and Orexin B are 33 and 28-amino acid long, respectively. While orexin A binds both orexin receptor 1 (OX1R) and 2 (OX2R), orexin B only binds OX2R (Sakurai et al., 1998).

Although centrally administered orexin enhanced sympathetic outflow, physical activity, metabolic rate, and thermogenesis in rodents, pharmacological antagonism or genetic ablation of orexin receptors in rodents resulted in reduced thermogenesis (Verty et al., 2010) and energy expenditure (Applegarth and Ross, 1975). These discrepancies may stem from differences in the experimental approaches. For instance, Verty et al. (2010) employed pharmacological antagonism of orexin receptors in rat hypothalamus alone without blocking orexin activity in other peripheral tissues, thereby informing a hypothalamic-specific role of orexin. In contrast, systemically ablating the orexin receptor, as done by Applegarth and Ross (1975), lacks tissue specificity regarding orexin functions, thereby challenging the interpretation of orexin roles in adipose tissues.

In adipose, specifically BAT, orexin-null mice had impaired thermogenesis as measured by reduced lipid and mitochondrial content in BAT, as well as reduced UCP1 expression (Sellayah et al., 2011). These outcomes were linked to the inability of brown adipocytes to differentiate, as marked by reduced expression of transcriptional regulators *Ppar- 1, Pgc-1 ,* and *Pgc-1* mRNA levels in BAT preadipocytes (but not other peripheral tissues) from orexin-null mice. Orexin administration in orexin-null neonatal mice rescued these outcomes, confirming the need for orexin for BAT differentiation and thermogenesis (Sellayah et al., 2011). More recently, however, orexin-induced thermogenesis in BAT was challenged by Jia et al. (2022), who observed a reduction in BAT thermogenesis after OX2R agonist [Ala11, D-Leu15]-OxB (OB-Ala) micro-injection into BAT in mice (Jia et al., 2022), as measured by reduced thermogenic gene expression (UCP1, *Ppar- 1*, and *Cidea*) in BAT. This was accompanied by reduced expression of -3 adrenoceptor, the receptor for the sympathetic neurotransmitter NE, as well as lower immunopositivity for TH (the rate-limiting enzyme for catecholamine production, including NE, and a marker for sympathetic nerves and their activation state) in BAT. This was observed after chronic OB-Ala administration in BAT, suggesting OX2R agonism may reduce sympathetic innervation. Furthermore, although OX2R mRNA expression itself was absent in BAT and ing-scWAT, primary DRG neurons expressed OX2R (by qPCR and in situ hybridization by ribonucleic acid-scope (RNAscope)), and 6-hydroxyopamine (6-OHDA)-mediated sympathetic denervation of BAT reduced TH but not OX2R levels (measured by western blot), confirming that OB-Ala effects on thermogenesis are mediated by its action on DRG afferents in BAT. However, whether orexin itself is synthesized peripherally remains unclear.

CONCLUSION

The nervous system has evolved with numerous effectors (neuropeptides, neurotransmitters) that can act on the nerves themselves, or on support cells or target tissues. These effectors may also be modulating/modifying or inhibiting other effector molecules. This derives exquisite complexity in nervous system control of metabolism, including sensory neuropeptide actions in adipose, but the specific functions of adipose neuropeptides are just beginning to be unraveled. Specialized sensory nerve terminals in BAT or WAT, neurovascular interactions, sensory-sympathetic nerve crosstalk, and interoceptive stimuli that activate sensory axons and stimulate neuropeptide release are all active areas of ongoing investigation that should yield new discoveries and a deeper understanding of the role of this arm of the PNS in energy balance and metabolic health.

AUTHOR CONTRIBUTIONS

Gargi Mishra (first) and Kristy L. Townsend (corresponding) are the sole authors of this manuscript.

DECLARATION OF COMPETING INTERESTS

The authors declare no conflict of interest.

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