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Anatomy and Function of Autonomic Innervation of the Liver

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ABSTRACT: The liver is one of the most important regulatory organs, and its extensive influence upon homeostatic balance is well-documented. However, the role of autonomic innervation in the control and regulation of hepatic function is poorly understood relative to the other organs of the digestive system. Furthermore, the neuroanatomical layout of the liver remains an incomplete puzzle with various missing pieces. The scarcity of information concerning such a critical organ presents an interesting conundrum for the scientific and biomedical communities alike, especially given the prevalence of liver degeneracy and disease in the modern clinical setting. A more complete understanding of the precise anatomy of hepatic autonomic innervation, as well as the role it plays in both the normal function and diseased state of the organ, could aid in the development of novel and improved treatments for various debilitating and potentially lethal conditions. The purpose of this review is to compile the breadth of knowledge regarding hepatic autonomic innervation, to discuss the limitations of previous research, and to suggest future directions for a field that shows great potential for continued advancement.

KEYWORDS: liver; autonomic innervation; hepatic innervation

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INTRODUCTION

The liver is a key regulatory organ of both glucose and lipid metabolism, and various studies have shown that autonomic innervation plays a role in the regulation of hepatic function (Akiyoshi, Gonda, & Terada, 1998; Berthoud, 2004; Kalsbeek et al., 2010; McCuskey, 2004; Perez-Tilve et al., 2010; Tunderaher, Farr, & Adeli, 2017; Verma, Antony, Ogunnaike, Hoek, & Vadigepalli, 2018; Yi, la Fleur, Fliers, & Kalsbeek, 2010). This liver-brain axis is under-studied, with many unknowns regarding its structure, function, and scope (Akiyoshi et al., 1998; Berthoud, 2004; Kalsbeek et al., 2010; McCuskey, 2004; Perez-Tilve et al., 2010; Taher et al., 2017; Verma et al., 2018; Yi et al., 2010). Additionally, in liver transplant cases, autonomic innervation of hepatic tissue is significantly reduced, yet the liver continues to function at a level that deviates only moderately from normal (Tiniakos, Lee, & Burt, 1996; Yi et al., 2010). The study of the liver-brain axis is further complicated by large anatomical differences in nerve distribution between rodents and higher mammals, particularly in the parenchyma (Akiyoshi et al., 1998; McCuskey, 2004; Verma et al., 2018). Despite these obstacles, the study of the neural map of the liver provides a link between the organ's many functions and the influence of the autonomic nervous system.

This review summarizes knowledge concerning the form and function of hepatic sensory and motor innervation acquired over the last five decades. Among the topics covered are the anatomical neural map of the rodent liver, which notably lacks intrahepatic ganglia (Berthoud, 2004; Berthoud and Neuhuber, 2000; Taher et al., 2017), as well as the influence of the sympathetic and parasympathetic nervous system upon various metabolic and regulatory pathways. While substantial evidence implicates the hypothalamus and autonomic nervous system in various modifications of liver activity, the precise mechanisms and interplay of the nervous, endocrine, and hepatic systems have yet to be fully elucidated. Understanding of the liver-brain axis is still far from complete, but technological advances may allow many of the topics covered here to be revisited and explored in far greater depth than was previously possible. These advances, in turn, could potentially lead to the discovery of novel, neural-based treatments for chronic liver disease.

BACKGROUND

The Neural Map of the Rat Liver

This discussion begins with analysis of the rat liver, the most well-studied of the mammalian livers. Although it provides the most complete picture of the anatomical layout and physiological role of autonomic innervation in the hepatic setting, the rat liver is far from the level of completion seen in the rest of the gastrointestinal tract. Despite these shortcomings, several major populations of neurons travelling between the sympathetic and parasympathetic centers and hepatic tissues have been identified. Sympathetic afferent innervation travels through the dorsal root ganglia to the dorsal gray horn of the spinal cord (Baik et al., 2017; Gardemann, Puschel, & Jungermann, 1992; Jensen, Alpini, & Glaser, 2013; Taher et al., 2017; Tiniakos et al., 1996). Vagal afferent innervation reaches the nucleus of the solitary tract via the nodose ganglion (Baik et al., 2017; Gardemann et al., 1992; Jensen et al., 2013; Taher et al., 2017; Tiniakos et al., 1996). Efferent innervation of the liver is achieved via two major motor plexuses. The anterior plexus is located at the hilum alongside the hepatic artery, while the posterior plexus is found near the portal vein and bile duct system (Baik et al., 2017; Gardemann et al., 1992; Jensen et al., 2013; Reilly, McCuskey, & McCuskey, 1978; Taher et al., 2017; Tiniakos et al., 1996). These plexuses carry parasympathetic fibers originating from the vagus, especially the dorsal motor nucleus (Baik et al., 2017; Gardemann et al., 1992; Jensen et al., 2013; Taher et al., 2017; Tiniakos et al., 1996). Intrahepatic ganglia, however, have not been found, with only nearby paraganglia displaying vagal innervation (Berthoud, 2004; Berthoud & Neuhuber, 2000; Taher et al., 2017). This omission is highly unusual for a major organ and presents a challenge for future studies that attempt to locate these missing ganglia. Sympathetic efferent neurons also innervate the liver, originating in the intermediolateral spinal column and passing through the celiac and superior mesenteric prevertebral ganglia (Baik et al., 2017; Gardemann et al., 1992; Jensen et al., 2013; Taher et al., 2017). Figure 1, seen below, gives a visual representation of the known paths between the Central Nervous System (CNS) and hepatic structures in the rat.

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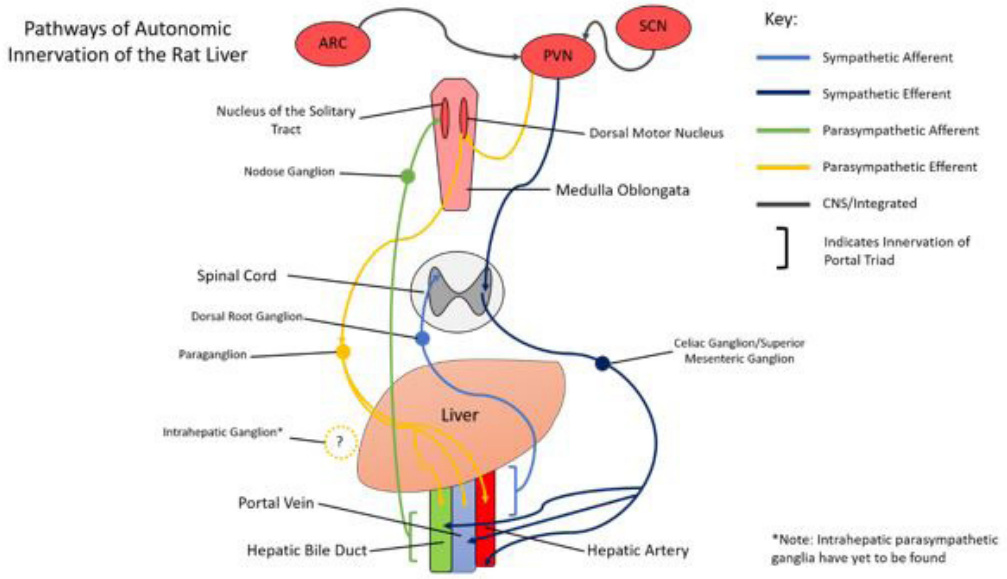

Figure 1: The origins and pathways of hepatic autonomic innervation in the rat liver.

Vagal afferent innervation reaches the NTS via the nodose ganglion. Vagal efferent innervation originates in the paraventricular nucleus (PVN), passes through the dorsal motor nucleus, and reaches the paraganglia before terminating primarily in the portal triad. Sympathetic afferents innervate the vessels and bile ducts, carrying sensory information to the dorsal horn of the spinal cord through the dorsal root ganglia. Sympathetic efferent innervation comes from the PVN and synapses in the celiac and superior mesenteric ganglia. The arcuate nucleus (ARC) and suprachiasmatic nucleus (SCN) exert CNS influence over the PVN, impacting both sympathetic and parasympathetic responses. Intrahepatic ganglia have not been found (Baik et al., 2017; Berthoud, 2004; Berthoud and Neuhuber, 2000; Gardemann et al., 1992; Jensen et al., 2013; Reilly, McCuskey & McCuskey, 1978; Taher et al., 2017; Tiniakos et al., 1996).

Afferent Tracing Techniques

Berthoud and others elucidated the afferent autonomic innervation of the rat liver using various tracing techniques. Retrograde and anterograde tracing methods have both been used to discover the origin and termination sites of vagal afferents (Berthoud, 2004; Kalsbeek et al., 2010). In the former, both horseradish peroxidase injections of the common hepatic branch of the vagus and True Blue injections of the hepatic artery and portal vein revealed fibers extending to the left nodose ganglion (Berthoud, 2004). Additional horseradish peroxidase injections of the hilum and bile duct regions revealed innervation in the right nodose ganglion, which is unusual because these nerves do not connect to the common hepatic branch (Berthoud, 2004). Anterograde tracing has been accomplished with DiI injections, but these studies have revealed that most of the common hepatic branch descends to innervate other gastrointestinal structures, such as the duodenum (Berthoud, 2004). Calretinin and P2X2/X3 vesicular glutamate transporter both show promise as more specific tracers, but neither have been extensively applied to the hepatic setting (Berthoud, 2004). Of the little innervation that reaches the liver proper, most is found around the bile ducts and portal triad (Akiyoshi et al., 1998; Berthoud, 2004; Kalsbeek et al., 2010). Neither technique has thus far revealed any hepatic vagal innervation in the rat parenchyma (Akiyoshi et al., 1998; Berthoud, 2004; Kalsbeek et al., 2010; McCuskey, 2004; Verma et al., 2018).

Researchers have achieved tracing of the spinal dorsal root ganglionic afferents via the use of calcitonin-gene-related peptide (CGRP) as a marker (Berthoud, 2004). HRP injections into liver tissue near intrahepatic bile ducts detected the presence of this substance primarily around the bile ducts, with very little innervation of the parenchyma (Berthoud, 2004).

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CGRP immunohistochemistry returned similar results (Akiyoshi et al., 1998; Berthoud, 2004).

Immunohistochemistry and Variability Between Species

Researchers have also used immunohistochemistry on other neuropeptide, neurotransmitter, and hormonal markers to map autonomic innervation in the livers of both rats and higher mammals. These studies have revealed more information regarding the innervation of the rat liver, but also how it differs from the innervation patterns in humans and other mammals such as the dog or guinea pig (Akiyoshi et al., 1998). Immunohistochemical techniques have revealed aminergic, peptidergic, and cholinergic innervation in the mammalian liver (Akiyoshi et al., 1998). The former two types of innervation were traced using various target substances, including tyrosine hydroxylase (TH), neuropeptide Y (NPY), substance P (SP), vasoactive intestinal polypeptide (VIP), calcitonin-gene-related peptide (CGRP), and galanine (GAL) (Akiyoshi et al., 1998; Burt et al., 1989). These markers showed the greatest response surrounding the portal system and bile ducts in the rat liver (Akiyoshi et al., 1998; Burt et al., 1989; McCuskey, 2004). Nevertheless, these forms of innervation were also present in all other species tested, indicating a highly conserved innervation pattern, at least in the case of these neurons (Akiyoshi et al., 1998; McCuskey, 2004). Acetylcholinesterase, used to indicate cholinergic innervation, gave a similar multi-species response (Akiyoshi et al., 1998; McCuskey, 2004). Higher mammals displayed TH, NPY, and SP innervation in the parenchyma of the liver, which is conspicuously absent in the livers of rats and other small rodents such as hamsters (Akiyoshi et al., 1998; Burt et al., 1989; Metz & Forssmann, 1980). Further confirmation of this trend has been provided by glyoxylic acid-paraformaldehyde fluorescent microscopy, especially of the guinea pig liver (Metz & Forssmann, 1980). The tree shrew, a small primate, demonstrates extensive adrenergic innervation extending out from the triads and into the lobules themselves (Forssmann & Ito, 1977). Researchers have theorized that this additional sympathetic innervation contributes to the function of larger livers (Azanza, Aisa, Junquera, & Castiella, 1989). The innervation itself is mainly found along the spaces of Disse surrounding the fat storing stellate cells (Forssmann & Ito, 1977; McCuskey, 2004; Streba, Vere, Ionescu, Streba, & Rogoveanu, 2014). Further study of the parenchymal innervation of higher mammals in both normal and pathological settings is required for a better understanding of this phenomenon. A promising target for future immunohistochemical techniques is PGP 9·5 (Lee, Ahmed, Hines, & Burt, 1992). This is a neuronal axoplasmic protein expressed by parenchymal nerves and shows potential for reliable marking (Lee et al., 1992).

Interestingly, in species displaying interlobular innervation, neural penetration of the parenchyma occurs very late in development (Kandilis, Papadopoulou, Koskinas, Sotiropoulos, & Tiniakos, 2015). Unlike other organs of the gastrointestinal tract, the liver does not receive innervation from the neural crest (Kandilis et al., 2015). The liver remains poorly innervated until near term, with only portal innervation present (Kandilis et al., 2015). Researchers have speculated that because the liver is mainly involved in hematopoiesis for much of its fetal life, the additional regulatory innervation for metabolic function would be unnecessary (Kandilis et al., 2015).

In rats and hamsters, portal innervation is believed to be the primary CNS control mechanism, a trait they share with less complex vertebrates such as frogs (Akiyoshi et al., 1998; Azanza et al., 1989; McCuskey, 2004). Where these small livers lack aminergic innervation in the parenchyma, gap junctions are found to propagate the electrical signal throughout the liver lobules (Beckh, Fuchs, Balle, & Jungermann, 1990; Jensen et al., 2013; McCuskey, 2004; Verma et al., 2018). Researchers have speculated that gap junctions and sympathetic innervation share an inverse relationship (Forssmann & Ito, 1977; McCuskey, 2004). Gap junctions in the rat liver consist of six connexin-32 units (Streba et al., 2014). Evidence for the function of these gap junctions is found in the propagation of intracellular Ca^{2+} responses, which requires non-unidirectional concentration gradients and cellular exchange (Verma et al., 2018). Ca^{2+} is vital for many functions of the liver, and monitoring of these responses in the live rodent model has revealed that they travel in a wavelike motion from the pericentral region (PC) region of the liver lobule to the periportal region (PP) (Verma et al., 2018). The fact that this propagation is opposite the blood flow of the liver suggests that there is zonation, or organized differences in cell signaling, among hepatocytes (Sluka et al., 2016; Verma et al., 2018). This zonation, however, is under-studied and not yet fully understood (Verma et al., 2018).

Challenges with Denervation Studies

While structural mapping of the rat liver has produced both successes and challenges, functional mapping of the

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liver may prove even more puzzling. The issues primarily arise from the close association of hepatic innervation to that of other areas of the gastrointestinal tract, as well as the difficulty in differentiating between varieties of hepatic afferents and efferents (Berthoud, 2004; Yi et al., 2010). Selective vagotomies, for example, are nearly impossible because the common hepatic branch of the vagus also innervates the duodenum, and any physiological changes that occur cannot necessarily be isolated to a hepatic cause (Berthoud, 2004). The use of this technique is therefore limited in reliability, but has been proven effective in the study of sympathetic efferent using toluidine blue labelling and pseudorabies virus (Berthoud, 2004). Selective vagal deafferentation and defferentation are also impractical methods in that the two types of fibers are extremely difficult, if not impossible, to separate. (Berthoud, 2004; Yi et al., 2010). Most afferent fibers elsewhere in the body are reliably denervated by treatment with capsaicin, which binds vanilloid receptors and triggers apoptosis (Berthoud, 2004). Hepatic vagal afferents, however, do not always respond to capsaicin treatment and thus there is no way of knowing if the deafferentation procedure was successful (Berthoud, 2004; Yi et al., 2010).

Selective sympathectomies and spinal deafferentation share similar issues, especially unwanted damage to nearby vagal pathways (Berthoud, 2004; Yi et al., 2010). Capsaicin is again an unreliable treatment, as spinal afferents run alongside other unrelated neurons in their journey to the dorsal root ganglia (Berthoud, 2004). Therefore, the effects of a supposed "spinal deafferentation" could be attributed to various other forms of neural damage and separate effector organ pathways. As previously mentioned, some successful studies of sympathetic innervation were carried out with toluidine blue and pseudorabies virus, but since only one such procedure has been performed successfully, the applications are extremely limited in scope (Berthoud, 2004).

In advanced species, interlobular innervation has been targeted with 6-OH dopamine (Burt et al., 1989; Forssmann & Ito, 1977). However, this technique is as unreliable as its counterparts, because it does not degrade all nerve fibers in the parenchyma, with only some fibers breaking down to myelin bodies (Forssmann & Ito, 1977). Furthermore, the denervation is not permanent, regenerating within 60-90 days (Forssmann & Ito, 1977).

The extreme difficulties in functional mapping of the hepatic system should be considered when discussing the specific functions of CNS innervation (Berthoud, 2004; Kalsbeek et al., 2010). While there is a general agreement as to the regulatory functions of many pathways, much of the CNS role in liver function is poorly understood, and knowledge is still evolving. Until more accurate methods of isolating liver innervation are discovered, there will most likely be a good deal of nervous function that remains under-studied.

The Hypothalamus: The Primary Brain-Liver Integration Center

The primary integration center of the liver, as with most organs under autonomic regulation, is the hypothalamus (Kalsbeek et al., 2010; Taher et al., 2017; Yi et al., 2010). This key regulatory center of the brain directly influences both lipid and glucose metabolism (Kalsbeek et al., 2010; Taher et al., 2017; Yi et al., 2010). The former process is controlled by the orexigenic and anorexigenic populations of the arcuate nucleus (ARC) that mediate very low-density lipoprotein (VLDL) production by the liver (Taher et al., 2017). The orexigenic populations secrete two neuropeptides that function antagonistically to one another (Taher et al., 2017). NPY increases hepatic VLDL production by stimulating the activity of stearoyl CoA reductase-1 (SCD-1), adenosine diphosphate (ADP) ribosylation factor-1 (ARF-1), and lipin-1, while galanine-like peptide (GALP) increases lipid metabolism by increasing the expression of the lipid transporters carnitine palmitoyl transferases (CTPs) I and II via sympathetic stimulation (Jensen et al., 2013; Rojas et al., 2015; Taher et al., 2017). This is an unusual function for an orexigenic pathway, but its presence allows the orexigenic center to both up and down-regulate lipid metabolism (Taher et al., 2017). The anorexigenic populations secrete pro-opiomelanocortin, the basis of a key lipid pathway in the liver-brain axis (Perez-Tilve et al., 2010; Taher et al., 2017). Pro-opiomelanocortin (POMC) stimulates second-order neurons to produce melanocortins 3 and 4 (MC3/MC4) (Perez-Tilve et al., 2010; Taher et al., 2017). Figure 2 provides a simplified version of the central melanocortin pathway. Blockage of this pathway reduces lipogenesis and high-density lipoprotein (HDL) uptake, and if left unchecked can lead to liver steatosis, the buildup of fat in liver tissue (Kandilis et al., 2015; Perez-Tilve et al., 2010; Taher et al., 2017).

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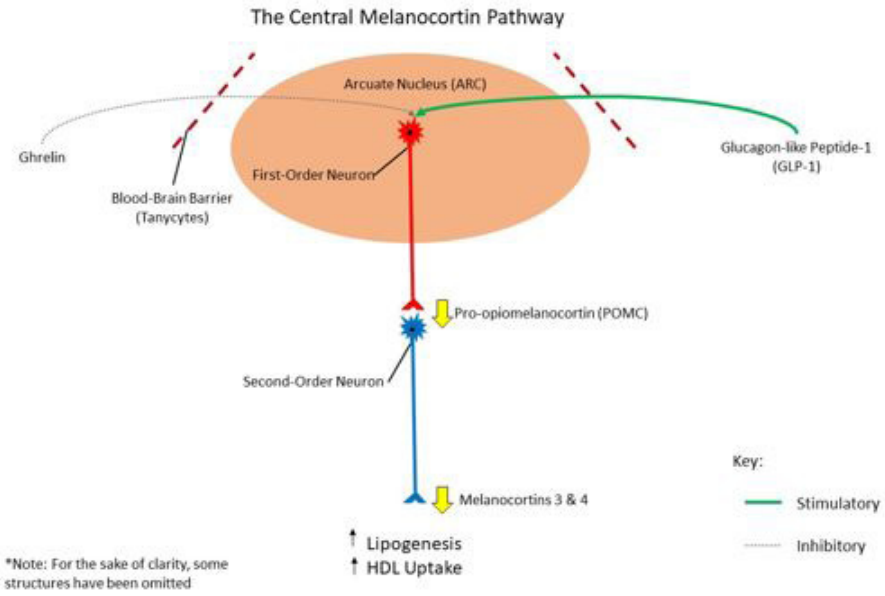

Figure 2: The central melanocortin pathway and the influence of hormones.

This neural pathway is mostly responsible for anorexigenic regulation of lipid metabolism, although POMC populations also play a role in glucose regulation. Anorexigenic neurons in the ARC produce POMC, which stimulates second order neurons to produce melanocortins 3 and 4. These peptides promote lipogenesis and HDL uptake. The hormones ghrelin and glucagon-like peptide-1 can cross the blood brain barrier at the median eminence via specialized cells called tanycytes. These hormones have antagonistic functions, with glucagon-like peptide-1 stimulating the central melanocortin pathway and ghrelin serving an inhibitory function (Coll & Yeo, 2013; Kandilis et al., 2015; Perez-Tilve et al., 2010; Taher et al., 2017).

Recent studies have shown that hormonal influences on the melanocortin pathway have a major impact on the concentration of high-density lipoprotein cholesterol (HDL-C) in the plasma (Perez-Tilve et al., 2010). These influences can be both up and down-regulatory, as indicated by the actions of ghrelin and glucagon-like peptide-1 (GLP-1) (See Figure 2, above) (Perez-Tilve et al., 2010). These hormones access the ARC through the median eminence, a region of the blood-brain barrier controlled by modified glial cells called tanycytes (Coll & Yeo, 2013). Ghrelin is an inhibitor of the central melanocortin pathway, and treatment with ghrelin leads to increased circulating HDL-C (Perez-Tilve et al., 2010). Glucagon-like-peptide is antagonistic to ghrelin and stimulates the central melanocortin pathway, lowering circulating HDL-C (Jensen et al., 2013; Perez-Tilve et al., 2010). These changes in plasma cholesterol occur without any associated changes in body weight in the rat model, even if the melanocortin receptors are destroyed or impaired (Perez-Tilve et al., 2010). Therefore, this pathway is likely responsible for HDL-C uptake rather than production (Perez-Tilve et al., 2010).

The hypothalamus is also involved in afferent lipid sensing, with the ability to reduce hepatic glucose production to prevent resting hyperglycemia (Pocai, Obici, Schwartz, & Rossetti, 2005). Central inhibition of fat oxidation stimulates the NTS and the dorsal motor nucleus to down-regulate gluconeogenesis and the construction of the associated enzymes, thereby lowering hepatic glucose production (Pocai et al., 2005). This pathway is mediated by ATP-dependent K^+ channels (K_{ATP}), and may require an intact hepatic vagus, as severing this branch leads to resting hyperglycemia (Pocai et al., 2005). The ARC provides a second hypothalamic pathway for lipid sensing (Pocai et al., 2005). A decrease in CTP1 mitochondrial enzyme activity leads to a build-up of long-chain fatty acyl-CoA (LC-CoA) (Pocai et al., 2005). This build-up signals an abundance of nutrients that eventually shifts metabolism from carbohydrates to lipids and reduces hepatic glucose production (Pocai et

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al., 2005), while ARC action is signaled by an increase in C-Fos expression following CTP1 inhibition (Pocai et al., 2005). Neither this pathway nor the NTS mechanism seems to require vagal afferents to function, and K_{ATP} channels can be stimulated hormonally by leptin and insulin (Pocai et al., 2005).

The role of the hypothalamus in hepatic glucose metabolism is closely tied to its larger role in the circadian rhythms of the body, which are controlled by the suprachiasmatic nucleus (SCN) (Jensen et al., 2013; Kalsbeek et al., 2010). This hypothalamic center has projections to both the sympathetic and parasympathetic periventricular nucleus (PVN), the integrating center responsible for CNS-mediated glucose regulation (Kalsbeek et al., 2010). The neuronal signals from the PVN take the form of various neuroendocrine and neuropeptide secretions (Kalsbeek et al., 2010).

Another proposed parasympathetic pathway for glucose regulation involves POMC, but there is a significant conflict in the data regarding this function (Kalsbeek et al., 2010). POMC neurons have been shown to respond to leptin, insulin, and serotonin (Coll & Yeo, 2013; Smith, 2018). These hormones work in opposition of NPY to reduce hepatic glucose production. Central insulin increases hepatic insulin sensitivity to reduce gluconeogenesis (Coll & Yeo, 2013; Konig, Bulik, & Holzhutter, 2012; Smith, 2018). Unlike downstream melanocortin pathways, however, inhibition of this pathway does lead to obesity (Kalsbeek et al., 2010). Individuals who lack central insulin and leptin receptors, as well as those who lack 5-hydroxytryptamine 2C receptors (5-HT2CRs) for serotonin have an increased incidence of obesity and hyperphagia (Coll & Yeo, 2013). Disruption of hypoxia-inducible factor (HIF) and cellular autophagy in POMC populations also leads to obesity (Coll & Yeo, 2013).

Other hormones and neuropeptides of the hypothalamus perform functions related to both feeding behavior and glucose metabolism, but since these are not always correlated with one another, each is considered a separate process (Kalsbeek et al., 2010). Orexin, for example, has ambiguous effects on obesity, but has been shown to increase hepatic glucose uptake (Kalsbeek et al., 2010). Melanin-concentrating hormone (MCH), while critical to the initiation of feeding behavior, does not appear to have any effect on glucose metabolism (Kalsbeek et al., 2010). By contrast, pituitary adenylate cyclase activating peptide (PACAP), when administered to the brain, increases circulating glucose levels and also reduces feeding behavior (Kalsbeek et al., 2010). This neuropeptide may have additional effects on the counterregulation of hypoglycemia and energy storage in the form of white adipose tissue (Kalsbeek et al., 2010). VIP has broad effects upon metabolism and feeding that may impact hepatic function (Kalsbeek et al., 2010).

The final three hypothalamic hormones discussed below have some glucose-related function, but their precise impacts and pathways remain ambiguous. The first is thyrotropin-releasing hormone (TRH), which can induce hyperglycemia, but its exact mechanism is unknown (Kalsbeek et al., 2010). Arginine-vasopressin (AVP) promotes hepatic glucose production via gluconeogenesis and glycogenolysis, and modifies insulin and glucagon release (Kalsbeek et al., 2010), but it has not been shown to directly impact glucose metabolism (Kalsbeek et al., 2010). This hormone is of interest due to its secondary effects on lipid metabolism (Kalsbeek et al., 2010). It can both up and down-regulate lipogenesis and lipogenolysis, creating a possible direct link between hypothalamic glucose and lipid metabolism (Kalsbeek et al., 2010). The final hormone, oxytocin, reduces food intake (Kalsbeek et al., 2010). An oxytocin-deficient model animal will become obese, but once again, there is no direct link between this function and hepatic glucose metabolism (Kalsbeek et al., 2010). These three hormones are not alone in ambiguity. In fact, a large quantity of PVN secretions have functions that remain unclear (Kalsbeek et al., 2010).

Neuro-endocrine Interplay in Hepatic Regulation

CNS control of hepatic glucose and lipid metabolism extends beyond the major hypothalamic pathways to include various endocrine and receptor-mediated pathways that aid in systemic regulation of circulating glucose levels and fat storage (Berthoud, 2004; Forssmann & Ito, 1977; Jensen et al., 2013; Mizuno & Ueno, 2017; Taher et al., 2017; Yi et al., 2010). Furthermore, these peripheral pathways appear to function semi-normally even without much of the liver-brain axis intact, as with liver transplant patients (Tiniakos et al., 1996; Yi et al., 2010).

The liver contains various mechanisms for glucose sensing, neuronal monitoring of circulating glucose via glucokinase (GK) and glucose transporters (GLUT) (Jensen et al., 2013; Mizuno & Ueno, 2017; Yi et al.,

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2010). Liver receptor X (LRX) and carbohydrate response element-binding protein (ChREBP) are intrahepatic glucose monitors with unknown signaling pathways that may involve the autonomic nervous system (Yi et al., 2010). The former controls the secretion of adropin, which is a paracrine and endocrine mediator of glucose and lipid metabolism (Yi et al., 2010). Hepatic insulin-sensitizing substance (HISS) is secreted by the liver following parasympathetic stimulation (Kandilis et al., 2015; Yi et al., 2010). This substance also increases skeletal muscle glucose uptake (Yi et al., 2010). Parasympathetic denervation disrupts this pathway and can reduce total glucose disposal by as much as 50% (Kandilis et al., 2015). Interestingly, blockage of M3 cholinergic receptors did not lead to similar effects, indicating that the vagus may act through alternative pathways as opposed to direct acetylcholine stimulation (Kandilis et al., 2015). Central insulin can modify gluconeogenesis outright, though the mechanism is still largely ambiguous (Kimura et al., 2016). The pathway is thought to involve the disinhibition of IL-6 and STAT-3, both of which reduce the expression of gluconeogenic enzymes (Kimura et al., 2016). IL-6 and STAT-3 are normally inhibited by vagal innervation of the α7-nicotinic cholinergic receptors (α7-nAchR) of Kupffer cells (Kimura et al., 2016). Central insulin detection leads to suppression of the vagus and release of these factors (Kimura et al., 2016). In obese and insulin-resistant individuals, the vagal inhibition is less successful (Kimura et al., 2016).

The portal vein is primarily responsible for hepatic glucose sensing and is especially critical in responding to systemic hypoglycemia (Jensen et al., 2013; Yi et al., 2010). As a hypoglycemic state is often the result of fasting, portal sensors play a key role in initiating a feeding response (Jensen et al., 2013; Yi et al., 2010). The portal response, however, does not appear to reduce food intake upon its inhibition and is incapable of detecting rapid-onset hypoglycemia (Jensen et al., 2013). A pathway involving neural-hormonal crosstalk and transcriptional regulation is simply too slow to respond in such a short time frame (Konig et al., 2012). Liver transplant patients have been shown to have a higher prevalence of systemic hypoglycemia due to a reduced counterregulatory portal response (Jensen et al., 2013).

Lipid-sensing is another key function of the alternative liver pathways and has been shown to work closely with the gastrointestinal tract to maintain an optimal balance of free fatty acids (FFAs) in the blood (Jensen et al., 2013; Mizuno & Ueno, 2017; Taher et al., 2017; Yi et al., 2010). An increase in FFAs either through diet or through enhanced lipolysis of abdominal adipose triggers a response in the hepatic vagus that increases general satiety, but if the increase in FFAs is too great, overstimulation can lead to insulin resistance and reduced insulin clearance (Yi et al., 2010). This pathway is likely mediated by peroxisome-proliferator-activator receptors (PPARs) in the liver (Yi et al., 2010). Insulin itself is also active in hepatic lipid sensing, with centralized insulin responses leading to decreased VLDL production and increased hepatic lipid storage, and peripheral insulin responses having an antagonistic function (Taher et al., 2017). In addition, insulin is often aided by leptin, which if deficient can lead to insulin resistance, and by adipokines, factors secreted by brown adipose tissue (BAT) (Kandilis et al., 2015; Taher et al., 2017).

In times of energy shortage, the liver can respond by secreting its own mediator of lipid metabolism, fibroblast growth factor-21 (FGF-21) (Berthoud, Munzberg, & Morrison, 2017; Clemmensen et al., 2017; Coll & Yeo, 2013). This hormone increases fat oxidation and ketogenesis to produce energy during crisis. This hormone also responds to glucagon and GLP-1 stimulation (Clemmensen et al., 2017).

Various other endocrine inputs can stimulate the hepatic vagus or spinal afferents. Generally, the vagus acts in satiety and promotes gluconeogenesis, while the spinal nerves act in scarcity and promote glycogenolysis (Jensen et al., 2013; Streba et al., 2014; Yi et al., 2010). Interleukin-1, for example, is a cytokine that reduces feeding behavior during illness and acts through hepatic vagal stimulation (Berthoud, 2004; Jensen et al., 2013; Yi et al., 2010). Other interleukins may signal hepatocytes to produce immune proteins (Jensen et al., 2013; Streba et al., 2014). Vagal afferents have also been shown to respond to cholecystokinin (CCK) released from I cells in the duodenum (Jensen et al., 2013). This hormone reduces hepatic glucose production and is inhibited by a high-fat diet. While the hypothalamus plays a role in both pathways due to their endocrine nature, even a denervated liver can still express these functions (Yi et al., 2010). In liver transplant patients, for example, the majority of functions are preserved (Tiniakos et al., 1996; Yi et al., 2010). Yet it should be noted that a complete denervation is superior to an imbalance, and that completely denervated patients have the most trouble mobilizing large stores of glucose in the case of a fight-or-flight response (Kandilis et al., 2015; Yi et al., 2010).

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Liver Innervation and Blood Flow

The influence of the autonomic nervous system upon hepatic function extends beyond metabolism to include the regulation of blood flow and solute concentration (Forssmann & Ito, 1977; Tiniakos et al., 1996). The liver is a heavily supplied organ, and in the event of an imbalance, it acts to maintain plasma homeostasis in response to autonomic innervation. This function makes the liver an extremely valuable peripheral osmoreceptor. The well-innervated walls of the hepatic blood vessels can trigger a pressor reflex in which sympathetic efferent stimulate the vessels to constrict and raise blood pressure (Jensen et al., 2013; Streba et al., 2014). This activity is mediated by afferent osmoreceptors such as transient receptor channel protein vanilloid 4 (TRPV4), which senses hypoosmotic environments through changes in ion current. The pressor reflex is impaired in liver transplant patients that cannot receive the influx of norepinephrine from adrenergic innervation (Jensen et al., 2013; Kandilis et al., 2015; Mizuno & Ueno, 2017). Another reflex mediated by hepatic innervation is the hepatorenal reflex, a form of communication between the portal vein and the kidneys. The portal vein is well-suited to sensing changes in plasma ion content because the concentration of dissolved solutes in the portal vein is much higher than in systemic circulation (Jensen et al., 2013; Mizuno & Ueno, 2017). If the portal vein senses an increase in the concentration of sodium ions portal innervation acts to dampen sympathetic stimulation of the kidneys. This innervation leads to increased Na+ excretion and restores ionic balance (Jensen et al., 2013).

The large amount of blood funneled into the liver also makes the liver a key target in the case of hemorrhage. When a large amount of blood is lost from circulation, the body turns to the vast blood supply of the liver as a counterregulatory mechanism (Gardemann et al., 1992; Jensen et al., 2013). Sympathetic innervation of the hepatic artery and its branches stimulates vasoconstriction, reducing blood flow to the liver in times of crisis (Beckh et al., 1990; Gardemann et al., 1992; Jensen et al., 2013; Mizuno & Ueno, 2017). This process is highly dependent upon endothelin, with TL_A and TL_{B1} receptors inducing the contraction of the smooth muscle (Streba et al., 2014). Higher mammals possessing aminergic innervation of the spaces of disse also experience contraction of the sinusoids (Burt et al., 1989; Gardemann et al., 1992; Jensen et al., 2013; Mizuno & Ueno, 2017). This process expels blood into the systemic circulation to restore blood volume and is mediated by catecholamines and substance P (Gardemann et al., 1992; Jensen et al., 2013). When the crisis has passed, vagal innervation works to relax the sinusoids (Gardemann et al., 1992; Jensen et al., 2013). Acetylcholine and vasoactive intestinal peptide induce relaxation and allow the liver to resume its normal filtration of the blood (Gardemann et al., 1992; Jensen et al., 2013; Streba et al., 2014). Liver transplant patients, who lack these regulatory controls, are at high risk for hypovolemic shock due to reduced portal flow (Gardemann et al., 1992; Jensen et al., 2013; Kandilis et al., 2015). In fact, application of norepinephrine and epinephrine to a denervated liver results in an arterial buffer phenomenon, where portal flow is lost and hepatic artery flow increases (Gardemann et al., 1992; Jensen et al., 2013; Kandilis et al., 2015).

Liver Innervation and Bile Secretion

The liver's network of bile ducts is innervated in many species, although usually in conjunction with nearby blood vessels. Therefore, hepatic autonomic innervation has an extensive impact on the flow and secretion of bile through the biliary system. Specialized epithelial cells, known as cholangiocytes, modify bile through the absorbance and secretion of bile salts and bicarbonate. These, in turn, are under sympathetic and parasympathetic control (Baik et al., 2017; Mizuno & Ueno, 2017). Cholinergic action is mediated by M3 cholinergic receptors on the cholangiocytes, and stimulation of these induces cholangiocyte proliferation and an increase in secretion of bicarbonate (Mizuno & Ueno, 2017).

Cholangiocytes also possess α and β adrenergic innervation, with the α-1 and α-2 receptors functioning antagonistically to one another (Mizuno & Ueno, 2017). The α1-adrenergic receptor pathway normally decreases ductal secretion, but this function can be reversed by exposure to phenylephrine, a factor that also promotes cholangiocyte proliferation (Jensen et al., 2013). By comparison, α2-adrenergic receptor stimulation down-regulates the Gαq pathway responsible for aiding phenylephrine-induced proliferation (Jensen et al., 2013). The antagonism of these two pathways is temporarily interrupted in the event of biliary injury, when both cooperate to increase expression of cyclical adenosine monophosphate (cAMP) and protein kinase B (Akt) to hasten the repair process (Jensen et al., 2013). In addition to the adrenergic sympathetic pathways, the cholangiocytes also respond to a dopaminergic pathway

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that functions similarly to α2 stimulation (Jensen et al., 2013).

Cirrhotic Livers and Neuronal Regulation of Tissue Regeneration

The sympathetic nervous system has been shown to be both beneficial and disruptive to the repair of cirrhotic liver injury (Baik et al., 2017). Sympathetic innervation exerts its influence through the regulation of hepatic stellate cells (HSC) and oval cells (HOC) (Jensen et al., 2013). HSCs are normally responsible for producing extracellular matrix and storing vitamin A (Jensen et al., 2013; Streba et al., 2014). However, in the event of liver damage, these proliferate as myofibroblasts and synthesize collagen (Jensen et al., 2013). While this function itself is not necessarily harmful, a buildup of collagenous scar tissue can eventually impair liver function and cause abnormal regrowth, a cycle of continued damage demonstrated in Figure 3 (Jensen et al., 2013; Mizuno & Ueno, 2017). HSCs are stimulated by aminergic innervation and may also aid their own proliferation by secreting catecholamines themselves (Figure 3) (Jensen et al., 2013).

This activity is responsible for the initial spike in catecholamines documented soon after liver damage, even though the growth nodules themselves tend to be denervated (Jensen et al., 2013; Kiba, 2002; Lee et al., 1992; Tiniakos et al., 1996). The proliferation of HSCs combined with aminergic stimulation blocks the growth of HOCs, which are necessary for the regeneration of hepatocytes (Figure 3) (Jensen et al., 2013; Kandilis et al., 2015). Much of the abnormal growth seen in cirrhotic livers is thought to be caused by the impairment of these cells (Jensen et al., 2013). Sympathetic stimulation also tends to exacerbate the damage by reducing blood supply to the liver (Jensen et al., 2013; Kiba, 2002). Thus, inhibition of the hepatic sympathetic fibers may be a potential treatment for cirrhosis in the future (Jensen et al., 2013).

Pathogenesis occurs through a cycle of damage and sympathetic response. Initial trauma leads to a surge in catecholamines, which causes the proliferation of stellate cells and inhibits oval cells. Stellate cells produce collagenous scar tissue, leading to abnormal regrowth. Constriction of the hepatic artery leads to further damage, and the cycle continues. Patients are at risk for developing hepatocellular carcinoma if abnormal growth persists (Baik et al., 2017; Jensen et al., 2013; Kandilis et al., 2015; Kiba, 2002; Mizuno & Ueno, 2017).

The parasympathetic nervous system influences liver regeneration through the hepatic thymidine kinase and aspartate transcarbamoylase pathways (Jensen et

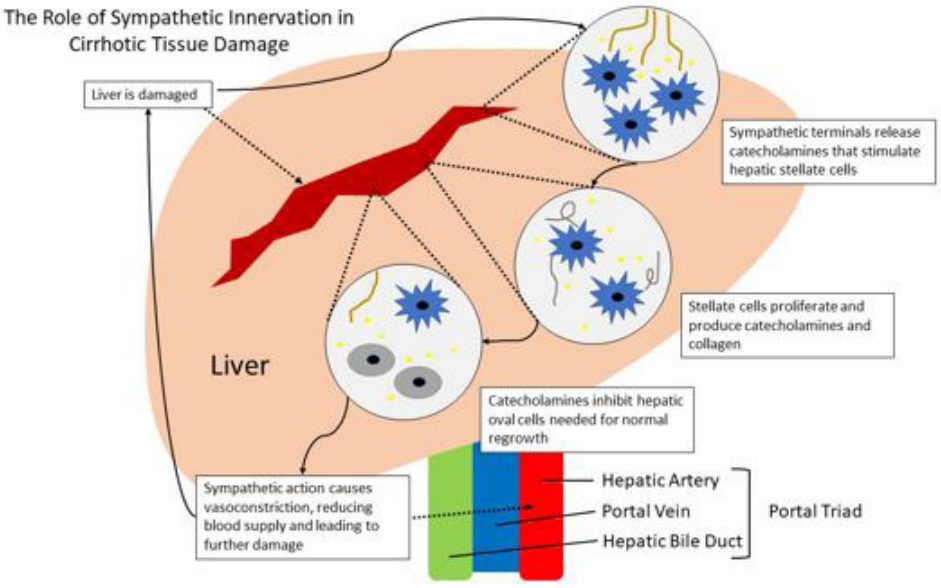

Figure 3: Sympathetic stimulation leads to abnormal regeneration in cirrhotic livers.

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al., 2013). Loss of subdiaphragmatic vagal innervation impairs liver regeneration, but this connection is confounded by other factors such as reduced food intake and impaired gastric emptying (Kiba, 2002). A ventromediolateral hypothalamic lesion, which increases vagal activity, was shown to have the opposite effect, increasing hepatic regeneration (Kiba, 2002). The hypothalamus plays a role in mediating liver apoptosis. Though the details of this role are somewhat ambiguous, the hypothalamus is thought to have an impact on cholinergic innervation (Kiba, 2002).

Liver innervation may not only be regulatory to tissue regeneration, but also necessary for it (Mizuno & Ueno, 2017). Studies have shown that regeneration of the rat liver is possible as long as the fibers of the porta hepatis remain intact (Kiba, 2002). Furthermore, in observations of advanced cirrhosis and hepatocellular carcinoma, the infiltration of sympathetic innervation and subsequent stimulation of α1 adrenergic receptors on Kupffer cells were shown to promote carcinogenesis (Huan et al., 2017). Kupffer cells release Interleukin-6 (IL-6) and transforming growth factor β (TGF-β), both of which are associated with poor prognosis in hepatocellular carcinoma patients (Boilly, Faulkner, Jobling, & Hondermarck, 2017; Huan et al., 2017). Sympathetic denervation improved the prognosis significantly, and evidence from other studies indicates neural-immune interplay in the generation of tumors (Boilly et al., 2017; Huan et al., 2017). Carcinogenesis displays many similarities with epimorphic regeneration of limbs and organs (Boilly et al., 2017). Both processes require the invasion of macrophages, induction of inflammation by interleukins, and secretion of growth factors, all of which are aided by sympathetic innervation (Boilly et al., 2017; Streba et al., 2014). In many aggressive cancers, tumor cells secrete neurotrophic growth factor (NGF) to promote axonogenesis and the innervation of the tumor tissue (Boilly et al., 2017). This benefits the cancer in two ways. First, innervation stimulates β-adrenergic and muscarinic receptors expressed by cancer cells, inducing rapid and aggressive proliferation (Boilly et al., 2017). Second, access to a neural trunk allows cancers to metastasize (Boilly et al., 2017). Therefore, denervations, especially sympathetic denervations, are considered new targets for cancer therapy (Boilly et al., 2017; Huan et al., 2017). The critical role liver innervation plays in both normal and abnormal tissue growth cannot be discounted.

Other Liver Diseases and their Impacts

While cirrhosis is a highly prevalent liver condition that has been shown to have both structural and functional impacts upon the organ, other maladies of the liver have also been shown to present alterations in liver neural structure. Among these are hepatitis B and C (Baik et al., 2017). Both conditions reduce liver innervation as well as the production of S100B mRNA (Baik et al., 2017). S100B is a calcium-binding protein found in the cytoplasm of glial cells and serves as an indicator for portal innervation (Baik et al., 2017). An absence of S100B-coding RNA indicates a loss of innervation, and this trend has also been reported for liver cirrhosis as well as hepatocellular carcinoma (Baik et al., 2017; Kandilis et al., 2015). This observation may seem counterintuitive, as innervation is critical to the degeneration of these conditions, and indeed various studies have found that there is an increase in hepatic innervation present, but both sides of this debate have reported similar problems with accurate quantification (Baik et al., 2017). Regardless of form, the abnormal growth of nodules, tumors, and other structural features of disease results in abnormal innervation. As with cirrhosis, fibrotic growth of the HSCs is speculated to be a contributing factor to liver denervation in other conditions, but evidence shows that once mRNA expression is altered, it becomes independent of further fibrosis (Baik et al., 2017).

DISCUSSION

This paper has offered an overview of the current literature surrounding the anatomy and physiology of autonomic innervation of the liver and has explored the interaction between the CNS and hepatic tissue to maintain homeostasis and regulate metabolism. However, this review would be incomplete without an analysis of the issues that remain unresolved, as well as the directions future studies may take to answer these questions. First, it is worth noting that much of the information contained in this review was drawn from studies that are many years, even decades old. There thus exists a lack of recent literature regarding hepatic innervation. The paucity of information concerning the liver-brain axis would suggest one of two possibilities: either that limited progress has been made, or that the discouraging lack of knowledge regarding the topic has sidelined it in favor of other targets. In either case, the fact that relatively little recent literature exists means that much of the current knowledge surrounding liver innervation is in dire need of an update. Study of these topics was likely

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limited by the technology of the past, but with modern advances in microscopy, tissue sectioning, and labelling, new studies could be undertaken to not only validate the predecessors but also discover new information. Among the most important issues to be addressed are the elusive intrahepatic ganglia. The idea that an organ as large, complex, and critical as the liver would lack such vital neural structures seems absurd, yet no such formation has been found to date (Berthoud, 2004; Berthoud and Neuhuber, 2000; Taher et al., 2017). An inability to find a structure is hardly compelling evidence that it is not present at all, especially given the dated methods used before. Thus, the search for intrahepatic ganglia must be continued.

The physiology of liver innervation has more gaps in the literature than the anatomy of the neurons themselves. More specifically, the current knowledge understands "what" sympathetic and vagal stimulation of hepatic tissue is likely responsible for, but "how" the mechanisms operate is largely unknown. For example, there is a suspected parasympathetic pathway of NPY function, but so far only the sympathetic arm has been elucidated in appreciable detail (Kalsbeek et al., 2010; Smith, 2018). TRH is known to induce hyperglycemia, but the mechanism behind this blood sugar spike is not available (Kalsbeek et al., 2010). This mechanism could be neural or endocrine, and involve autonomic pathways from the CNS, but only further study will be able to say for certain. Perhaps the strangest and most puzzling problem of all is how the liver functions without CNS innervation. Liver transplant patients have hepatic tissues that operate almost normally (Tiniakos, Lee, & Burt, 1996; Yi et al., 2010). If the liver-brain axis is as vital as some data suggest, why are denervated livers able to perform most of the same actions a normal liver can? The ambiguity surrounding the anatomy, function, and necessity of hepatic autonomic innervation makes this area a vital field of future study.

If any scientific progress is to be made toward a more complete understanding of liver innervation, more powerful, accurate, and thorough screening and denervation technologies must be brought to bear. Tracers such as calretinin and P2X2/X3 vesicular glutamate transporter have potential (Berthoud, 2004), but have been used to label liver tissue in few, if any, laboratory trials. Of even greater importance is the need for a reliable way to obliterate certain populations of hepatic neurons without harming unrelated nerves. This method would allow the functions of specific groups of neurons to be isolated according to type, origin, and secretions. Chemical and biological agents that specifically target sympathetic hepatic neurons may be especially important for treating liver cirrhosis and cancer, since sympathetic stimulation is partly responsible for the progression of these diseases (Baik et al., 2017; Huan et al., 2017; Jensen et al., 2013). Such technologies are within our capacity to develop and doing so will reveal the location of intrahepatic ganglia as well as elucidate the various CNS pathways that are currently incomplete.

CONCLUSION

The connection between the liver and the central nervous system is a vastly under-studied arena with various unknown structures, functions, and mechanisms (Akiyoshi et al., 1998; Berthoud, 2004; Kalsbeek et al., 2010; McCuskey, 2004; Perez-Tilve et al., 2010; Taher et al., 2017; Verma et al., 2018; Yi et al., 2010). A large part of this knowledge gap is due to the inability of previous technologies to properly isolate and map liver innervation (Berthoud, 2004; Yi et al., 2010). As biomedical sciences continue to advance and our understanding of the neural structure and function of the liver improves, these questions may be answered, thereby revealing more of the widespread impact of autonomic hepatic innervation in both normal physiology and disease states. Such an under-studied yet critically important organ provides an exciting frontier for continued research and the potential for intriguing new discoveries. This paper provides a brief analysis of past and current data, taking special note of areas that require a great deal of further study. Revisiting the issue of autonomic influence upon hepatic function could prove incredibly useful to both the scientific and medical community.

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REFERENCES

1. Akiyoshi, H., Gonda, T., & Terada, T. (1998). A comparative histochemical and immunohistochemical study of aminergic, cholinergic and peptidergic innervation in rat, hamster, guinea pig, dog and human livers. *Liver, 18*(5), 352-359.

2. Azanza, M. J., Aisa, J., Junquera, C., & Castiella, T. (1989). The autonomic innervation of the liver and gallbladder of Rana ridibunda. *Histol Histopathol, 4*(4), 405-410.

3. Baik, S. J., Kim, T. H., Yoo, K., Moon, I. H., Choi, J. Y., Chung, K. W., & Song, D. E. (2017). Decreased S100B expression in chronic liver diseases. *Korean J Intern Med, 32*(2), 269-276. doi:10.3904/kjim.2015.296

4. Beckh, K., Fuchs, E., Balle, C., & Jungermann, K. (1990). Activation of glycogenolysis by stimulation of the hepatic nerves in perfused livers of guinea pig and tree shrew as compared to rat: differences in the mode of action. *Biol Chem Hoppe Seyler, 371*(2), 153-158.

5. Berthoud, H. R. (2004). Anatomy and function of sensory hepatic nerves. *Anat Rec A Discov Mol Cell Evol Biol, 280*(1), 827-835. doi:10.1002/ar.a.20088

6. Berthoud, H. R., Munzberg, H., & Morrison, C. D. (2017). Blaming the Brain for Obesity: Integration of Hedonic and Homeostatic Mechanisms. *Gastroenterology, 152*(7), 1728-1738. doi:10.1053/j.gastro.2016.12.050

7. Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci, 85*(1-3), 1-17. doi:10.1016/s1566-0702(00)00215-0

8. Boilly, B., Faulkner, S., Jobling, P., & Hondermarck, H. (2017). Nerve Dependence: From Regeneration to Cancer. *Cancer Cell, 31*(3), 342-354. doi:10.1016/j.ccell.2017.02.005

9. Burt, A. D., Tiniakos, D., MacSween, R. N., Griffiths, M. R., Wisse, E., & Polak, J. M. (1989). Localization of adrenergic and neuropeptide tyrosine-containing nerves in the mammalian liver. *Hepatology, 9*(6), 839-845.

10. Clemmensen, C., Muller, T. D., Woods, S. C., Berthoud, H. R., Seeley, R. J., & Tschop, M. H. (2017). Gut-Brain Cross-Talk in Metabolic Control. *Cell, 168*(5), 758-774. doi:10.1016/j.cell.2017.01.025

11. Coll, A. P., & Yeo, G. S. (2013). The hypothalamus and metabolism: integrating signals to control energy and glucose homeostasis. *Curr Opin Pharmacol, 13*(6), 970-976. doi:10.1016/j.coph.2013.09.010

12. Forssmann, W. G., & Ito, S. (1977). Hepatocyte innervation in primates. *J Cell Biol, 74*(1), 299-313.

13. Gardemann, A., Puschel, G. P., & Jungermann, K. (1992). Nervous control of liver metabolism and hemodynamics. *Eur J Biochem, 207*(2), 399-411.

14. Huan, H. B., Wen, X. D., Chen, X. J., Wu, L., Wu, L. L., Zhang, L., . . . Xia, F. (2017). Sympathetic nervous system promotes hepatocarcinogenesis by modulating inflammation through activation of alpha1-adrenergic receptors of Kupffer cells. *Brain Behav Immun, 59*, 118-134. doi:10.1016/j.bbi.2016.08.016

15. Jensen, K. J., Alpini, G., & Glaser, S. (2013). Hepatic nervous system and neurobiology of the liver. *Compr Physiol, 3*(2), 655-665. doi:10.1002/cphy.c120018

16. Kalsbeek, A., Bruinstroop, E., Yi, C. X., Klieverik, L. P., La Fleur, S. E., & Fliers, E. (2010). Hypothalamic control of energy metabolism via the autonomic nervous system. *Ann N Y Acad Sci, 1212*, 114-129. doi:10.1111/j.1749-6632.2010.05800.x

17. Kandilis, A. N., Papadopoulou, I. P., Koskinas, J., Sotiropoulos, G., & Tiniakos, D. G. (2015). Liver innervation and hepatic function: new insights. *J Surg Res, 194*(2), 511-519. doi:10.1016/j.jss.2014.12.006

18. Kiba, T. (2002). The role of the autonomic nervous system in liver regeneration and apoptosis--recent developments. *Digestion, 66*(2), 79-88. doi:10.1159/000065594

19. Kimura, K., Tanida, M., Nagata, N., Inaba, Y., Watanabe, H., Nagashimada, M., . . . Inoue, H. (2016). Central Insulin Action Activates Kupffer Cells by Suppressing Hepatic Vagal Activation via the Nicotinic Alpha 7 Acetylcholine Receptor. *Cell Rep, 14*(10), 2362-2374. doi:10.1016/j.celrep.2016.02.032

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UNDERGRADUATE RESEARCH JOURNAL

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20. Konig, M., Bulik, S., & Holzhutter, H. G. (2012). Quantifying the contribution of the liver to glucose homeostasis: a detailed kinetic model of human hepatic glucose metabolism. *PLoS Comput Biol, 8*(6), e1002577. doi:10.1371/journal.pcbi.1002577

21. Lee, J. A., Ahmed, Q., Hines, J. E., & Burt, A. D. (1992). Disappearance of hepatic parenchymal nerves in human liver cirrhosis. *Gut, 33*(1), 87-91.

22. Li, L., de La Serre, C. B., Zhang, N., Yang, L., Li, H., & Bi, S. (2016). Knockdown of Neuropeptide Y in the Dorsomedial Hypothalamus Promotes Hepatic Insulin Sensitivity in Male Rats. *Endocrinology, 157*(12), 4842-4852. doi:10.1210/en.2016-1662

23. McCuskey, R. S. (2004). Anatomy of efferent hepatic nerves. *Anat Rec A Discov Mol Cell Evol Biol, 280*(1), 821-826. doi:10.1002/ar.a.20087

24. Metz, W., & Forssmann, W. G. (1980). Innervation of the liver in guinea pig and rat. *Anat Embryol (Berl), 160*(3), 239-252.

25. Mizuno, K., & Ueno, Y. (2017). Autonomic Nervous System and the Liver. *Hepatol Res, 47*(2), 160-165. doi:10.1111/hepr.12760

26. Perez-Tilve, D., Hofmann, S. M., Basford, J., Nogueiras, R., Pfluger, P. T., Patterson, J. T., . . . Tschop, M. H. (2010). Melanocortin signaling in the CNS directly regulates circulating cholesterol. *Nat Neurosci, 13*(7), 877-882. doi:10.1038/nn.2569

27. Pocai, A., Obici, S., Schwartz, G. J., & Rossetti, L. (2005). A brain-liver circuit regulates glucose homeostasis. *Cell Metab, 1*(1), 53-61. doi:10.1016/j.cmet.2004.11.001

28. Reilly, F. D., McCuskey, P. A., & McCuskey, R. S. (1978). Intrahepatic distribution of nerves in the rat. *Anat Rec, 191*(1), 55-67. doi:10.1002/ar.1091910106

29. Rojas, J. M., Bruinstroop, E., Printz, R. L., Alijagic-Boers, A., Foppen, E., Turney, M. K., . . . Niswender, K. D. (2015). Central nervous system neuropeptide Y regulates mediators of hepatic phospholipid remodeling and very low-density lipoprotein triglyceride secretion via sympathetic innervation. *Mol Metab, 4*(3), 210-221. doi:10.1016/j.molmet.2015.01.004

30. Sluka, J. P., Fu, X., Swat, M., Belmonte, J. M., Cosmanescu, A., Clendenon, S. G., . . . Glazier, J. A. (2016). A Liver-Centric Multiscale Modeling Framework for Xenobiotics. *PLoS One, 11*(9), e0162428. doi:10.1371/journal.pone.0162428

31. Smith, J. K. (2018). Exercise, Obesity and CNS Control of Metabolic Homeostasis: A Review. *Front Physiol, 9,* 574. doi:10.3389/fphys.2018.00574

32. Streba, L. A., Vere, C. C., Ionescu, A. G., Streba, C. T., & Rogoveanu, I. (2014). Role of intrahepatic innervation in regulating the activity of liver cells. *World J Hepatol, 6*(3), 137-143. doi:10.4254/wjh.v6.i3.137

33. Taher, J., Farr, S., & Adeli, K. (2017). Central nervous system regulation of hepatic lipid and lipoprotein metabolism. *Curr Opin Lipidol, 28*(1), 32-38. doi:10.1097/MOL.0000000000000373

34. Tiniakos, D. G., Lee, J. A., & Burt, A. D. (1996). Innervation of the liver: morphology and function. *Liver, 16*(3), 151-160.

35. Verma, A., Antony, A. N., Ogunnaike, B. A., Hoek, J. B., & Vadigepalli, R. (2018). Causality Analysis and Cell Network Modeling of Spatial Calcium Signaling Patterns in Liver Lobules. *Front Physiol, 9*, 1377. doi:10.3389/fphys.2018.01377

36. Yi, C. X., la Fleur, S. E., Fliers, E., & Kalsbeek, A. (2010). The role of the autonomic nervous liver innervation in the control of energy metabolism. *Biochim Biophys Acta, 1802*(4), 416-431. doi:10.1016/j.bbadis.2010.01.006