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Neural Connections Between the Hypothalamus and the Liver

NAOKI UYAMA,* ALBERT GEERTS, AND HENDRIK REYNAERT
Laboratory for Molecular Liver Cell Biology, Vrije Universiteit Brussel, Brussels, Belgium

ABSTRACT

After receiving information from afferent nerves, the hypothalamus sends signals to peripheral organs, including the liver, to keep homeostasis. There are two ways for the hypothalamus to signal to the peripheral organs: by stimulating the autonomic nerves and by releasing hormones from the pituitary gland. In order to reveal the involvement of the autonomic nervous system in liver function, we focus in this study on autonomic nerves and neuroendocrine connections between the hypothalamus and the liver. The hypothalamus consists of three major areas: lateral, medial, and periventricular. Each area has some nuclei. There are two important nuclei and one area in the hypothalamus that send out the neural autonomic information to the peripheral organs: the ventromedial hypothalamic nucleus (VMH) in the medial area, the lateral hypothalamic area (LHA), and the periventricular hypothalamic nucleus (PVN) in the periventricular area. VMH sends sympathetic signals to the liver via the celiac ganglia, the LHA sends parasympathetic signals to the liver via the vagal nerve, and the PVN integrates information from other areas of the hypothalamus and sends both autonomic signals to the liver. As for the afferent nerves, there are two pathways: a vagal afferent and a dorsal afferent nerve pathway. Vagal afferent nerves are thought to play a role as sensors in the peripheral organs and to send signals to the brain, including the hypothalamus, via nodosa ganglia of the vagal nerve. On the other hand, dorsal afferent nerves are primary sensory nerves that send signals to the brain via lower thoracic dorsal root ganglia. In the liver, many nerves contain classical neurotransmitters (noradrenaline and acetylcholine) and neuropeptides (substance P, calcitonin gene-related peptide, neuropeptide Y, vasoactive intestinal polypeptide, somatostatin, glucagon, glucagon-like peptide, neurotensin, serotonin, and galanin). Their distribution in the liver is species-dependent. Some of these nerves are thought to be involved in the regulation of hepatic function as well as of hemodynamics. In addition to direct neural connections, the hypothalamus can affect metabolic functions by neuroendocrine connections: the hypothalamus-pancreas axis, the hypothalamus-adrenal axis, and the hypothalamus-pituitary axis. In the hypothalamus-pancreas axis, autonomic nerves release glucagon and insulin, which directly enter the liver and affect liver metabolism. In the hypothalamus-adrenal axis, autonomic nerves release catecholamines such as adrenaline and noradrenaline from the adrenal medulla, which also affects liver metabolism. In the hypothalamus-pituitary axis, release of glucocorticoids and thyroid hormones is stimulated by pituitary hormones. Both groups of hormones modulate hepatic metabolism. Taken together, the hypothalamus controls liver functions by neural and neuroendocrine connections.

Key words: neural connection; liver; hypothalamus; autonomic nerve; innervation; efferent nerve; afferent nerve; sensory nerve; neurotransmitter; neuropeptide; neuroendocrine pathway; pancreas; pituitary gland; adrenal gland; thyroid

In the 1960s, Shimazu et al. showed that autonomic nerves from the hypothalamus control glycogen metabolism in the liver (Shimazu and Fukuda, 1965; Shimazu et al., 1966; Shimazu, 1967). Since then, the anatomical and functional aspects of the autonomic nerves in the liver, which are derived from the hypothalamus, have been further investigated. As for the anatomical aspects, anterograde and retrograde transneural tracing methods and advanced immunohistochemistry have contributed to analyzing and characterizing the neural pathways between the hypothalamus and the liver. Electrical stimulation and surgical denervation techniques and drugs to inhibit autonomic nerves have contributed to exploring the relationship between autonomic nerves and liver functions. An increasing amount of evidence has shown that the innervation of the liver plays a key role in regulating liver metabolism and hemodynamics. In addition, advanced im-

*Correspondence to: Naoki Uyama, Laboratory of Molecular Liver Cell Biology, Vrije Universiteit Brussel, Laarbeeklaan 103 1090 Brussels, Belgium. Fax: 32-2-477-4412.
E-mail: nuyama@vub.ac.be

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TABLE 1. Nucleus and area in the hypothalamus

Area		Nucleus	Function
Lateral			Sending parasympathetic signals to the body via autonomic nerves; anabolic response: glycogen synthesis, lipogenesis; appetite loss
Medial	VMH	Ventromedial hyopothalamic nucleus	Sending sympathetic signals to the body via autonomic nerves; catabolic response: gluconeogenesis, lipolysis; appetite gain.
Periventricular	PVN	Periventricular hyopothalamic nucleus	Receiving information from other parts of the hypothalamus such as SCN and ARN; sending information to autonomic nerves as well as the pituitary gland
	SCN	Suprachiasmatic nucleus	Playing a role as the central clock; receiving information from retina; sending some signals to the PVN.
	ARN	Arcuate nucleus	Sending signals to the PVN and other nuclei in the hypothalamus; sensitive to insulin, leptin, and glucose in the blood; playing a key role in the energy metabolism

munohistochemistry and electron microscopy have revealed the distribution of nerves containing classical neurotransmitters (noradrenaline and acetylcholine) and neuropeptides [substance P, calcitonin gene-related peptide (CGRP), neuropeptide Y, vasoactive intestinal polypeptide (VIP), somatostatin, glucagon, glucagon-like peptide, neurotensin, serotonin, and galanin] in the liver. Some of these classical neurotransmitters and peptides are thought to be involved in the regulation of liver hemodynamics as well as liver function. In addition, accumulated evidence has shown that the hypothalamus can control liver metabolism by other neuroendocrine pathways. In the first half of this article, we will describe two aspects: the neural pathways between hypothalamus and liver and the nerve distribution within the liver. In the second part, we will discuss the neuroendocrine pathways between the hypothalamus and the liver.

ANATOMY AND FUNCTION OF HYPOTHALAMUS

The hypothalamus is the gray matter flanking the third ventricle below the thalamus. It is phylogenetically very ancient, being the most rostral part of the reticular formation and linking the most primitive parts of the forebrain, the olfactory and limbic lobes, with the caudal neuraxis. The hypothalamus has important functions in homeostasis and survival. Its homeostatic functions include the regulation of food and water intake, thermoregulation, the sleep and wake cycle, sexual behavior patterns, defense mechanisms, and the regulation of the intermediate metabolism in the body.

The hypothalamus can be divided into three areas, named lateral, medial, and periventricular. These three areas and some of their important nuclei are summarized in Table 1. The lateral hypothalamic area (LHA) and the medial hypothalamic area have extensive connections with the brain stem and the telencephalon and regulate the autonomic function. The LHA is thought to be involved in the parasympathetic flow, because stimulation of this area diminishes the appetite and elicits a series of anabolic responses, including increased secretion of insulin, which affect lipogenesis in adipose tissue (de Jong et al., 1977; Inoue et al., 1977; Gutstein and Parl, 1978; Milam et al., 1980; Yoshimatsu et al., 1984). On the other hand, the ventromedial hypothalamic nucleus (VMH), which is a major part of the medial hypothalamic area, is considered to be involved in the sympathetic outflow (Inoue et al., 1977; Niijima et al., 1984; Yoshimatsu et al., 1984; Saito et al., 1989). Stimulation of this nucleus increases the appetite and activates a series of catabolic responses, including hyperglycemia, lipolysis in adipose tissue, and increased secretion of glucagon. Therefore, the functions of the LHA and the VMH are reciprocal.

The periventricular hypothalamic area consists of several nuclei, including the periventricular hypothalamic nucleus (PVN) and the suprachiasmatic nucleus (SCN). The PVN, which is a key nucleus in the periventricular hypothalamic area, receives much of its input from the other areas or nuclei in the hypothalamus. In this nucleus, there are two types of cells. The first cell type controls the autonomic nervous system and regulates the outflow of the sympathetic and parasympathetic innervation of visceral organs, including the liver, the pancreas, and the adrenal glands (Kannan et al., 1987, 1989; van Dijk et al., 1994). The other type of cells constitutes neurosecretory neurons, which affect the pituitary gland and control the secretion of various hormones (Freund-Mercier et al., 1981). Some of these cells, which control the posterior lobe of the pituitary gland, are magnocellular neurosecretory cells (Sokol et al., 1976). These cells are also located in the supraoptic nucleus in the LHA (Sokol et al., 1976). Magnocellular neurosecretory cells extend axons down to the stalk of the pituitary, into the posterior lobe of pituitary gland, and secrete the neurohormones, oxytocin and vasopressin. The other type of cells in this nucleus, which control the anterior lobe, are parvocellular neurosecretory cells (Freund-Mercier et al., 1981). Unlike magnocellular neurosecretory cells, they do not extend axons to the pituitary gland. Instead, they secrete hypophysiotropic hormones into hypothalamus-pituitary portal circulation, which runs down the stalk of the pituitary, branches in the anterior lobe, and communicates with target cells in the anterior lobe. After stimulation of target cells by hypophysiotropic hormones, the anterior lobe secretes six hormones: growth hormone, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, and prolactin. These pituitary hormones act directly on their target cells or stimu-

late other endocrine glands to secrete hormones, which brings about changes in the body such as the volume and composition of body fluid, body growth, reproduction, and response to stress. Thus, the PVN regulates general conditions by stimulating autonomic nerves and by releasing various hormones from the pituitary gland.

The SCN, another important nucleus in the periventricular hypothalamic area, lies just above the optic chiasm. This nucleus plays the role of biological clock in the body (Moore and Eichler, 1972; Stephan and Zucker, 1972; Karatsoreos et al., 2004). This nucleus receives direct retinal innervation and projects to a number of areas in the hypothalamus. By organizing the circadian rhythm in hormone secretion and by autonomic control of our organs, the SCN prepares the body for physiological changes associated with the sleep-wake cycle. Furthermore, the SCN has been reported to control basal levels of plasma glucose, resulting in a clear 24-hr rhythm, with a rise at the end of the light period (La Fleur et al., 1999).

NEURAL CONNECTIONS BETWEEN HYPOTHALAMUS AND LIVER

There are two types of neurons connecting the hypothalamus and the liver: efferent and afferent nerves. Efferent nerves consist of sympathetic and parasympathetic nerves. Their anatomical and functional aspects have been well characterized. Efferent neural pathways from three major areas in the hypothalamus are involved in the autonomic regulation of the liver. These three major areas are the VMH in the medial hypothalamic area (Shimazu, 1987, 1996), the LHA (Shimazu, 1987, 1996), and the PVN in the periventricular hypothalamic area (Fig. 1) (Buijs et al., 2003). On the other hand, afferent sensory nerves in the liver are assumed to consist of vagal afferent nerves and spinal afferent nerves (Fig. 2), although sensory functions of the liver have remained largely speculative.

Efferent Nerves

Ventromedial hypothalamic nucleus: sympathetic nerve pathway. The major descending projections from the VMH are directed to the ventrolateral and ventromedial portions of the medullary reticular formation. From this reticular formation in the lower brainstem, projections can be traced to the intermediolateral cell column in the thoracolumbar spinal cord. From the intermediolateral cell column in the thoracolumbar spinal, preganglionic neurons extend their cell bodies to the collateral ganglia within the abdominal cavity. From the ganglia, postsynaptic nerves enter the liver with the afferent vascularture (Shimazu, 1987, 1996). Although there is no hard evidence about the original ganglion of sympathetic nerves arriving in the liver, it has been assumed to be the celiac ganglion. Stimulation of this pathway causes glucose output from the liver through rapid activation of the key glycogenolytic enzyme, glycogen phosphorylase, which results in hyperglycemia and a marked reduction of glycogen content in the liver (Shimazu, 1981, 1983, 1987). In addition, stimulation of VMH causes an increase in the activity of phosphoenolpyruvate carboxykinase (PEPCK), a key gluconeogenic enzyme, and marked suppression of pyruvate kinase (PK), a key glucolytic enzyme, of the liver (Shimazu and Ogasawara, 1975).

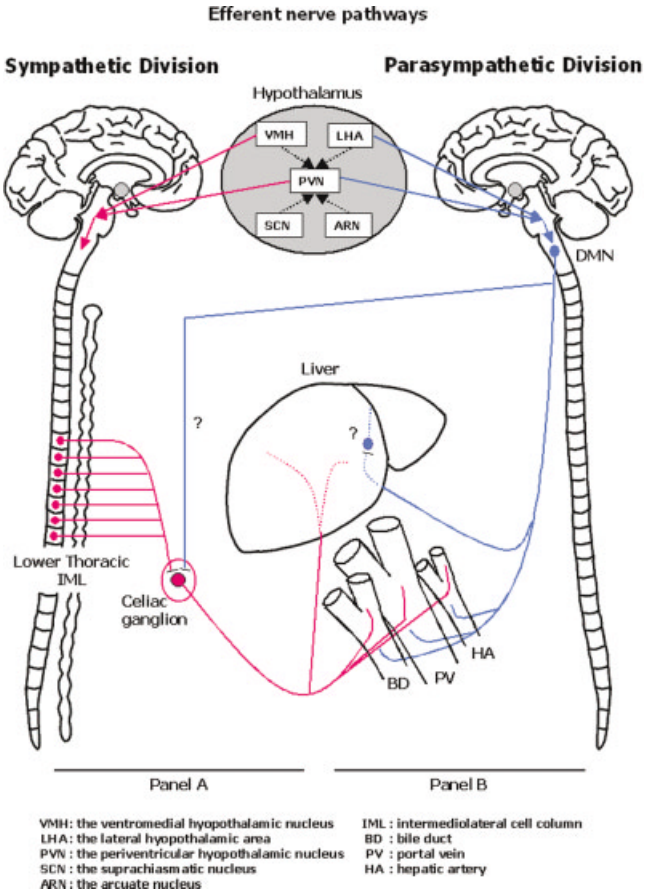

Fig. 1. Scheme of efferent nerve pathways between hypothalamus and the liver. A shows the sympathetic pathway (red line); B, the parasympathetic pathway (blue line). **A:** In the hypothalamus, the PVN and VMH play a key role in sending sympathetic signals to the liver. The descending projections from VMH and PVN are directed to the intermediolateral cell column in the thoracolumbar spinal cord via the reticular formation in the lower brain stem. Preganglionic neurons send signals to the liver via the celiac ganglia. Noradrenergic nerves are located in the vasculature as well as in the parenchyma. **B:** In the hypothalamus, the PVN and LHA play a key role to send parasympathetic signals to the liver. The outgoing connections of the LHA are for the major part directly aimed at the parasympathetic cell groups of the nucleus vagus/ambiguous complex and at the nearby medullary reticular cell group. The dorsal motor vagal and ambiguous nuclei supply efferent preganglionic parasympathetic nerves to the liver. These nerves form the hepatic branch of vagal nerve. Although there is little information about the intrahepatic parasympathetic ganglion and parasympathetic innervation, it is suspected that the vagal nerve indirectly controls functions of the liver by affecting the celiac ganglion or microganglia near the celiac artery. ARN, arcuate nucleus; IML, intermediolateral cell column; BD, bile duct; PV, portal vein; HA, hepatic artery. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

Lateral hypothalamic area: parasympathetic nerve pathway. The outgoing connections of the LHA are directly aimed at the parasympathetic cell groups of the nucleus vagus/ambiguous complex and at the nearby medullary reticular cell group. The dorsal motor vagal and ambiguous nuclei supply efferent preganglionic parasympathetic nerves to the liver, which constitute the hepatic branch of the vagal nerve (Shimazu, 1987, 1996). This

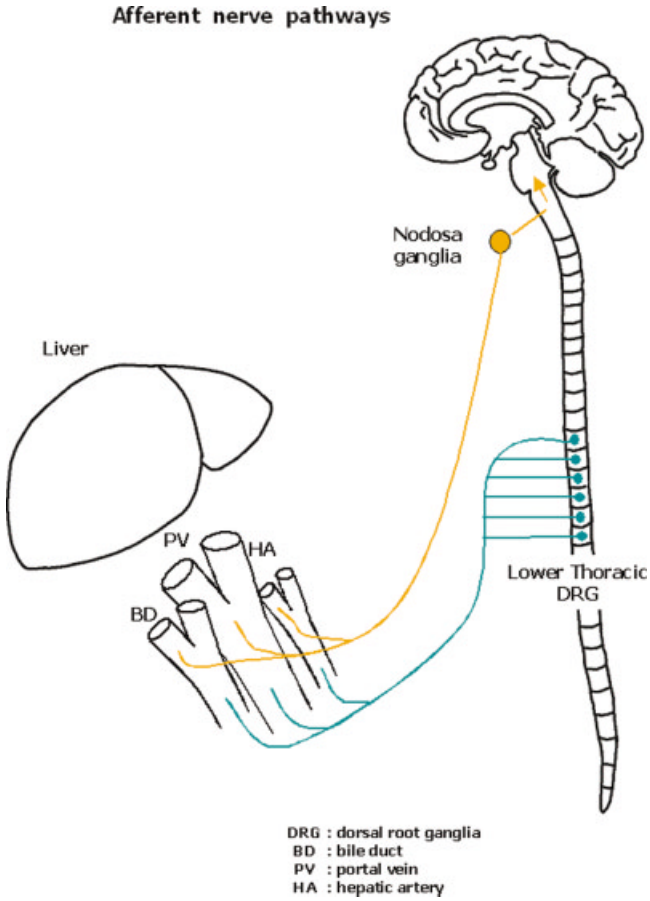

Fig. 2. Scheme of afferent nerve pathways between hypothalamus and the liver. Orange line shows the vagal afferent pathway from the liver and the green line shows the spinal afferent nerve pathway. Vagal afferent nerves terminate in the bile ducts and hepatic hilus, but not in the liver parenchyma. They are thought to play a key role as sensors in peripheral organs and send the signals to the brain, including the hypothalamus, via nodosa ganglia of the vagal nerve. Spinal afferent nerves are thought to be primary sensory nerves. They send nociceptive information to the brain via lower thoracic dorsal root ganglia. DRG, dorsal root ganglia. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

parasympathetic nerve is assumed to synapse on the postsynaptic nerves at the intrahepatic ganglia (Mikhail and Saleh, 1961; Reilly et al., 1978).

Stimulation of the LHA leads to hepatic glycogen synthesis through activation of the enzyme, glycogen synthase in the liver (Shimazu and Amakawa, 1975; Shimazu, 1981, 1983, 1987). In addition, stimulation of LHA results in a decrease or complete inactivation of PEPCK activity and PK activity (Shimazu and Ogasawara, 1975). Therefore, signals from LHA downregulate gluconeogenesis in the liver. However, there are only two reports that document the intrahepatic ganglia. Retrograde tracing experiments and Fluorogold staining experiments, which discriminate the autonomic ganglia and neurons, do not prove the existence of intrahepatic ganglia (Powley and Berthoud, 1991). Therefore, significant direct vagal cholinergic innervation of the liver is doubtful. Instead, it is suspected that the vagal nerve indirectly controls functions of the liver by affecting the celiac ganglia or microganglia near the celiac artery (Berthoud and Powley, 1993; Berthoud and Neuhuber, 1996). Indeed, vagal preganglionic terminals are found in the celiac ganglia as well as microganglia near the celiac artery. A large part of the mechanism remains obscure. Therefore, more detailed analysis is needed for the pathway of the parasympathetic innervation of the liver.

Periventricular hypothalamic nucleus: sympathetic and parasympathetic nerve pathways. The pathway between the PVN and the liver has been shown by a retrograde transneural viral tracing method. The descending PVN pathway projects to the liver via an intermediolateral nucleus in the spinal cord as well as via a dorsal nucleus vagus in the brain stem (Buijs et al., 2003). Therefore, the PVN pathway can be involved in both sympathetic and parasympathetic regulation of the liver. In addition, this retrograde transneural viral tracing method highlights the internal communications in the hypothalamus from the VMH to the PVN, from the SCN to the PVN, and from the arcuate nucleus, which is involved in the energy metabolism, to the PVN (Fig. 1) (La Fleur et al., 1999; Buijs et al., 2003). Thus, the PVN integrates the information from other hypothalamic and autonomic control of the liver function.

Afferent Nerves From Liver

There are two afferent nerve pathways: vagal afferent nerve pathway and spinal afferent nerve pathway (Fig. 2). Afferent vagal fibers enter the liver with characteristic terminal-like structures at three locations in the liver hilus: peribiliary glands in the larger intra- and extrahepatic bile ducts, paraganglia, and portal vein adventitia. They do not terminate in the intralobular area (Magni and Carobi, 1983; Barja and Mathison, 1984; Berthoud and Neuhuber, 1996). These afferent nerves are thought to function as sensors for circulating cytokines and metabolites such as glucose (Sakaguchi and Iwanaga, 1982), interleukin-1 (Niijima, 1996), amino acids (Torii and Niijima, 2001), lipids (Randich et al., 2001), and nicotine (Niijima et al., 2001).

Some afferent hepatic nerves are provided by the lower thoracic (Th 7-Th 12) dorsal root ganglia (Magni and Carobi, 1983; Barja and Mathison, 1984). These nerves are thought to transmit nociceptive information from the liver to the brain and contribute to the occurrence of pathological pain states such as inflammation and nerve injury. We do not describe this issue in detail, since it is described in an accompanying article, "Anatomy and Function of Sensory Hepatic Nerves."

NERVE DISTRIBUTION IN LIVER TISSUE

Immunohistochemistry has revealed the distribution of nerves in liver tissue. Intrahepatic distribution of these nerves is highly species-dependant. Among mammals, man has the highest density of intralobular nerves. These nerves contain classical neurotransmitters (noradrenaline and acetylcholine) as well as neuropeptides (substance P, CGRP, neuropeptide Y, VIP, somatostatin, glucagon, glucagon-like peptide, neurotensin, serotonin, and galanin). Some neuropeptidergic nerves have shown to be colocalized with neurotransmitters in both adrenergic and cholinergic nerves. Functions of some of these neurotransmit-

TABLE 2. Distribution of adrenergic, cholinergic, and neuropeptidergic nerves in the liver*

	Periportal region			
	HA	PV	BD	Parenchyma
Adrenergic nerves				
Rat	++	+	+	−
Mouse	++	+	+	−
Hamster	++	+	+	
Guinea pig	+++	++	+	++
Cat	++	+	+	−
Dog	+++	++	+	+
Man	++	+	+	+
Cholinergic nerves				
Rat	+++	+	++	−
Hamster	++	+	+	−
Guinea pig	+	+	+	−
Dog	++	+	+	−
Man	++	+	+	−
Substance P nerves				
Rat	+	−	−	−
Hamster	+	+	−	−
Guinea pig	++	+	+	+
Cat	++	+	++	+
Dog	+	+	+	+
Man	+	++	+	+
CGRP nerves				
Rat	+	++	++	−
Hamster	+	++	++	−
Guinea pig	+	+	++	−
Cat	+	+	+	−
Dog	+	+	++	−
Man	+	−	++	−
Neuropeptide Y nerves				
Rat	+	−	−	−
Mouse	+	−	−	−
Hamster	++	−	−	−
Guinea pig	++	++	+	+++
Cat	+++	++	++	++
Dog	++	++	+	++
Man	+	++	−	++
VIP nerves				
Rat	+	+	−	−
Hamster	+	+	+	−
Guinea pig	+	+	+	−
Cat	+	+	+	−
Dog	+	−	+	−
Man	+	+	+	−
Somatostatin nerves				
Cat	++	++	++	++
Man	++	++	++	++

*+++, numerous; ++, moderate; +, few; −, absent.

ter- and neuropeptide-containing nerves are thought to play a role as sensors or regulate hepatic function as well as hemodynamics. The distribution of these neurotransmitter- and neuropeptide-containing nerves is summarized in Table 2 and Figure 3.

Adrenergic Nerves in Liver

Anatomical aspects of adrenergic nerves in the liver are described in detail in the accompanying article "Anatomy of Effernt Hepatic Nerves." As for the function of noradrenaline, it has been reported that noradrenaline can affect the glucose metabolism of hepatocytes via α_1-adrenergic receptors (Arinze and Kawai, 1983; Garceau et al., 1984; Takahashi et al., 1996). In addition, noradrenaline can be involved in amino acid metabolism (Noda et al., 1983) and mitogenic activity (Cruise et al., 1985). Although it has been demonstrated that hepatic branch sympathectomy had no effect on liver regeneration, it has been suggested that adrenergic agents regulate liver regeneration via α_1-adrenergic receptors (Cruise et al., 1987). Recently, noradrenaline has been shown to stimulate the proliferation and collagen synthesis of hepatic stellate cells (Oben et al., 2003c). Destruction of noradrenergic fibers or pharmacologic antagonism of noradrenergic signaling through α_1-adrenergic receptors, on the other hand, inhibited the development of liver fibrosis (Dubuisson et al., 2002). Furthermore, sympathetic activity modulates progenitor cell accumulation in damaged livers. Blockade with α_1-adrenergic receptor antagonists enhanced hepatic progenitor cell accumulation associated with a decrease in the number of hepatic stellate cells (Oben et al., 2003a). These data suggested a role for the sympathetic nerve system in liver fibrosis and regeneration. Adrenergic agents probably also play a role in apoptosis. Indeed, it has been demonstrated that noradrenaline suppressed apoptosis in hepatocytes (Hamasaki et al., 2001) and that apoptosis was inhibited *in vivo* by β_2-adrenergic receptor agonists (Andre et al., 1999).

Cholinergic Nerve in Liver

Anatomical aspects of cholinergic nerves in the liver are described in detail in the article "Anatomy of Effernt Hepatic Nerves." As to the function of acetylcholine, it has been reported that acetylcholine affects metabolic functions, including glucose production via type 3 muscarinic receptors (Vatamaniuk et al., 2003). In addition, type 1, type 2, and type 3 muscarinic receptors were found in biliary epithelial cells, portal vein, and cholangiocytes, respectively (Eglen and Whiting, 1990; Alvaro et al., 1997; Elsing et al., 1997). Type 3 muscarinic receptor was also found in hepatic progenitor cells (Cassiman et al., 2002). Acetylcholine will act on these cells and affect bile secretion, portal blood flow, and regeneration.

In comparison to normal liver, the distribution of acetylcholine-positive nerves in cirrhotic liver is different (Akiyoshi and Terada, 1998; Akiyoshi et al., 1998). In the fibrous septa, numerous acetylcholinesterase-positive nerve fibers were observed. Their terminals were observed in close contact to myofibroblasts and mast cells. In cirrhotic nodules, nerve terminals were situated in close contact to myofibroblasts in the periseptal sinusoids. In addition, acetylcholine has been shown to stimulate the proliferation and collagen production of hepatic stellate cells (Oben et al., 2003b). Therefore, cholinergic nerves might play a role in liver fibrogenesis.

Distribution of Peptidergic Nerves in Liver

It has been reported that there are many kinds of neuropeptide-containing nerves such as substance P, CGRP, neuropeptide Y, VIP, somatostatin, glucagon, glucagon-like peptide, neurotensin, serotonin, and galanin. Among these neurotransmitter-containing nerves, substance P-, CGRP-, neuropeptide Y-, VIP-, and somatostatin-containing nerves are well characterized.

Immunohistochemistry has shown that many neuropeptide-containing nerve fibers are present in the liver, including CGRP, somatostatin, neuropeptide Y, VIP, and substance P. CGRP-, neuropeptide Y-, VIP-, and sub-

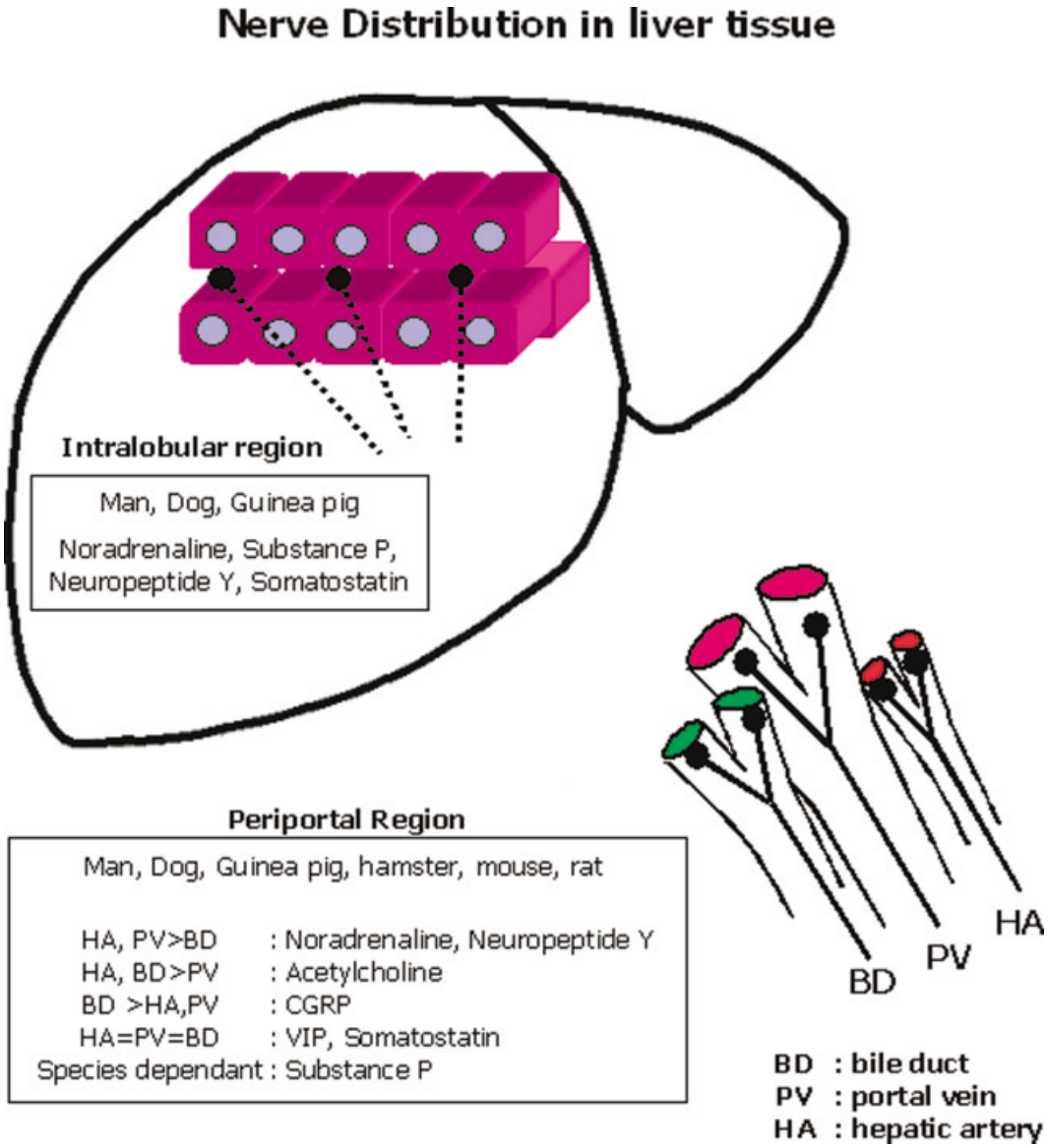

Fig. 3. Scheme of nerve distribution in liver tissue. Adrenergic, cholinergic, substance P-, CGRP-, neuropeptide Y-, VIP-, somatostatin-containing nerves are found in periportal area of rat, hamster, guinea pig, cat, dog, and human liver. VIP-, somatostatin-containing nerves are evenly distributed in hepatic artery, portal vein, and bile duct. Adrenergic, cholinergic, and neuropeptide Y-containing nerves are more abundant around hepatic artery and portal vein than around bile duct. On the other hand, CGRP-containing nerves predominantly exist around bile duct. Adrenergic, cholinergic, and neuropeptidergic nerves are not found in liver parenchyma of rat, mouse, and hamster. Adrenergic, somatostatin-, neuropeptide Y-, and substance P-containing nerves are distributed in liver parenchyma of man, dog, cat, and guinea pig.

stance P-positive nerves are distributed in the periportal area of man, cat, dog, and guinea pig liver. Neuropeptide Y-, somatostatin-, and substance P-positive, but not CGRP- or VIP-positive, nerves were also demonstrated in the intralobular area. These nerves are in close contact with hepatic sinusoidal cells. On the other hand, in golden hamster and rat liver, CGRP-, substance P-, and neuropeptide Y-containing nerves were found in the periportal area, but not in the intralobular area. There is also a big difference in the intrahepatic distribution of neuropeptide-containing nerves among species.

Substance P-containing nerves in liver. Substance P-positive nerves are richly distributed in the periportal and intralobular regions in human, guinea pig, dog, and cat liver (Feher et al., 1992; Akiyoshi et al., 1998) and their terminals were in close contact with hepatic stellate cells (Tanikawa, 1995; Ueno and Tanikawa, 1997). In rat liver, substance P-positive nerves were only present in the periportal region. Substance P, a member of neurotransmitter tachykinins, is highly concentrated in the superficial layer of the dorsal horn in the spinal cord (Takahashi and Otsuka, 1975; Hokfelt et al., 1976; DiFiglia et al., 1982). It is thought to transmit nociceptive information and contribute to the occurrence of pathological pain states such as inflammation and nerve injury. Indeed, substance P is contained in unmyelinated primary affer-

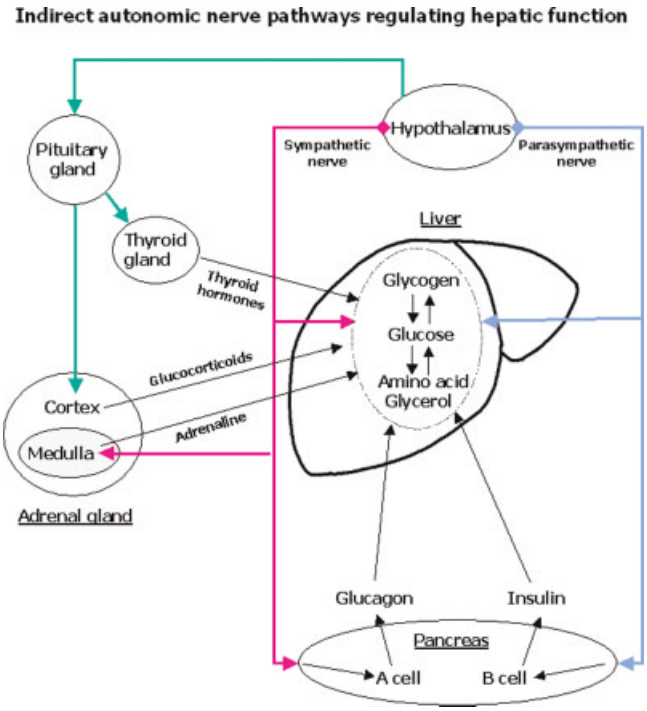

Fig. 4. Scheme of pathways regulating glucose metabolism of the liver. Red and blue thick arrow lines show sympathetic and parasympathetic nerves, respectively, that connect the hypothalamus and visceral organs. Green thick arrow line shows hypothalamus-pituitary neuroendocrine pathway. Liver autonomic nerves directly affect glucose metabolism in the liver, whereas autonomic nerves in the pancreas and adrenal gland indirectly influence glucose metabolism by releasing insulin and adrenaline from the pancreas and the adrenal glands. Growth hormone, ACTH, and thyroid hormones, which are controlled by the hypothalamus-pituitary axis, can also indirectly affect glucose metabolism in the liver. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

ent nerves, which mediate nociception (Nussbaumer et al., 1989). As described above, combination of retrograde tracing experiments and immunohistochemistry for substance P has shown that nerves in the liver derived from thoracic dorsal ganglia contain substance P (Barja and Mathison, 1984). In addition, extrinsic denervation and capsaicin treatment, which is known to cause degeneration of a certain number of primary sensory neurons, resulted in a decreased number of substance P-positive nerve terminals in the liver (Cai et al., 1983; Barja and Mathison, 1984; Burt et al., 1989). Therefore, substance P-containing nerves in the liver are likely to be primary sensory nerves from the dorsal root ganglia.

Calcitonin gene-related peptide-containing nerves in liver. It has been reported that CGRP-positive nerves are distributed in the periportal area but not in the intralobular area of the mammalian livers (Sasaki et al., 1986; Akiyoshi et al., 1998; Stoyanova and Gulubova, 1998). More specifically, CGRP-positive nerves were more associated with bile ducts than with the hepatic artery and portal vein (Carrier and Connat, 1995; Akiyoshi et al., 1998). CGRP is also known as a neurotransmitter in unmyelinated primary afferent nerves, which mediate nociception (Skofitsch and Jacobowitz, 1985; Goehler et al., 1988). Substance P-containing afferent nerves often contain CGRP (Skofitsch and Jacobowitz, 1985; Goehler et al., 1988). Similar to substance P, CGRP is localized in the neurons of spinal ganglia, and extrinsic denervation and capsaicin treatment deplete CGRP-containing nerve fibers in the liver (Sasaki et al., 1986; Goehler and Sternini, 1996). In addition, anterograde tracing experiments have shown that CGRP-positive nerves in the liver derive from dorsal root ganglia (Berthoud and Neuhuber, 1996). Therefore, CGRP-positive nerves are also thought to be primary sensory nerves.

Neuropeptide Y-containing nerves in liver. In rat liver, neuropeptide Y-positive nerves are found only in the periportal region. On the other hand, in human, guinea pig, dog, and cat liver, neuropeptide Y-positive nerves are richly distributed in the periportal and intralobular regions (Goehler and Sternini, 1991; Akiyoshi et al., 1998; Stoyanova and Gulubova, 1998). In particular, neuropeptide Y-positive nerves are more abundant around portal vein branches and hepatic artery branches than around bile ducts in the periportal area. Moreover, liver is reported to be the major source of circulating neuropeptide Y (Taborsky et al., 1994). As for the origin of neuropeptide Y-positive nerves, double immunostaining for neuropeptide Y and tyrosine hydroxylase has revealed that tyrosine hydroxylase immunoreactive nerves are coinciding with those containing neuropeptide Y (Uddman et al., 1985; Feher et al., 1991). Systemic administration of the sympathetic neurotoxin, 6-hydroxydopamine, leads to virtually complete elimination of both tyrosine hydroxylase and neuropeptide Y immunoreactive neurons (Goehler and Sternini, 1991). In addition, neuropeptide Y release from the dog liver was observed during sympathetic nerve stimulation (Taborsky et al., 1994). Therefore, neuropeptide Y-containing nerves are thought to represent a subpopulation of the sympathetic nerves of the liver. However, it has been shown that the density of neuropeptide Y-containing nerve fibers in the liver and around the biliary tree was greater than that of tyrosine hydroxylase-immunoreactive nerves (Feher, 1998). Therefore, some of the neuropeptide Y-containing nerve fibers may not be of sympathetic origin. More detailed investigation is needed.

Information about the function of neuropeptide Y in the liver is scarce. Neuropeptide Y potentiates noradrenaline-induced vasoconstriction of the rat portal vein branches (Dahlof et al., 1985; Pernow et al., 1986) and even produced stronger vasocontraction than noradrenaline alone in the dog hepatic artery (Corder and Withrington, 1988). However, there are also some studies showing no reactivity of neuropeptide Y on the contraction of the portal vein (Lundberg et al., 1984; Corder and Withrington, 1988). Therefore, more experiments concerning this issue are needed. Recently, neuropeptide Y has been shown to promote the proliferation of hepatic stellate cells, although the mechanism remains to be elucidated (Oben et al., 2003c). This study provides evidence for a possible direct effect of neuropeptide Y on hepatic stellate cells and liver fibrosis.

Vasoactive intestinal polypeptide-containing nerves in liver. In the human, cat, and rat liver, VIP-positive nerves are distributed around the portal vein, hepatic artery, and bile ducts in the periportal area, but

TABLE 3. Effects of autonomic nerves and hormones on glycogen and glucose metabolism

	Glycogen		Glucose	
	Synthesis	Degradation	Gluconeogenesis	Glucolysis
Sympathetic nerve		Direct effect	Direct effect	
Catecholamines		Direct effect	Indirect effect	
Glucagon		Direct effect	Direct effect	
Glucocorticoids	Direct effect		Direct effect	
Thyroid hormone		Direct effect	Direct effect	
Parasympathetic nerve	Direct effect			Direct effect
Insulin	Direct effect			Direct effect

not in the intralobular area (el Salhy et al., 1993; Akiyoshi et al., 1998). The number of VIP-positive nerves is much lower than that of substance P and neuropeptide Y. In the liver, hepatocytes have been reported to express receptors for VIP. VIP acts as a comitogenic factor on hepatocytes (Kar et al., 1996). In addition, there is evidence that VIP regulates biliary bicarbonate secretion: VIP-containing fibers are located near bile ducts (Ueno et al., 1991), VIP infusion significantly increases human biliary bicarbonate output (Nyberg et al., 1989), and VIP stimulates the bicarbonate secretion in cultured cholangiocytes via a cAMP-independent pathway (Cho and Boyer, 1999). However, more detailed investigations are needed.

Somatostatin-containing nerves in liver. Somatostatin-positive nerves are richly distributed around the portal vein and hepatic artery in the periportal region, as well as in the perisinusoidal region in human and cat normal liver. The terminals of these nerves are observed in close contact with sinusoidal liver cells (Feher et al., 1992; Stoyanova and Gulubova, 1998). It has been shown that the number of somatostatin-positive nerve was decreased in patients with alcoholic cirrhosis (Stoyanova and Gulubova, 2000). In cat liver, cutting the extrinsic hepatic nerves produces obvious changes in the number and distribution of substance P-positive nerves, but not in the number of somatostatin-positive nerves (Feher et al., 1992). Therefore, the majority of somatostatin-positive nerves are not thought to be primary sensory nerves. More detailed analysis is required.

Somatostatin is a 14 amino acid peptide that is widely distributed in the body. High concentrations are found in the gut, brain, and pancreas. It has a lot of physiological functions, including modulation of neurotransmission, protein secretion, smooth muscle cell contractility, intestinal motility, and immune cell function. The effects of somatostatin are mediated via five receptors. In the liver, somatostatin receptors are found in cholangiocytes (somatostatin receptor 1, 2, 3, and 4) (Tietz et al., 1995; Alpini et al., 1998; Gong et al., 2003) and in activated hepatic stellate cells (somatostatin receptor 1, 2, and 3) (Reynaert et al., 2001). In cholangiocytes, somatostatin inhibits both basal and secretin-induced exocytosis and cAMP increase (Alpini et al., 1998). In addition, in in vivo experiments, somatostatin inhibited basal and secretin-induced ductal bile secretion (Tietz et al., 1995; Alpini et al., 1998; Gong et al., 2003) as well as the proliferation of cholangiocytes induced by bile duct ligation. Recently, somatostatin receptor 2 expressed by cholangiocytes was revealed to play a key role in the bile secretion by using knockout mice (Gong et al., 2003). Somatostatin inhibited endothelin-1-induced contraction of activated rat hepatic stellate cells via activation of somatostatin receptor 1 (Reynaert et al., 2001). This suggests that somatostatin influences directly the sinusoidal blood flow. Thus, somatostatin receptors are expressed by cholangiocytes and hepatic stellate cells. Somatostatin affects bile secretion and may control microcirculation in the liver.

Other neuropeptidergic nerves in liver. In comparison to the above five neuropeptidergic nerves, there is much less information about the distribution of other neuropeptidergic nerves, including glucagon- (Sasaki et al., 1984; Carlei et al., 1988; el Salhy et al., 1993), glucagon-like peptide- (el Salhy et al., 1993), serotonin- (el Salhy et al., 1993; Carrier and Connat, 1995), neurotensin- (el Salhy et al., 1993), and galanin-containing nerve (Mundinger et al., 1997; Akiyoshi et al., 1998; Taborsky et al., 1999). Furthermore, the distribution of these nerves is a controversial issue. Further studies are required.

INDIRECT AUTONOMIC NERVE PATHWAYS REGULATING HEPATIC FUNCTION

In addition to direct control, autonomic nerves can influence indirectly liver function via intervention of pancreas (hypothalamus-pancreas axis) and adrenal gland (hypothalamus-adrenal axis) (Table 3; Fig. 4). Sympathetic outflow from hypothalamus stimulates release of catecholamines (adrenaline and noradrenaline) from the medulla of the adrenal glands and release of the glucagon from α-cells in the pancreas. On the other hand, parasympathetic signals from hypothalamus stimulate the release of insulin from β-cells in the pancreas. These three hormones are intensely involved in the regulation of glucose homeostasis (Table 3).

Hypothalamus-Pancreas Axis

Glucagon acts directly on the liver via its receptor. The receptors are coupled to G-proteins and promote increase in intracellular cAMP via activation of adenylate cyclase or elevated cytosolic calcium (Brechler et al., 1992; Robles-Flores et al., 1995). As a result of phospholipid breakdown, inositol trisphosphate is formed. Accumulated cAMP consecutively activates protein kinase A, phosphorylase kinase, and glycogen phosphorylase, which induces glycogen degradation. Furthermore, both enzyme of protein kinase A and phosphorylase kinase inactivates glycogen synthase by its phosphorylation (Ramachandran et al., 1983; Ciudad et al., 1984; Akatsuka et al., 1985). Therefore, glucagon plays a role as stimulator of glycogen degradation. During fasting, protein kinase A induced by glucagon

stimulates lipolysis in adipose tissue by activation of hormone-sensitive lipase (Yeaman, 1990). Lipolysis releases glycerol from adipose tissues, which is a substrate for the production of glucose in the liver. In addition, accumulated cAMP induced by glucagon stimulates the expression of the genes for the key gluconeogenic enzymes PEPCK and glucose-6-phosphatase (G6Pase) (Band and Jones, 1980a, 1980b; Beale et al., 1984; Striffler et al., 1984; Iynedjian et al., 1985; Christ et al., 1988; Yoon et al., 2001) and suppresses expression of the genes for the key enzyme of the glucolytic pathway, namely, pyruvate kinase (Pilkis and Claus, 1991). As a result, it stimulates gluconeogenesis in the liver.

Insulin sends intracellular signals by activating its receptor and insulin receptor substrate, which activates phosphatidylinositol 3-kinase (PI3) kinase (White and Kahn, 1994). The PI3 kinase-dependent signaling pathway consecutively leads to the activation of 3-phosphoinositide-dependent protein kinase-1/2 and protein kinase B (Cohen et al., 1997; Alessi and Cohen, 1998; Anderson et al., 1998). Protein kinase B inactivates glycogen synthase kinase, resulting in the inactivation of glycogen synthase (Cohen et al., 1997). PI3 kinase activates glycogen synthesis. In addition, PI3 kinase is also upstream of a mechanism that results in the activation of a cAMP phosphodiesterase (Cohen et al., 1997). Therefore, PI3 kinase leads to a lower concentration of cAMP, which induces glycogen degradation via activation of glycogen phosphorylase and inactivation of glycogen synthase. Taken together, insulin plays a role as a stimulator of glycogen synthesis. As for the gluconeogenesis, insulin suppresses in the liver the expression of gluconeogenic gene, PEPCK and G6Pase (Beale et al., 1984; Christ et al., 1988). In addition, in the adipose tissue, insulin stimulates uptake of glucose and induces lipogenesis by decreasing the activity of hormone-sensitive lipase in adipose tissue (Yeaman, 1990) and inducing lipogenic enzymes. Thus, these functions of insulin are antagonistic to glucagon.

Hypothalamus-Adrenal Axis

Adrenaline, which is predominantly released from the adrenal glands, can also directly affect lipid, protein, and carbohydrate metabolism. During physiological and stressful conditions (i.e., hypoglycemia), adrenaline can affect hepatic glucose production. Binding of adrenaline with β-adrenergic receptors increases intracellular cAMP and activates protein kinase A. This signal leads to the glycogen degradation. The α-adrenergic receptor is coupled to phospholipase C via an activating G-protein (Baek et al., 1993), leading to the production of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol. Ins(1,4,5)P_3 stimulates the release of Ca^{2+} from the endoplasmic reticulum. Ca^{2+} and diacylglycerol activate protein kinase C, which inactivates glycogen synthase, thereby downregulating the glycogen synthesis (Urcelay et al., 1993). In addition, the Ca^{2+}-calmodulin complex activates phosphorylase kinase and calmodulin-dependent protein kinase, which activates the glycogen phosphorylase and inactivates glycogen synthase, respectively. Thus, the Ca^{2+}-calmodulin complex downregulates glycogen synthesis and upregulates glycogen degradation. As for gluconeogenesis, adrenaline does not directly stimulate gluconeogenesis in the liver (Chu et al., 1997, 1998). However, adrenaline can stimulate lipolysis of adipose tissue and proteolysis of muscle (Miles et al., 1982; Sacca et al., 1983). Adrenaline increases substrates for gluconeogenesis such as glycerol, thereby affecting gluconeogenesis indirectly.

INVOLVEMENT OF HYPOTHALAMUS-PITUITARY AXIS PATHWAY IN LIVER FUNCTION

The hypothalamus-pituitary axis, which consists of neuroendocrine pathways from the hypothalamus, can also regulate liver functions (Table 3; Fig. 4). As mentioned in the previous section, the hypothalamus sends signals to the pituitary gland, which releases eight different hormones. Among them, ACTH is thought to be intensely involved in the regulation of liver glucose metabolism.

ACTH

ACTH released from the pituitary gland stimulates the release of glucocorticoid hormones, cortisol and corticosterone, from the cortex of the adrenal glands. Administration of glucocorticoids in vivo causes activation of glycogen synthase and inactivation of phosphorylase, resulting in glycogen synthesis (Laloux et al., 1983). Under fasting conditions, glucagon stimulates gluconeogenesis in the liver by inducing the expression of the genes for the key gluconeogenic enzymes PEPCK and G6Pase (Lamers et al., 1982; Lange et al., 1994; Schmoll et al., 1999). In addition, glucocorticoids lead to lipolysis in adipose tissue and proteolysis in the skeletal muscle by inhibiting glucose uptake by these tissues, resulting in release of glycerol from adipose tissue and amino acids from muscles (Livingston and Lockwood, 1975; Kawai and Kuzuya, 1981; Smith et al., 1990). In turn, glycerol and amino acids are used as substrates for the production of glucose in the liver. Effects of glucocorticoids on peripheral tissue can also contribute to gluconeogenesis in the liver.

Thyroid Hormone

Thyroid hormone is also involved in the glucose metabolism of the liver (Arrondo et al., 1981; Muller et al., 1982). Thyroid stimulating hormone stimulates the release of thyroid hormones from the thyroid. The thyroid hormone, 3,5,3′-triiodothyronine, acts on the liver mediated by thyroid hormone receptor that belongs to the nuclear hormone receptor superfamily. 3,5,3′-triiodothyronine stimulates glycogen degradation via the intracellular cAMP pathway (Arrondo et al., 1981; Shaheen et al., 1982b; Muller and Seitz, 1983; Nebioglu et al., 1990) and induces key enzymes for gluconeogenesis, PEPCK and G6Pase (Muller et al., 1982; Shaheen et al., 1982a). It also enhances the ability of glycogen degradation and gluconeogenesis by catecholamines and glucagon (Shaheen et al., 1982b; Muller and Seitz, 1983; Nebioglu et al., 1990).

Growth Hormone

Studies on the effect of growth hormone on liver metabolism are scarce. Growth hormone is thought to increase indirectly the production of glucose in the liver. In adipose tissue, growth hormone increases the sensitivity of the adipocytes to the lipolytic action of the catecholamines (Beauville et al., 1992) and decreases its sensitivity to the lipogenic action of insulin (Teglund et al., 1998; Yin et al., 1998). This action leads to the release of free fatty acids and glycerol into the blood. Therefore, the increased amount of glycerol reaching the liver acts as a substrate for gluconeogenesis.

CONCLUSION

In this article, we have reviewed recent progress in the neural connections between hypothalamus and liver. Although there is accumulating evidence that neural connections between the hypothalamus and the liver contribute to the regulation of liver functions, there are many aspects that have not been well characterized yet.

The following issues remain to be resolved. One, the neural pathways, by which efferent autonomic nerves, especially parasympathetic nerve, affect the liver function. Two, the function of hepatic afferent nerves in the liver. Three, the distribution of neuropeptidergic nerves in diseased liver. Four, the function of hepatic neurotransmitter- or neuropeptide-containing nerves in normal and diseased liver. Five, the involvement of neuroendocrine pathway in the normal and diseased liver function.

In general, there are many articles that describe anatomical and functional findings of these neural pathways in normal liver. However, anatomical and functional studies on these neural pathways in diseased liver, including fatty liver, fibrotic liver, resected liver, acutely injured liver, are scarce. Recently, it has been revealed that adrenergic and cholinergic nerves are involved in the accumulation of progenitor cells and stellate cells in acutely damaged liver. This document gives us interesting aspects on the function of autonomic nerves in damaged or regenerating liver. In the future, more studies on the relationship between liver disease and these neural pathways are required.

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