

Research Article

Hepatoportal Leptin Sensors and Their Reflex Effects on Autonomic Outflow in the Rat

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Afferent nerve signals were recorded from a peripheral cut end of the small nerve bundle of the hepatic branch of the vagus nerve in anesthetized rats. An injection of leptin (100 pg, 0.1 mL) into the portal vein facilitated the afferent activity. The response was dose dependent. Further, an intravenous (IV) injection of leptin (1 ng, 0.1 mL) facilitated the efferent nerve activity of the sympathetic nerve to the adrenal gland and suppressed that of the celiac branch of the vagus nerve. In hepatic vagotomized rats, no change in efferent activity of the adrenal sympathetic nerve nor celiac branch of the vagus nerve was observed following iv administration of leptin. These observations suggest that leptin sensors in the hepatoportal region play a role in reflex modulation of autonomic outflow in relation to metabolic functions.

1. Introduction

Leptin is a satiety hormone secreted by white adipose tissue (WAT). Both central and peripheral administration of leptin reduce food intake and body weight [1, 2]. Leptin receptors are localized to the hypothalamus [3, 4] and choroid plexus [5]. Direct application of leptin has been shown to increase the activity of glucoreceptor neurons in the ventromedial hypothalamus (VMH) and inhibit the activity of glucose-sensitive and nonglucose-sensitive neurons in the lateral hypothalamus (LHA) [6]. This finding suggests that leptin is liberated from WAT into the bloodstream, transported into the hypothalamus through the choroid plexus, and bound to receptors in the hypothalamic neurons to modulate their activities. In addition to the direct action of blood-borne leptin in the hypothalamus, the existence of afferent signaling from the leptin sensors in the WAT of the epididymis and the sensors' role in reflex modulation of sympathetic nerve activity have been reported [7, 8]. It is reasonable to assume that leptin sensors exist in the hepatoportal region because the portal vein is a main pathway for leptin secreted from the visceral WAT to arrive in the portal venous blood. Further, it has been reported that several types of chemosensors, such as

glucose sensors [9], amino acid sensors [10], and interleukin-1 β sensors [11], exist in the hepatoportal region and send their information through the hepatic branch of the vagus nerve to the brain. The aim of the present study was to investigate the effect of leptin on afferent signals from leptin sensors in the hepatoportal region and the sensors' reflex effect on autonomic outflow.

2. Materials and Methods

Male Wistar rats weighing 250–300 g were used. All animals were housed in a room maintained at approximately 24°C and illuminated for 12 h (07:00–19:00). Food (type MF; Oriental Yeast Co., Tokyo) and water were freely available. All procedures were performed in accordance with The Japanese Physiological Society's guidelines for animal care. Experiments were conducted after the animals had been allowed to adapt to their housing conditions for one week. Food, but not water, was removed 12 h before the experiment. The animals were anesthetized by intraperitoneal injection of 1 g/kg urethane. After the laparotomy, a catheter was inserted into the portal vein or the inferior vena cava for

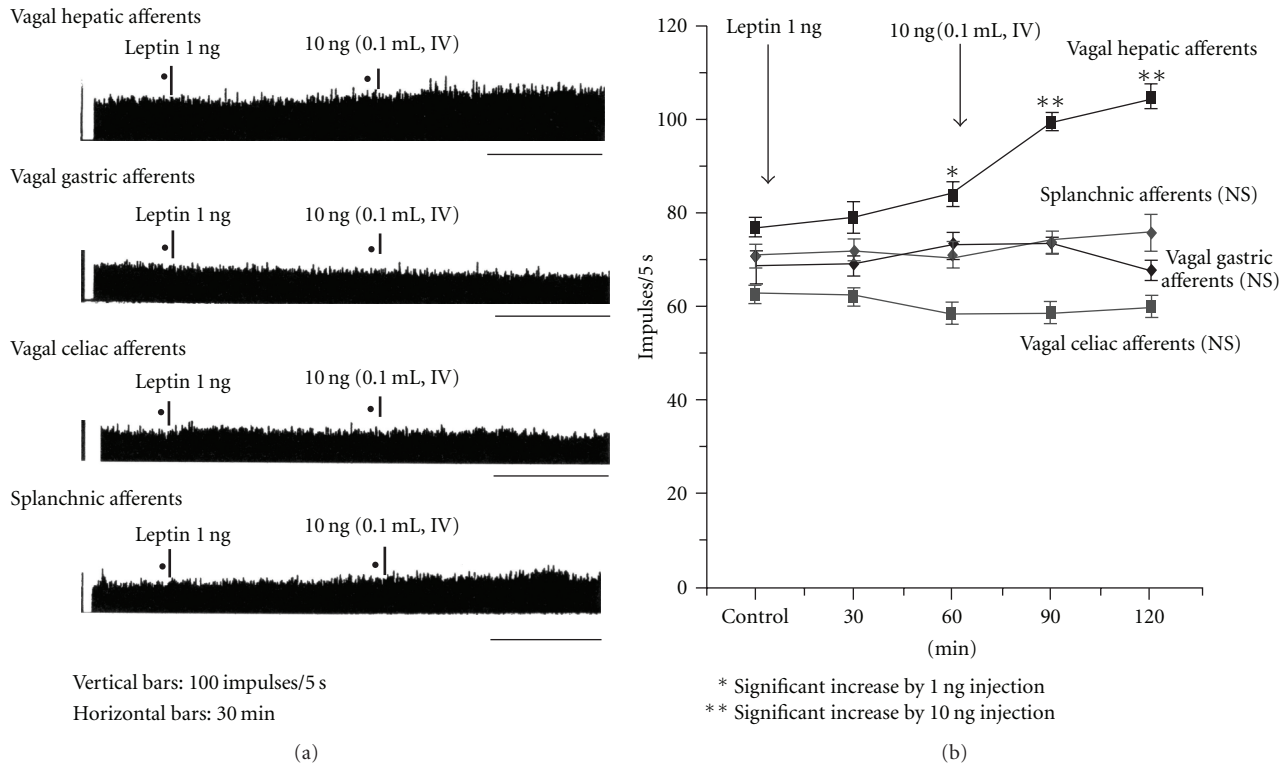


FIGURE 1: Effect of intravenous (IV) injection of leptin (1 ng and 10 ng, 0.1 mL) on the afferent discharge rate of the hepatic, gastric, or celiac branch of the vagus nerve, and that of the splanchnic nerve.

administration of leptin. With a dissection microscope, an isolated nerve filament from the peripheral cut end of the hepatic branch of the vagus nerve was placed on a pair of silver wire recording electrodes to record the nerve activity. Afferent signals were sometimes recorded from the gastric or celiac branches of the vagus nerve.

To record the efferent nerve activity of the sympathetic or vagal nerve branch, a nerve filament dissected from its central cut end was used. The recording electrodes were immersed in a mixture of liquid paraffin and petroleum jelly to prevent dehydration. The nerve activity was amplified in a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. All nerve activity were analyzed after conversion of raw data to standard pulses by a window discriminator, which separated discharges from background noise. The discharge rate was displayed on a pen recorder by means of a rate-meter with a reset time of 5 seconds. The effects of leptin injection on nerve activity were investigated by comparing the mean number of impulses per 5 seconds over 50 seconds (i.e., the mean value of 10 successive measured samples) before and after the injection. Data were expressed as the mean \pm SEM. Statistical significance was determined by analysis of variance (ANOVA) ($P < .05$). Recombinant leptin (SIGMA) was kept in a freezer (-20°C) and dissolved in physiological saline before use. The leptin solution was injected through a

catheter inserted into the inferior vena cava or portal vein. The animals' body temperatures were maintained by means of heating pads.

3. Results

3.1. Effect of IV Injection of Leptin on the Visceral Afferents. Figure 1 illustrates typical examples of the effects of two successive IV injections of leptin on the afferent nerve activities of the hepatic, gastric, and celiac branches of the vagus nerve, as well as of the splanchnic nerve. All recordings were made from the nerve filament dissected from the peripheral cut end of the nerve. The second IV injection (10 ng, 0.1 mL) was made 60 minutes after the first injection (1 ng, 0.1 mL).

Afferent discharge rates of vagal hepatic afferents just before, 30 minutes after, and 60 minutes after the first injection were 77.6 ± 1.9 , 79.7 ± 36.1 , and $84.7 \pm 2.3^*$ impulses per 5 seconds, respectively. Those just before, 30 minutes after, and 60 minutes after the second injection were 84.7 ± 2.3 , $100.2 \pm 1.9^*$, and $105.7 \pm 2.8^*$ impulses per 5 seconds, respectively (*represents significant increase, $P < .05$). These results indicate that IV injections of leptin at doses of 1 ng and 10 ng significantly increased the afferent discharge rate, and the responses were dose related. However, no significant changes in afferent discharge rates were observed

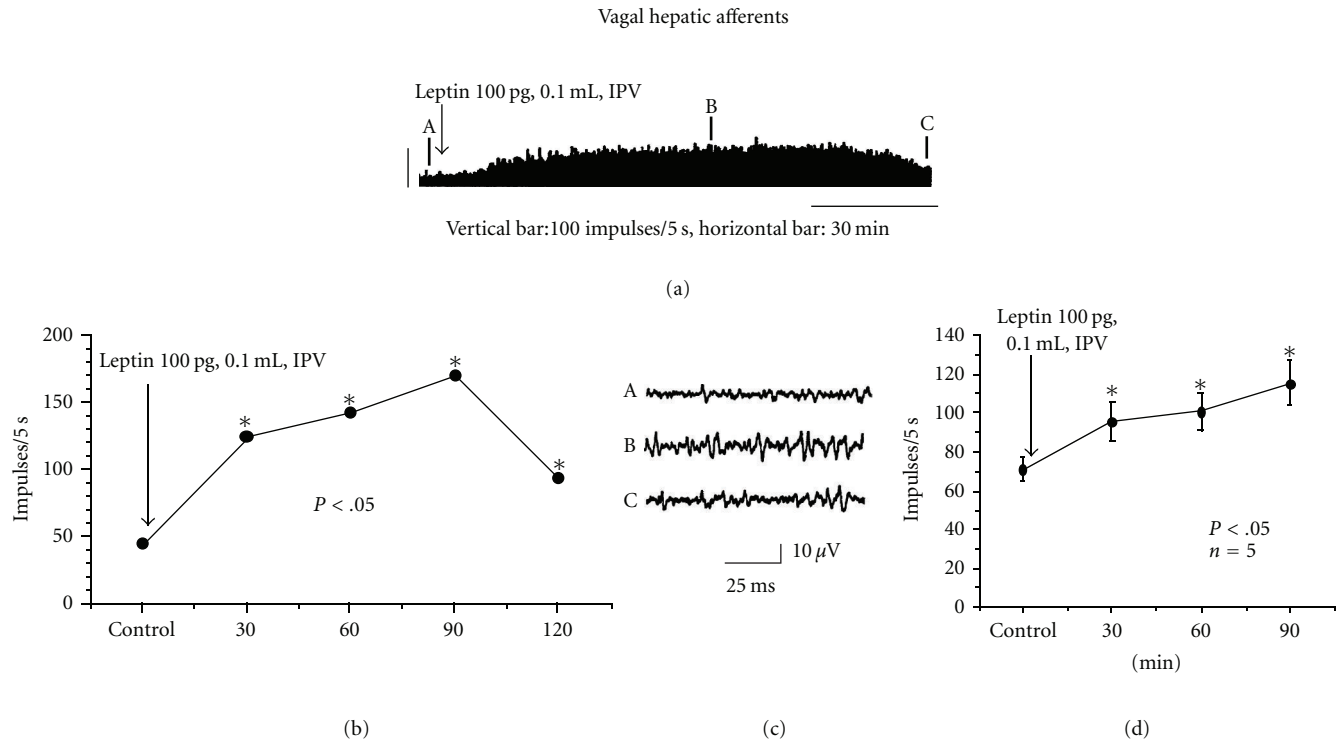


FIGURE 2: Effect of intraportal injection (IPV) of leptin 100 pg on afferent activity of the hepatic branch of the vagus nerve. (a) Time course of afferent activity before and after injection. Vertical bar: 100 impulses/5 seconds. The arrow shows the time of injection. (b) Afferent discharge rate just before 30, 60, 90, and 120 minutes after injection. (c) Afferent nerve activity (A) just before injection, (B) 60 minutes, and (C) 110 minutes after injection. (d) Effect of IPV injection of leptin (100 pg, 0.1 mL) on mean discharge rates of vagal hepatic afferents ($n = 5$). *Shows significant increase in discharge rates from the control value just before injection.

in the gastric or celiac branches of the vagus nerve, nor in the splanchnic nerve, after IV injections of leptin (1 ng and 10 ng). The results of these experiments clearly suggest the existence of leptin sensors at the terminals of vagal hepatic afferents.

3.2. Effect of Intraportal Injection of Leptin on Vagal Hepatic Afferents. To confirm the existence of leptin sensors in the hepatoportal region, the effect of leptin injection into the portal vein (IPV) on the afferent activity of the hepatic branch of the vagus nerve was investigated. Figure 2(a) shows an example of the effect of an IPV injection of leptin (100 pg, 0.1 mL) on the vagal hepatic afferents. As shown in the trace, an injection of leptin caused a gradual and long-lasting increase in afferent activity, which reached a peak value approximately 60 min after the injection. The discharge rates just before and 30, 60, 90, and 120 minutes after IPV injection were 65.2 ± 2.2 , $113.6 \pm 4.1^*$, $128.5 \pm 2.9^*$, $149.8 \pm 5.8^*$, and $83.1 \pm 2.5^*$ impulses per 5 seconds, respectively (*indicates a significant increase, $P < .05$) (Figure 2(b)). Figure 2(c) presents the afferent action potentials at times A, B, and C in Figure 2(a). Figure 2(d) presents the mean discharge rates of five different preparations before and 30, 60, and 90 minutes after IPV injection of leptin (100 pg, 0.1 mL). The mean discharge rates were 71.0 ± 5.7 , $95.7 \pm 10.0^*$, $100.7 \pm 9.6^*$, and $115.3 \pm 11.7^*$ impulses per 5

seconds, respectively (*indicates a significant increase, $n = 5$, $P < .05$). Further, each of five preparations demonstrates a significant increase in afferent discharge rates 60 and 90 minutes after IPV injection (Table 1). It was further observed that an IPV injection of saline (0.1 mL) as a control resulted in no significant change in discharge rates; IPV injection of 10 pg leptin resulted in no significant increase. The least effective dose to increase afferent activity was 100 pg.

3.3. Reflex Effects from Hepatoportal Leptin Sensors to Autonomic Outflow. Reflex effects of IPV infusion of leptin on the efferent activity of the sympathetic branch to the adrenal medulla, pancreas, liver, epididymal WAT, interscapular brown adipose tissue (BAT), as well as that of the celiac and pancreatic branches of vagus nerve, were investigated. Figure 3 presents typical examples of the effects of IPV infusion of leptin 1 ng (0.1 mL) on sympathetic and vagal outflows. The injection activated sympathetic nerve activity and inhibited vagal nerve activity to the organs mentioned above. Figure 4 and Table 2 show the mean discharge rates of sympathetic or vagal nerve activities before and after IV injection of leptin (1 ng, 0.1 mL). These observations demonstrated that IPV or IV administration of leptin at a dose of 1 ng activates sympathetic and inhibits vagal outflow.

In subsequent experiments, the effects of IPV injection of leptin (1 ng, 0.1 mL) on the efferent nerve activity of the

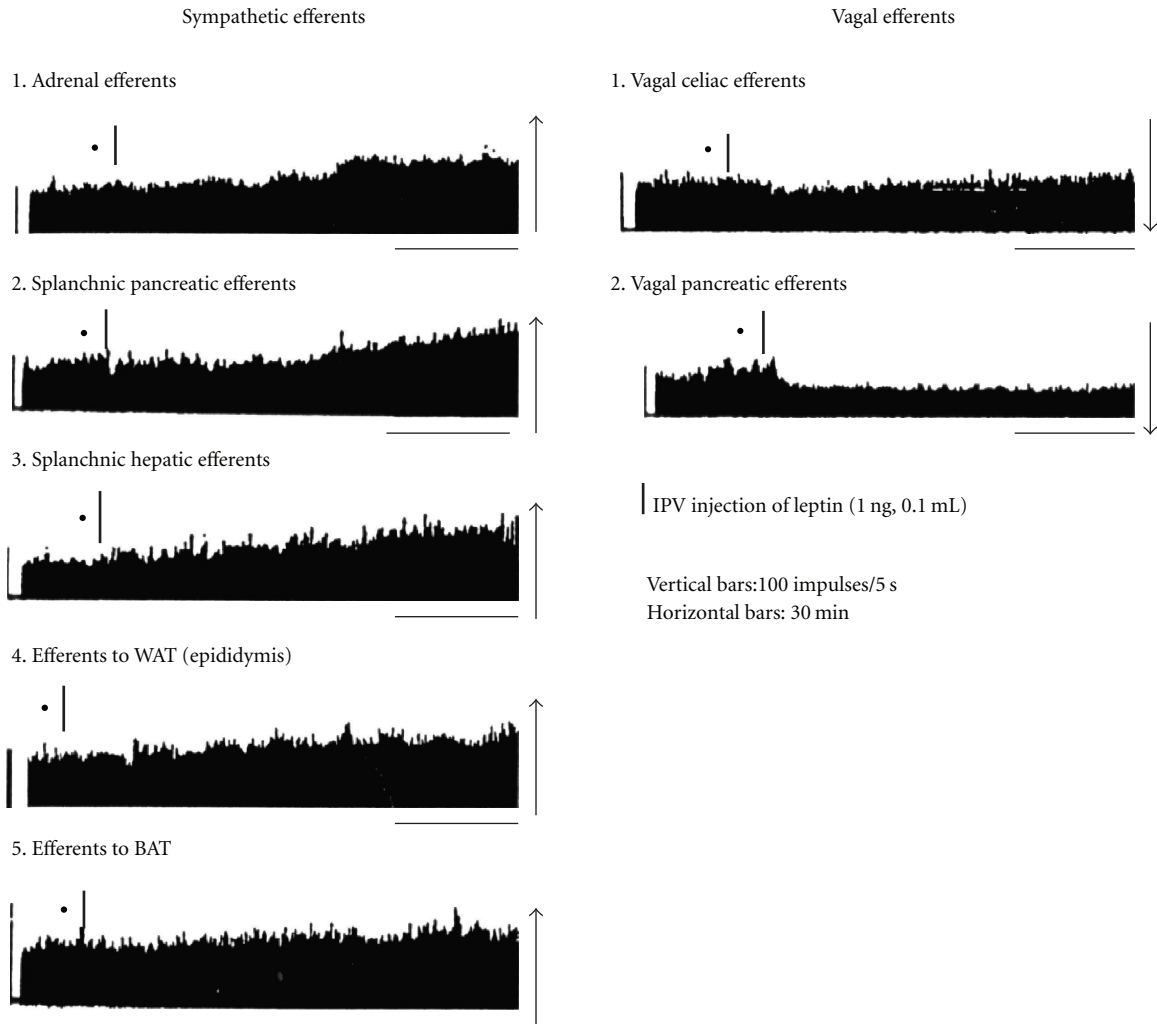


FIGURE 3: Reflex effects of IPV injection of leptin (1 ng, 0.1 mL) on efferent discharges of sympathetic and vagus nerve. Arrows indicate the time of injection.

TABLE 1: Firing rate response of vagal hepatic afferent fibers before and after intraportal injection of leptin (100 pg, 0.1 mL).

No.	Before	Impulses/5 seconds (mean \pm SEM)		
		30	60	90 minutes
1	54.0 \pm 3.1	61.2 \pm 1.9	70.6 \pm 2.8*	91.3 \pm 6.3*
2	73.2 \pm 2.2	89.1 \pm 3.0*	107.3 \pm 2.6*	122.1 \pm 1.2*
3	65.2 \pm 2.2	113.6 \pm 4.1*	128.5 \pm 2.9*	149.8 \pm 5.8*
4	88.5 \pm 2.4	116.9 \pm 2.7*	106.1 \pm 4.0*	126.2 \pm 3.3*
5	74.2 \pm 2.7	97.7 \pm 2.6*	90.8 \pm 2.2*	86.8 \pm 2.9*
Mean	71.0 \pm 5.7	95.7 \pm 10.0*	100.7 \pm 9.6*	115.3 \pm 11.7* ($n = 5, P < .05$)

(*Significant increase, $P < .05$).

adrenal sympathetic and vagal celiac nerves were compared in normal and hepatic vagotomized rats. As shown in the upper part of the left panel of Figure 5, leptin injection (1 ng, 0.1 mL) caused a significant increase in adrenal nerve activity in normal rats; however, no significant change in nerve activity occurred following IPV injection of leptin at the same dose in hepatic vagotomized rats. The lower part of the

left panel of the Figure 5 presents the effects of leptin on vagal celiac efferents. In the normal rat, leptin injection suppressed vagal nerve activity, although no significant change in nerve activity was observed following injection of leptin in hepatic vagotomized rats. The right panel of Figure 5 demonstrates that IV administration of leptin (1 ng, 0.1 mL) caused an increase in efferent activity of the sympathetic adrenal

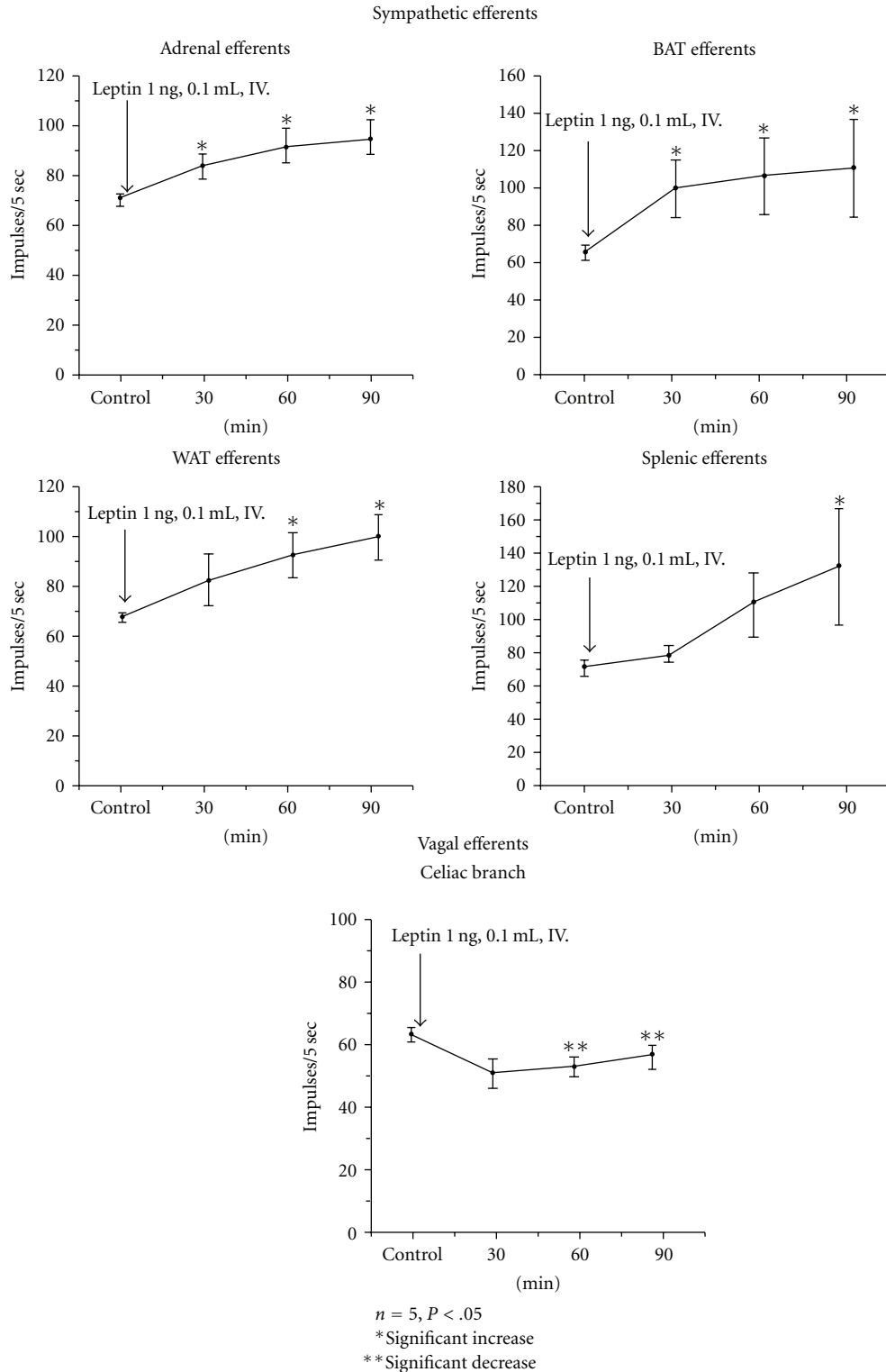


FIGURE 4: Reflex effects of IV injection of leptin (1 ng, 0.1 mL) on the mean discharge rates of sympathetic and vagus nerve.

efferents; further, in bilateral or hepatic vagotomized rats, no remarkable changes in efferent activity following IV injection of leptin at doses of 1 ng, 10 ng, 100 ng, or 1 μ g were observed. However, IV administration of a large amount of leptin (10 μ g) resulted in a remarkable increase in efferent activity.

This response might be the direct effect of blood-borne leptin on the hypothalamus. These observations indicate that IPV injection of leptin results in reflex activation of sympathetic outflow and reflex suppression of vagal outflow to visceral organs that regulate metabolic functions.

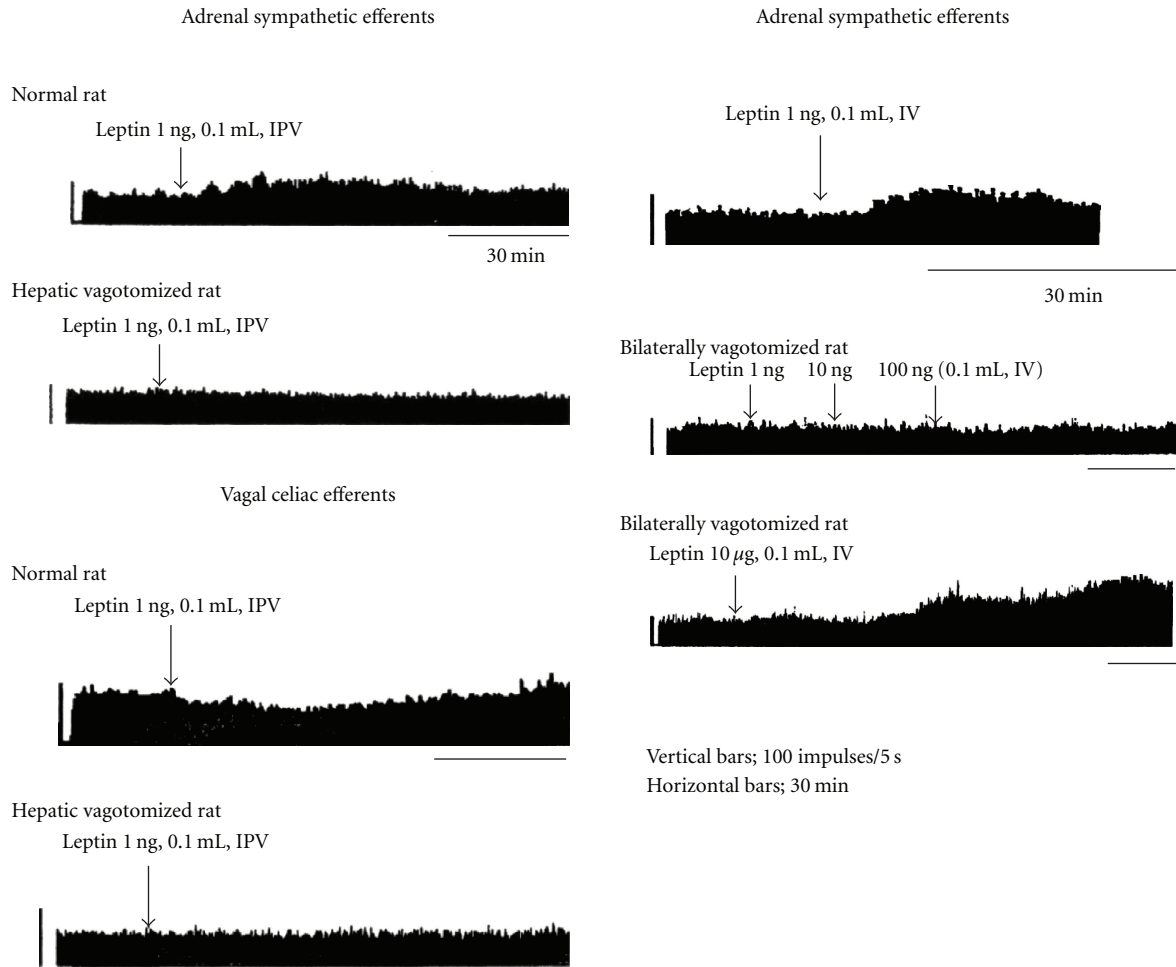


FIGURE 5: Effects of IPV or IV injection of leptin on the efferent activity of sympathetic nerve and vagus nerve in normal and hepatic or total vagotomized rat.

TABLE 2: Reflex responses of sympathetic and vagal efferents following IV injection of leptin (1 ng, 0.1 mL).

	Impulses/5 seconds \pm SE			
	Before injection	30 minutes	60 minutes	90 minutes
Sympathetic efferents				
Adrenal efferents ($n = 5$)	70.7 \pm 2.7	83.6 \pm 5.4*	92.0 \pm 6.8*	95.5 \pm 6.8*
WAT efferents ($n = 5$)	69.3 \pm 1.8	84.8 \pm 10.8	95.3 \pm 9.4*	102.3 \pm 9.6*
BAT efferents ($n = 5$)	65.0 \pm 3.7	100.2 \pm 16.0*	106.3 \pm 20.6*	110.7 \pm 26.2*
Splenic efferents ($n = 5$)	71.4 \pm 4.9	79.3 \pm 5.0	110.0 \pm 19.6	132.8 \pm 35.2*
Vagal efferents				
Vagal celiac efferents ($n = 5$)	63.5 \pm 2.4	50.6 \pm 4.7**	53.0 \pm 3.2**	56.2 \pm 3.9

(*Significant increase, **significant decrease, $P < .05$).

4. Discussion

It was stated in the introduction that both central and peripheral administration of leptin reduces food intake and body weight, and that the site of action of leptin is the hypothalamus [1, 2, 12]. It was also reported that leptin receptors are distributed within the hypothalamus and

choroid plexus [3–5], and that leptin directly stimulates activity of VMH (satiety enter) neurons and inhibits activity of LHA (feeding center) neurons [6], and stimulates/inhibits activity of ARC (dual center) neurons [13]. Interestingly, several lines of evidence support that leptin signaling in the central nervous system regulates autonomic outflow to white adipose tissue [14], brown adipose tissue [15], muscle and

liver [16]. In relation to this, the importance of the role played by the hepatic sympathetic nerves has been reported [7].

The most widely accepted hypothesis is that blood-borne leptin is transported into the brain by the choroid plexus and acts on the leptin receptors in the hypothalamus; chemical signals may thereby be translated into neural signals.

It was recently reported that leptin sensors exist in the WAT of the epididymis and send signals through afferent nerve fibers innervating WAT to the central nervous system; those afferent signals then evoke reflex activation of sympathetic nerve activity to the visceral organs, such as the adrenal medulla, pancreas, liver, WAT, and BAT, and evoke reflex inhibition of vagal nerve activity to the pancreas and liver [13, 14].

Further, it is possible to assume that peripheral leptin sensors also exist in the hepatoportal region, are sensitive to leptin liberated from the visceral WAT into the portal venous blood, and send signals to the brain via vagal hepatic afferent fibers.

The results of the present study demonstrated that an IPV or IV injection of leptin (100 pg to 1 ng, 0.1 mL) activated afferent nerve activity of the hepatic branch of the vagus nerve; an IV or IPV injection of leptin (1 ng, 0.1 mL) caused facilitation of sympathetic nerve activity to visceral organs such as the adrenal medulla, WAT, and BAT, and inhibition of vagal celiac and pancreatic nerve activity; the same amount of leptin injection was without effect in totally vagotomized or hepatic vagotomized rats; the doses of leptin (100 pg to 10 ng) used for IV or IPV injections were in the physiological range of plasma leptin concentrations (6 ± 1 ng/mL) [6]; IV or IPV injection of physiological saline was without effect on the afferent activity of the hepatic branch of the vagus nerve as well as on the reflex change in autonomic outflow; IV or IPV injection of a larger amount of leptin (1 μ g or 10 μ g) increased adrenal sympathetic nerve activity in totally or hepatic vagotomized rats. Further, the author observed that injections of leptin at doses of 0.5–1.0 μ g (1.5 μ l) into the lateral ventricle activated efferent activity of the adrenal sympathetic nerve (unpublished data).

In addition to the blood-borne leptin pathway and afferent signaling system from leptin sensors in the WAT, the present study demonstrated a third pathway of the leptin signaling system from hepatoportal leptin sensors to the brain. Leptin secreted from the abdominal visceral white adipose tissue into the portal vein may play an important role in reflex regulation of metabolic function. The existence of three different leptin signaling systems (the blood-borne system, afferent signaling system from WAT, and that from hepatoportal leptin sensors), is likely to represent a fail-safe alarm system.

The results of the present experiments demonstrate the reflex modulation of metabolic functions, including acceleration of lipolysis in WAT, increase in catecholamine secretion from the adrenal medulla, facilitation of glycolysis in the liver, and reduction in insulin release from the pancreas. The reflex center is in the hypothalamus or brainstem, and the afferent limb is made up of afferent fibers in the hepatic branch of the vagus nerve. The exact sites of

hepatoportal leptin sensors, as well as morphological and immunohistochemical properties of these sensors, remain to be studied.

5. Conclusion

The results of the present experiments indicate that afferent signals from leptin sensors in the hepatoportal region play a role in reflex regulation of metabolic functions through modulation of autonomic outflow.

Support for Work

ANBAS Corpotaion, Kita-ku, Osaka, 531-0072, Japan

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