CHAPTER 6. LEPTIN INJECTION INTO WHITE ADIPOSE TISSUE ELEVATES RENAL SYMPATHETIC NERVE ACTIVITY DOSE-DEPENDENTLY THROUGH THE AFFERENT PATHWAY IN RATS (Study 3)

The objectives of the Study 3 were to investigate whether leptin in WAT affected the sympathetic outflow to kidney. Therefore, this study assessed the effect of leptin injection into WAT on efferent renal SNA and BP in rats.

Research methods and procedures

Animals and diets

Twenty two male Sprague-Dawley rats fed standard chow (CE-2; Cler Japan, Tokyo, Japan), weighing about 400 to 450g, were used in the experiment. They were kept in a room maintained at 22 ± 1 ℃ with a 12h-12h light-dark cycle. Food and water were freely available until the days of the experiment. On the experimental days, food was removed 4 h before the experimental treatments.

Injection of leptin and measurements of renal SNA, MAP and HR

The general experimental methods were described in detail in Study 2. The animal was allowed to be stabilized for 60 min after placement of nerve electrodes. The baseline measurements of systemic arterial pressure, MAP, HR and renal SNA were made for 5 min just before injection. Recombinant murine leptin (IBL, Gunma, Japan) was kept in -20 °C and dissolved in physiological saline before use. The concentrations of the solution used for injection were 10 and 100 ng/kg. Leptin solution was injected into the left perirenal WAT through a fine needle (Terumo, 18G, Tokyo, Japan) with a small syringe (Terumo, 1 ml, Tokyo, Japan). After the injection of saline or leptin (10 ng/kg or 100 ng/kg), these parameters were measured in every 5 min for 60 seconds during 90 min experimental period.

Blood analysis

An arterial blood sample was obtained every 30 min for measurement of plasma levels of leptin and insulin, and blood levels of glucose and lactate. Analysis of blood samples was conducted similarly Study 2.

Statistical analysis

Data are reported as the mean ± SEM. In view of the interindividual variability of resting renal SNA, percent change from baseline was calculated for renal SNA. Change form baseline in the saline or leptin-treated rats was compared by Student's paired *t*-test. Differences between leptin-treated and saline-treated rats were assessed using a one-way ANOVA. $P<0.05$ was considered statistically significant.

Results

There was no significant difference between the control rats and leptin-treated rats in the basal renal SNA, MAP, HR, plasma levels of leptin and insulin, and blood levels of glucose and lactate (Table 6).

Leptin injections (10 and 100 ng/kg) into the left perirenal WAT increased the renal SNA dose-dependently. Fig. 6 showed the change of renal SNA after injections of leptin. An increase in renal SNA started within 15 min, then reached almost maximum at 60 min. The effects lasted for more than 90 min. After 90 min renal SNA reached 147 ± 18% (pre-injection level was expressed as 100%) with 10 ng/kg and 201 ± 34% with 100 ng/kg, respectively. Injection of saline did not change renal SNA.

MAP was not changed after saline or leptin injection into WAT. Plasma levels of leptin and insulin, and blood levels of glucose and lactate were not modified by the saline and leptin injection into WAT. HR gradually increased after injection of saline or leptin, but the magnitude of the increase was not significantly different between saline and leptin treatments (Table 6).

Table 6 Responses of renal SNA, MAP, HR, plasma leptin and insulin, and blood glucose and lactate to saline or leptin (10 or 100 ng/kg) injection in rats (Study 3)

	Time (min)	RSNA§ (pulse/sec)	MAP (mmHg)	HR (beats/min)	Leptin (ng/ml)	Insulin (μU/ml)	Glucose (mg/dl)	Lactate (mmol/l)
Saline (n=7)	0	100±17	68±4	415±20	5.3±0.9	4.2±2.0	106±7	1.5±0.1
	30	93±19	66±4	420±15	5.2±1.0	3.9±1.8	104±7	1.5±0.1
	60	104±15	69±4	428±15	5.2±1.0	4.0±1.8	107±7	1.5±0.1
	90	105±17	65±3	434±13	5.8±1.0	4.1±1.9	106±7	1.4±0.1
Leptin (10 ng/ml, n=8)	0	90±9	69±3	445±14	5.7±0.8	4.0±0.9	119±14	1.4±0.1
	30	125±13*	70±3	460±20	5.6±0.9	4.2±0.9	116±16	1.3±0.1
	60	161±23*	71±3	476±21	5.7±1.0	3.8±0.9	110±10	1.3±0.1
	90	165±25*	69±2	489±24	5.8±0.8	4.4±1.2	110±11	1.3±0.1
Leptin (100 ng/ml, n=7)	0	102±12	72±5	436±12	4.9±0.7	3.9±0.4	110±5	2.1±0.4
	30	170±19*	74±6	452±13	5.0±0.7	3.9±0.4	115±8	1.8±0.2
	60	185±22**	75±7	473±18	4.9±0.6	3.9±0.5	105±6	2.0±0.4
	90	189±28*	73±5	491±20*	4.9±0.7	3.9±0.5	109±6	2.1±0.4

§ Renal SNA

Values are means ± SEM. *P<0.05, **P<0.01 vs. time 0

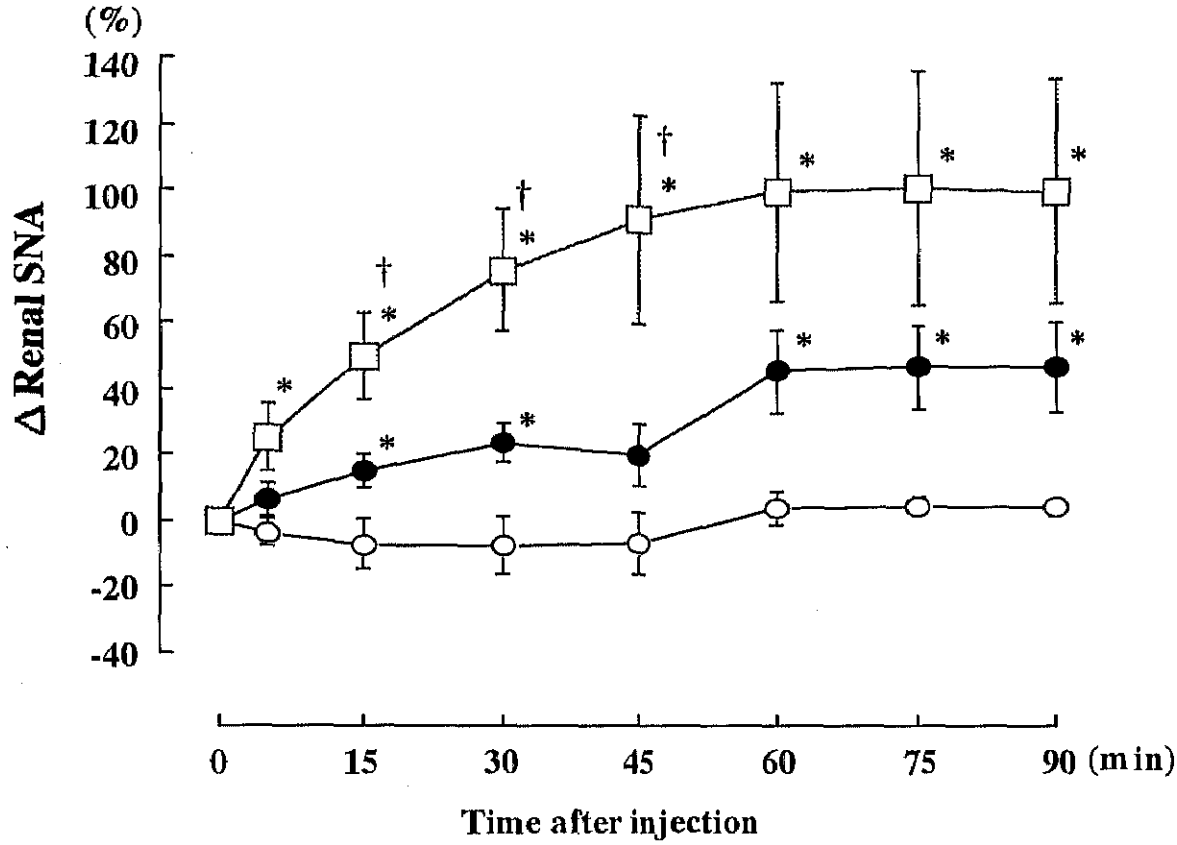

Fig. 6 **Percent changes from baseline in renal SNA after injection of saline (n=7, *open circle*) or leptin (10 ng/kg, n=8; *solid circle*, 100 ng/kg, n=7; *open square*) into white adipose tissue. Values are means ± SEM. * $P < 0.05$ vs saline group and † $P < 0.05$ vs. 10ng/kg leptin injection group (Study 3).**

Discussion

The observations obtained in the Study 3 clearly demonstrated that the local injection of leptin into perirenal WAT resulted in activation of renal SNA in dose-dependent manner without elevation of plasma leptin levels.

Recently, Niijima suggested that there were leptin sensors in WAT of epididymis. It was reported that injections of leptin into WAT activated afferent nervous activity in dose dependent manner (77). Furthermore, these local injection of leptin activated sympathetic outflow to WAT, liver, interscapular brown adipose tissue, adrenal medulla and pancreas (78). This study firstly demonstrated that leptin injection into WAT caused dose-dependent elevation of renal SNA. It was further shown that leptin injection into WAT did not elevate plasma leptin levels. In addition, other candidates for activation of the SNS, such as plasma insulin, glucose and lactate, were not changed by leptin injection either. Previous studies did not determine circulating levels of sympathetic nervous activator after leptin injection into WAT (77, 78). The present data strongly supported the idea that WAT have leptin sensors and the renal SNA was increased by activation of the afferent nerves from WAT.

Present data showed that leptin injection into WAT did not increase MAP in spite of elevation of renal SNA. In the previous studies, no change of BP by acute leptin infusion has been consistent (72, 90, 91). Generally, the renal SNA is a major factor for the long-term control of BP; elevation of renal SNA has pressor effects on renal BF, glomerular filtration rate, urinary sodium excretion and renin expression (85, 92). At present, the reason was not clear why MAP was not increased. However, one possible explanation is that bolus injection of leptin was insufficient to evoke MAP elevation as well as acute hyperleptinemia (72, 90, 91). Practically, chronic leptin infusion increased arterial pressure at 3 to 4 days after commencement of leptin infusion (71).

In conclusion of the Study 3, this study provided the additional evidences that there were leptin sensors in WAT. These data demonstrated that leptin injection into WAT increased renal SNA without the elevation of circulating leptin levels. These findings suggested that leptin activated the afferent nerves through the sensors in WAT, resulting in

elevation of renal SNA. The novel control pathway of renal SNA by leptin is possible to associate pathophysiology of FAT-related BP elevation.