

CHAPTER 5. ELEVATION OF RENAL SYMPATHETIC ACTIVITY AND BLOOD PRESSURE IN RATS ON HIGH-FAT DIET (Study 2)

The purpose of the Study 2 is to investigate the role of renal SNA in the pathophysiology of FAT related blood pressure elevation. Therefore, comparison of the basal levels of efferent renal SNA in FAT-fed and CHO-fed control rats was conducted. In addition, renal BF and urinary sodium excretion, controlled by renal SNA, were investigated in the rats.

Research methods and procedures

Animals and diets

Twenty four male Sprague-Dawley rats (FAT: n=11, CHO: n=13) aged 4 wk were purchased (Cler Japan, Tokyo, Japan). All of the experimental conditions were same as described in Study 1 during 20-21 wk of experimental period. Body weight and food intake were monitored in all rats throughout the experimental period. Five days before the experiment, the rats were moved to the metabolic cages, and 24 h urine was collected for analysis of urinary sodium excretion. On the experimental day, the rat was fasted for 4 h before the experimented treatments, and all of the experiments were conducted at same time.

Measurements of renal SNA, MAP, HR and renal BF

The rats were anesthetized by intraperitoneal injection of urethane (1 g/kg, Tokyo Kasei, Tokyo, Japan). After anesthesia, polyethylene catheters were inserted into the right femoral vein for the administration of gallamin triethiodide, the right femoral artery for the measurement of the systemic arterial pressure and HR, which was computed from the systemic arterial pressure pulses, and the left femoral artery for blood sampling. After the trachea was cannulated, the rats were paralyzed with gallamin triethiodide (initially 0.5 mg bolus, then 0.1 mg/h, Sigma, St. Louis, USA) and ventilated artificially by a respiratory pump (661; Harvard Apparatus, Boston, USA). The rats were fixed in a stereotaxic

frame, and the left renal nerve was exposed retroperitoneally through a left flank incision. Using a dissecting microscope, the distal cut end of the renal nerve was isolated from the surrounding tissue and was mounted on a pair of silver wire electrodes. Discharges of the renal nerve were amplified and filtered (low and high cut frequencies were 100 and 5 kHz, respectively). Hexamethonium chloride (Wako, Oosaka, Japan) was administered intravenously (10 mg/kg) to confirm that the activity recorded was post-ganglionic efferent SNA. A laser-Doppler flow meter probe (ALF21D, Advance, Tokyo, Japan) was attached to the surface of the left kidney for measurement of renal BF. The original nerve activity, systemic arterial pressure, MAP, HR and renal BF were monitored continuously and recorded on a magnetic tape (PC208A, Sony, Tokyo, Japan) for later analysis.

After the placement of nerve electrodes and physiological stabilization for 60 min, measurements of systemic arterial pressure, MAP, HR, renal BF and renal SNA were recorded for 5 min. Arterial blood samples were obtained immediately after the surgery to determine plasma leptin, insulin, glucose and lactate concentrations.

Multifiber nerve discharges were rectified (half wave) and smoothed (leaky integrator, time constant 50 ms). The smoothed signal was sampled every 0.1 s (MacLab, ADInstruments, Australia) then averaged for 5 min. The noise, that was determined after the animal was sacrificed by overdose of anesthetic was subtracted. The discharges were also evaluated by conversion to standard pulses by a window discriminator (EI-601G, Nihon Kohden, Tokyo, Japan) and counting every second for 5 min. The numbers of counted pulses were averaged. The threshold of the window was set just above the noise level recorded after the animal was sacrificed.

Blood and urine analysis

Plasma glucose and lactate levels were determined using the glucose analyzer BIOSEN 5030L (N.S.I., Tokyo, Japan). Plasma insulin and leptin levels were determined similarly Study 1. 24-h sodium excretion was calculated from urine volume and urinary sodium concentrations measured by flame photometry (ANA-135, Tokyo Photoelectric, Tokyo, Japan). And urinary sodium excretion rate was calculated by the

sodium excretion divided by the sodium intake.

Statistical analysis

Data are shown as the mean \pm SEM. Statistical evaluation was performed with Student's *t*-test. And $P < 0.05$ was considered statistically significant.

Results

Table 5 shows the characteristics of the experimental rats. After consuming the experimental diets for 20-21 weeks, body weight was slightly greater and abdominal adipose tissue weight was significantly greater in the FAT group than in the CHO group. The average energy intake was significantly higher in FAT compared with CHO. Blood glucose and plasma insulin levels were similar between the two dietary groups and leptin levels were higher in the FAT group.

MAP was significantly higher in the FAT group and HR was similar between both groups (Fig. 3).

Renal SNA calculated as either spikes per second and integrated voltage was significantly higher in the FAT group compared with the CHO group (Fig. 4).

Renal BF was significantly lower in the FAT group. In addition, the 24-h urinary sodium excretion rate was significantly lower in the FAT group (Fig. 5).

Table 5 Characteristics of rats after 20-21 weeks of CHO or FAT diet (Study 2)

	CHO		FAT	
	n	13	11	
Body weight, g		596 ± 8.7	624 ± 16.0	
Average energy intake, kcal/day		75.2 ± 1.0	85.9 ± 1.4**	
Abdominal fat weight [§] , g		41.6 ± 2.2	56.1 ± 3.8**	
Plasma insulin, µU/ml		167.2 ± 14.1	156.2 ± 16.8	
Blood glucose, mg/dl		219.7 ± 6.3	208.1 ± 7.0	
Plasma leptin, ng/ml		43.1 ± 3.9	73.3 ± 10.1**	

§ The total weight of retroperitoneal, mesenteric and epididymal fat-pads.

Values are means ± SEM. **P<0.01 vs. CHO group.

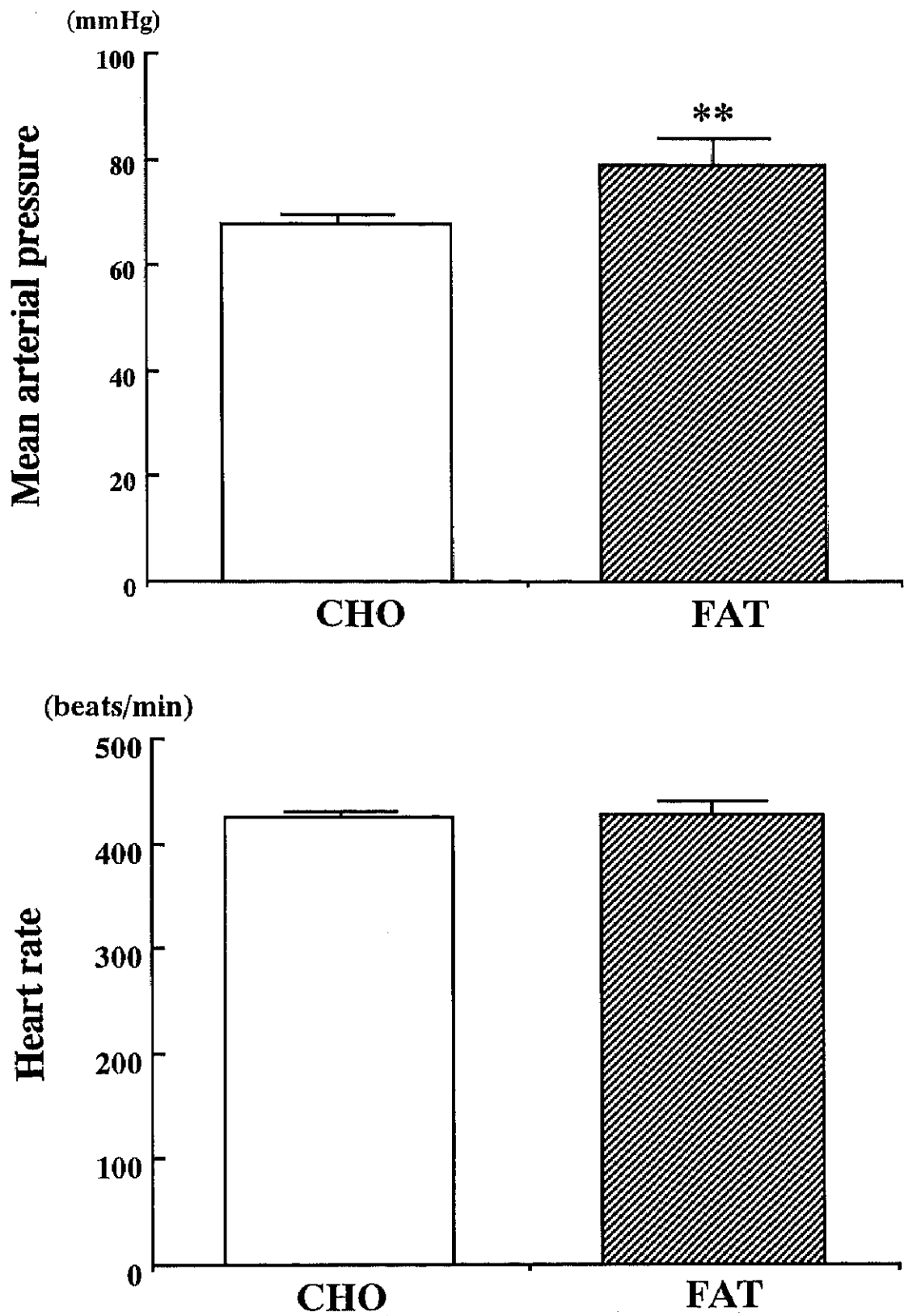


Fig. 3 Mean arterial pressure (top) and heart rate (bottom) of rats fed FAT (n=11) or CHO (n=13) for 20-21 weeks. Values are means \pm SEM. ** $P < 0.01$ vs. CHO group (Study 2).

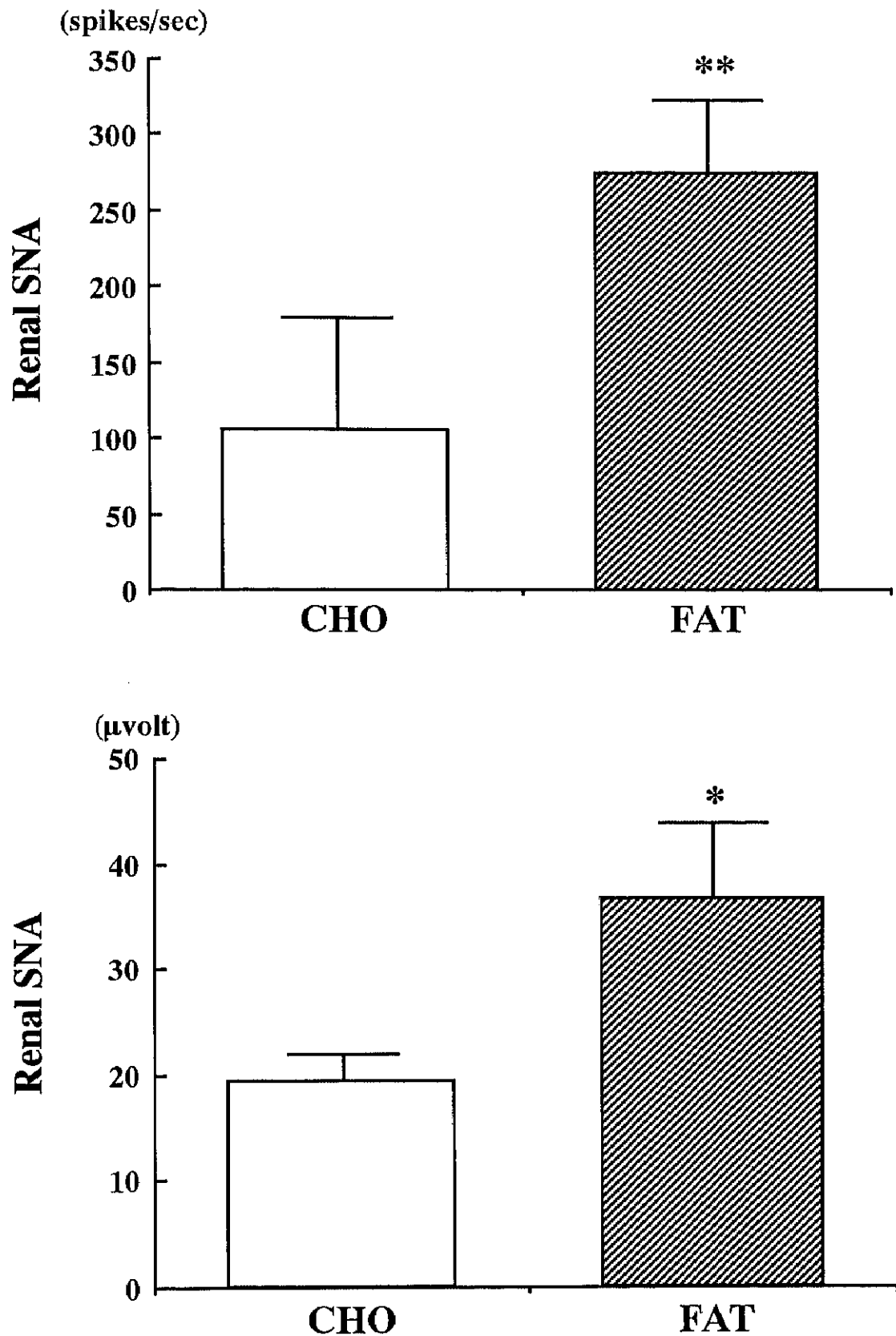


Fig. 4 Renal SNA measured by pulse counter (top) and by voltage integrator (bottom) in rats fed FAT (n=11) or CHO (n=13) for 20-21 weeks. Values are means \pm SEM. * $P < 0.05$, ** $P < 0.01$ vs. CHO group (Study 2).

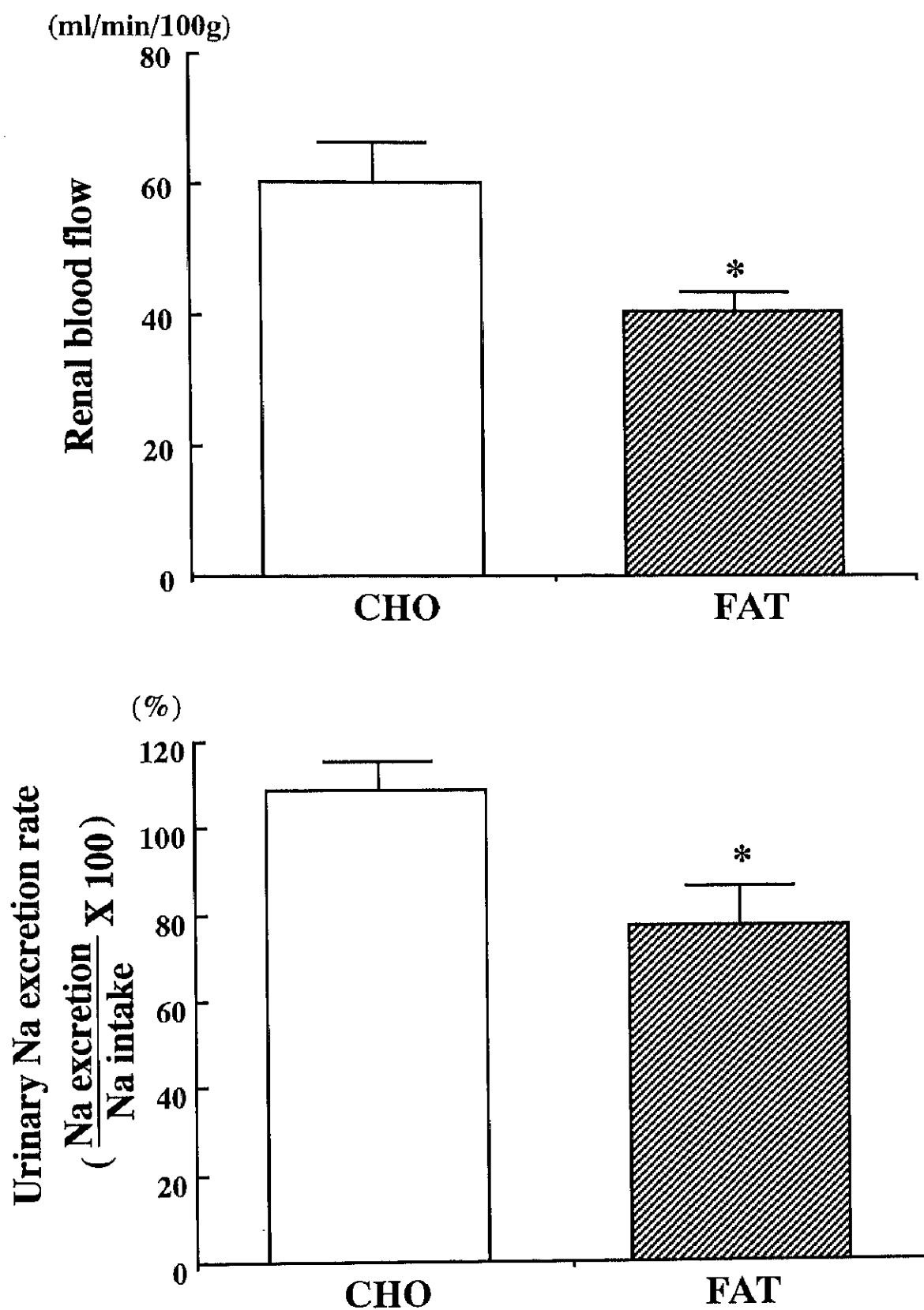


Fig. 5 Renal blood flow (top) and urinary sodium (Na) excretion rate (bottom) of rats fed FAT (n=11) or CHO (n=13) for 20-21 weeks. Values are means \pm SEM. * $P < 0.05$ vs. CHO group (Study 2).

Discussion

The Study 2 firstly demonstrated that the renal efferent sympathetic nerve discharge and BP were simultaneously higher in FAT-fed rats than in CHO-fed rats. The elevation of renal SNA would lower renal BF and urinary sodium excretion rate, resulting in increased sodium retention. Several researchers have suggested that renal denervation attenuates experimental hypertension and inhibits BP elevation (28-30). On the contrary, renal nerve stimulation reduced urinary volume and sodium excretion (85). Kassab et al. determined the hemodynamics and renal excretory responses to FAT in innervated and bilaterally renal-denervated chronically instrumented dogs (28). During 5 weeks of the diet period, arterial pressure significantly increased in innervated dogs, but not in bilaterally renal-denervated dogs. Furthermore, the decrease in sodium excretion in response to FAT was significantly greater in the innervated dogs than in the bilaterally renal-denervated dogs (28). On the other hand, sodium excretion was only transiently increased after denervation, although systemic arterial pressure was lowered by renal denervation in rats (29). In that study, it was not known whether the transient increase in sodium excretion contributed to lowered BP in renal denervated rats. As concerns natriuresis, proximal tubular sodium reabsorption is likely to be controlled by renal nerve (86). In spontaneously hypertensive rats, (Na⁺, K⁺)-ATPase activity in denervated kidney was lower than in contralateral kidney (86). These studies suggest that renal SNA plays a crucial role in BP regulation by control of urinary sodium excretion. Our data might also indicate that lowered renal BF and urinary sodium excretion rate in FAT-fed rats resulted from increased renal SNA. The urinary sodium excretion rate in 24-h, however, was lower by 30% in FAT-fed rats than in CHO-fed rats. Although this remarkable decrease in urinary sodium excretion rate in FAT-fed rats could contribute to BP elevation, it is difficult to believe that such aggravated natriuresis continued throughout the 20-21 wk experimental diet period. In the present study, we determined the 24-h urinary sodium excretion rate only one time. To obtain more reliable and reproducible data, repeated measurements of urinary sodium excretion rate may be necessary.

Although the importance of the renal nerve to BP regulation is well understood, only

a few studies have actually measured the renal SNA in the animal models of BP elevation (25, 26, 31, 32, 87). Measurement of renal efferent sympathetic nervous discharges one of reliable methods for investigating whether increased renal SNA participates in BP elevation. A study directly measured the basal levels of efferent renal SNA in genetic obesity (26). In genetically obese Zucker rats fed a high-salt diet (8.0% NaCl) for 2 wk, the basal efferent renal SNA and MAP were higher than in lean Zucker rats (26). Other studies investigated renal SNA in SHR (25, 87). In one study, basal renal SNA was similar to that of control WKY rats, despite MAP markedly increased in SHR (87). Another study showed marked increase in renal SNA in SHR fed a normal or a high-sodium diet compared with control WKY rats fed the same diets (25). Furthermore, Wong et al. investigated the effect of renal SNA on several different diets in rats (31), and showed that the rats fed a restricted diet, -40% of *ad libitum*, were decreased MAP compared with a standard chow *ad libitum*, a fructose and FAT fed rats, respectively. Renal SNA was lower in a restricted diet than in either an *ad libitum* or FAT, but not in fructose (31). BP elevation in a fructose might be caused by different mechanisms from renal SNA (17). Anyway, these studies suggested that renal SNA is an important factor of some types of BP elevation.

In the Study 2, the increase in BP and renal SNA may have been affected by either obesity or the FAT itself. Kaufman et al. investigated the effects of FAT on BP and SNA (17). They demonstrated that BP was higher concomitant with increase in body weight and urinary NE excretion in FAT fed rats compared with control rats. However, in rats fed a restricted FAT diet to avoid obesity, BP and urinary NE excretion were similar to control rats (17). In addition, no study has documented that non-obese FAT-fed elevates BP. Therefore, it might be suggested that elevation of BP and SNA were caused by obesity rather than the FAT alone. Another study measured NE turnover rates in the kidneys of rats with feeding FAT diet for 28 days. The results suggested that FAT did not affect NE turnover rate in the rat kidney (32). Another measured the basal levels of efferent renal SNA after 4 wk of FAT feeding in male Wister rats (31). In this *ad libitum* study, the renal SNA in FAT-fed rats was about 30% lower than in standard chow-fed rats,

but BP was similar to the standard chow-fed rats. Given these results, FAT in itself does not appear to increase renal SNA. In these studies, however, FAT was fed only about 4 wk and did not increase BP in the rats. Another possible cause for the elevation of renal SNA is increase in body weight and/or body fat accumulation. As described above, genetically obese rats have higher renal SNA compared with lean rats (26). 40% food restriction in rats decreased renal SNA compared with standard chow, 60% fructose diet and FAT (31). The weights of rats fed restricted diet were significantly reduced by 10 - 38% compared with other diets. These findings suggest that increases in body weight and/or body fat accumulation are important factors in the elevation of renal SNA. In the present study, FAT-fed rats showed a statistically significant increase in body fat accumulation. Thus, renal SNA was affected by the FAT-induced excess body fat accumulation.

Several acute and chronic studies suggest that increased plasma leptin elevates renal SNA (11, 68, 72). Since plasma leptin levels increased in FAT-fed rats, it is thought that circulating leptin activated the renal SNA in the present study. In addition, it is well known that insulin activates renal SNA through the CNS (80, 87). However, the present study found no increase in plasma insulin levels in FAT compared with CHO rats. Many previous studies have shown basal hyperinsulinemia in FAT fed rats (17, 88). We hypothesized that anesthetization by urethane disturbed glucose homeostasis (89). As described in Study 1, 16 wk of FAT feeding increased plasma insulin and glucose levels without anesthesia. Insulin would have a capability to elevate renal SNA (29). On the contrary, Morgan et al. failed to increase renal SNA by insulin administration (87). Furthermore, it is reported that 7 days insulin administration did not decrease in urinary sodium excretion, glomerular filtration rate and urine volume on the last day of hyperinsulinemia, suggesting not increase in renal SNA. (16). These studies suggested the contradictory effects of hyperleptinemia or hyperinsulinemia on renal SNA. Therefore, although insulin and leptin are main candidates for activation of SNS, whether or not renal SNA was affected by the hormones is not elucidated in this study.

In conclusion of the Study 2, FAT-induced obese rats were caused BP elevation,

which might be induced by increase in renal SNA. Elevated basal sympathetic activity in the kidney increases sodium retention in long-term FAT-fed rats, which would lead to BP elevation.