Effects of Adiponectin on the Renal Sympathetic Nerve Activity and Blood Pressure in Rats

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Adiponectin is an adipocytokine that modulates energy homeostasis and glucose metabolism. Here, we examined the effects of acute intravenous (iv) and lateral cerebral ventricular (LCV) injections of adiponectin on the renal sympathetic nerve activity (RSNA) and blood pressure (b/p) in urethane-anesthetized rats. Both iv and LCV injections of adiponectin induced dose-dependent suppressions of RSNA and b/p. Moreover, we found that bilateral lesions of the hypothalamic suprachiasmatic nucleus (SCN) abolished the effects of iv injection of adiponectin on RSNA and b/p. These findings suggest that adiponectin decreases the RSNA and b/p in a dose-dependent manner and that the SCN is implicated in mechanism of adiponectin actions on RSNA and b/p. These findings also suggest that the hypotensive-action activity of adiponectin is realized, at least partially, *via* changes in activities of autonomic nerves activity.
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Key words: adipocytokine; autonomic nervous system; cardiovascular action; hypotensive action; suprachiasmatic nucleus

Introduction

Adiponectin is an adipocytokine that was identified from a human adipose tissue cDNA library, and adipo Q, the mouse homologue of adiponectin, has been cloned and found to consist of 240 amino acids with a secretory signal sequence (amino acids 1–17) followed by collagenous (amino acids 45–111) and globular domains (amino acids 112–240) (1). Alterations in its activity have been implicated in obesity-related metabolic and vascular diseases (2). Hypertension is associated closely with various pathologic conditions, including obesity and insulin resistance. Previous studies have suggested that hypoadiponectinemia is involved in hypertension in humans (3) and that adiponectin deficiency contributes to the development of obesity-induced hypertension (4). Therefore, adiponectin might be an important modulator of cardiovascular functions. However, very little is known about the role of autonomic nerves in adiponectin-mediated control of blood pressure (b/p). Because renal sympathetic nerve activity (RSNA) plays a role in the regulation of b/p (5), we hypothesized that adiponectin might affect RSNA and b/p and investigated effects of adiponectin in urethane-anesthetized rats.

In addition, we found evidence that the hypothalamic suprachiasmatic nucleus (SCN), which is a master circadian oscillator, is involved in the control of autonomic and cardiovascular functions (6–13). For instances, autonomic and cardiovascular changes induced by light (9), odors (11, 12), L-carnosine (10), and Lactobacillus (13) were eliminated in the SCN-lesioned rats. Therefore, we investigated the effects of bilateral electrolytic lesions of the SCN on the changes induced by adiponectin injection.

Materials and Methods

Animals. This study used male Sprague-Dawley rats, weighing ~300 g. Rats were housed in a room maintained at 24 ± 1°C and illuminated for 12 hrs (08:00–20:00 hrs) daily. Food and water were freely available. Animals were

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adapted to this environment for at least 1 week before the experiment. All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of Osaka University.

General Animal Preparation. Animal maintenance and routine experimental procedures were performed as described previously (10). On the day of the experiment, rats were deprived of food for 4–6 hrs before surgery. Anesthesia was induced by intraperitoneal (ip) injection of 1 g kg^{-1} urethane. Once the rat was under the anesthesia, a polyethylene catheter was inserted into the left femoral vein for intravenous (iv) injections, into the left femoral artery for measurement of b/p, and into the right femoral artery for blood sampling. The animal was then cannulated intratracheally, fixed in a stereotaxic apparatus and maintained at 37.0°–37.5°C using an infrared lamp. Body temperature was measured using a thermometer inserted into the rectum. The animal was paralyzed with gallamine triethiodide (initially using 10 mg by iv, and thereafter, 4 mg hr^{-1} by iv), then ventilated artificially using a respiratory pump with a gas mixture of O_2 and room air (20% O_2). To reduce respiratory movements, a pneumothorax was applied. The end-expiratory partial pressure of carbon dioxide concentration was maintained at 3.0%–4.0% by adjusting the ventilation volume. We evaluated the adequacy of the depth of anesthesia by checking every half-hour throughout the experimental procedure to determine whether rapid variation of arterial blood pressure (±5 mm Hg) and heart rate (±10%) could be caused by paw pinches. Under a dissecting microscope, the left renal nerve was exposed retroperitoneally through a left-flank incision. The distal end of the nerve was ligated, cut, and then hooked up with a pair of silver-wire electrodes to record the efferent RSNA. The recording electrodes were immersed in a pool of liquid paraffin oil to prevent dehydration and electrical insulation.

The rat was allowed to stabilize for 30–60 mins after placement of the recording electrodes. The RSNA was amplified, filtered, and monitored by an oscilloscope, then stored on magnetic tape. Activity was sampled using a LEG-1000 system (Nihon Kohden Corporation, Tokyo, Japan), and data were obtained as described previously (10). The catheter in the left femoral artery was connected to a b/p transducer, and the output signal amplified using a b/p amplifier (AP641G, Nihon Kohden). Mean arterial pressure (MAP) was calculated from the b/p. Two needle electrodes were placed under the skin of the right arm and left leg for the electrocardiogram (ECG) to monitor heart rate (HR). The ECG signal was amplified using a bioelectric amplifier (AB-620G, Nihon Kohden). Blood pressure and ECG were monitored using an oscilloscope and sampled with a LEG-1000 system, then stored on a hard disk for off-line analysis.

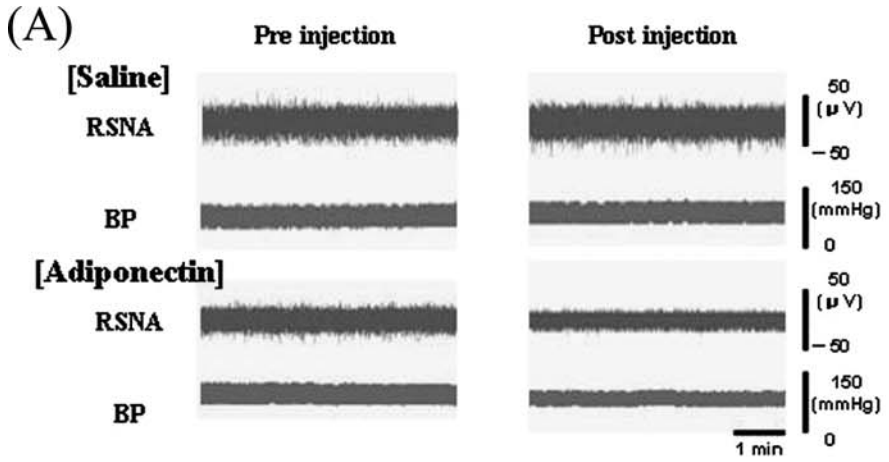
Intracerebroventricular Cannulation. As described previously (14), a brain cannula made of polyethylene tubing (PE-10; Clay Adams Division, Becton Dickenson Co., Parsippany, NJ) was inserted into the left lateral cerebral ventricle (LCV) under pentobarbital anesthesia (35 mg kg^{-1} ip), at least 1 week before the experiment. The following coordinates were used: 1.5 mm caudal to the bregma; 2.0 mm lateral to the midline; 3.0 mm below the skull surface (15).

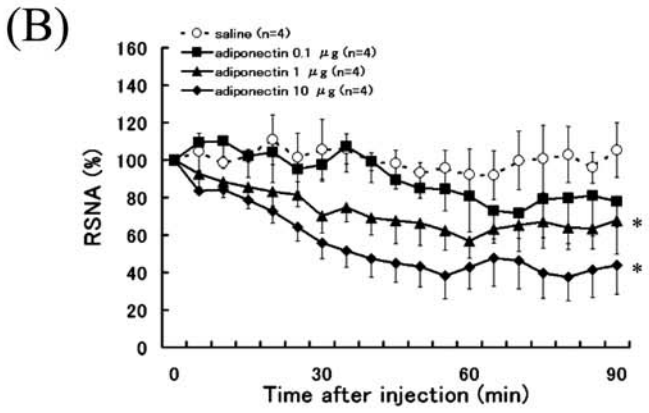
SCN Lesions. As described previously, bilateral electrolytic lesions of the SCN were performed on some rats ($n = 7$) 2 to 3 weeks before the adiponectin injection experiment (16). In brief, under pentobarbital anesthesia (35 mg kg^{-1}, ip), a stainless steel electrode was inserted into the SCN using the following coordinates: 1.2 mm posterior to the bregma; 0 mm from the midline; 9.0 mm from the skull surface, as per the atlas of Paxisons and Watson (15). A 1.0-mA anodal direct current was then passed through the electrode for 20 secs. Control rats ($n = 6$) received a sham-operation without the application of current. At the end of the experiment, the brain was removed and a histological examination performed using Cresyl-violet staining to verify placement of the bilateral lesions in the SCN. Only rats in which the lesions were accurately placed were included in the final data set.

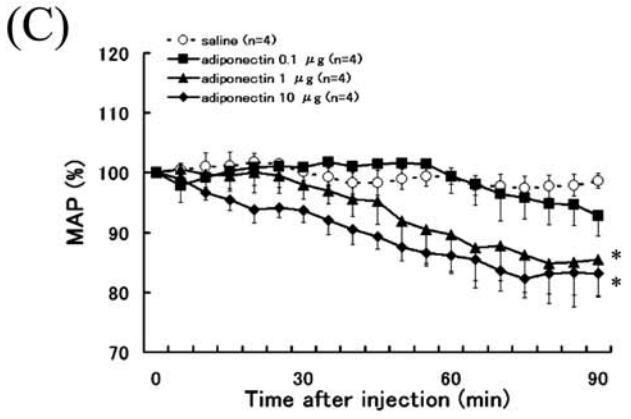
Experimental Protocol. Baseline measurements of RSNA, MAP, and HR were performed 5 mins before iv injection of 0.1, 1, or 10 μg of adiponectin in 0.1 ml saline, or saline (0.1 ml) and LCV injection of 0.01, 0.1; or 1 μg of adiponectin in 10 μl of artificial cerebrospinal fluid (aCSF), or aCSF (10 μl). Following injection, RSNA, MAP, and HR were recorded for 90 mins. The recombinant adiponectin used in this experiment was synthesized in the laboratory of the Department of Metabolic Medicine, Osaka University Graduate School of Medicine (17). To determine plasma levels of adiponectin and glucose, arterial blood was collected. At the conclusion of the experiment, hexamethonium chloride was administered (10 mg kg^{-1}, iv) to ensure that the recording was obtained from postganglionic efferent sympathetic nerve activity.

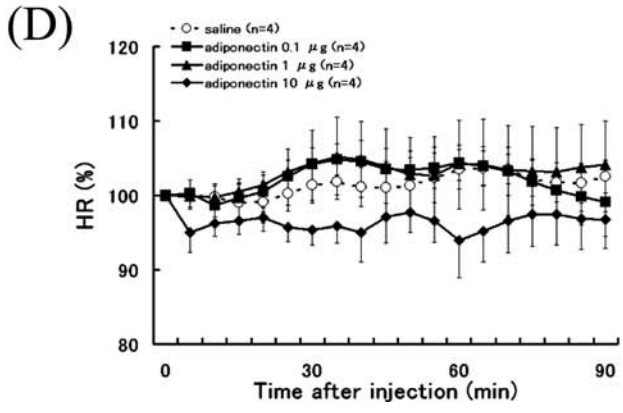
Determinations of Plasma Glucose and Adiponectin. Plasma levels of glucose and adiponectin after iv injection of adiponectin were determined under urethane anesthesia. Plasma concentrations of glucose or adiponectin were measured from arterial blood samples using a glucose analyzer (Fuji-DRI-CHEM, Fuji Photo Film Co., Tokyo, Japan) or enzyme-linked immunoabsorbent assay (ELISA) kits (Otsuka Pharmaceutical Co., Tokyo, Japan), respectively. Moreover, to examine effect of ganglionic blocker, hexamethonium chloride (5 mg kg^{-1}) was intravenously injected 10 mins before adiponectin injection (18).

Data Analyses. RSNA, MAP, and HR were measured every 5 mins following injection of adiponectin, saline, or aCSF, and these data were evaluated by digital signal processing. All data were expressed as means ± SEM. Differences between the basal values of the adiponectin and control groups were detected using the Mann-Whitney *U* test. Because preinjection values varied between individuals, percentages of change from the baseline values were calculated for RSNA, MAP, and HR. Friedman test was performed for comparisons with Time 0 in the groups


(A)
Pre injection
Post injection
[Saline]
RSNA
BP
[Adiponectin]
RSNA
BP
50
(μV)
−50
150
(mmHg)
0
1 min


(B)
saline (n=4)
adiponectin 0.1 μg (n=4)
adiponectin 1 μg (n=4)
adiponectin 10 μg (n=4)
RSNA (%)
Time after injection (min)


(C)
saline (n=4)
adiponectin 0.1 μg (n=4)
adiponectin 1 μg (n=4)
adiponectin 10 μg (n=4)
MAP (%)
Time after injection (min)


(D)
saline (n=4)
adiponectin 0.1 μg (n=4)
adiponectin 1 μg (n=4)
adiponectin 10 μg (n=4)
HR (%)
Time after injection (min)

treated with adiponectin, saline, or aCSF. Statistical analyses of differences between the before and after injections were performed using the Student's paired *t* test.

Results

Typical recordings of RSNA and b/p before and 90 mins following iv injection of saline or adiponectin are presented in Figure 1A. Comparison with saline injection indicated that adiponectin (10 μg) suppressed both RSNA and b/p at 90 mins following injection (postinjection). Time courses of RSNA and MAP after iv injection of adiponectin or saline are presented in Figures 1B and C. Following injection, the suppressive effects of adiponectin (10 μg) on RSNA and MAP (Figs. 1B and C, respectively) became marked by 15–25 mins; maximum suppression occurred at 75–85 mins. The lowest levels attained were 42.2% ± 14.4% spikes/5 secs (RSNA) and 82%.3 ± 3.2% mm Hg (MAP). In contrast, there was no significant effect on either RSNA or MAP levels at 90 mins following saline injection. Intravenous injections of lower doses of adiponectin (1 μg, but not 0.1 μg) resulted in a significant decrease in RSNA and MAP (Figs. 1B and C, respectively); maximum suppressive response occurred 90 mins postinjection. Neither saline nor adiponectin (0.1, 1, and 10 μg) significantly affected the HR after iv injection (Fig. 1D). The significance of differences with 0 time in the groups for values obtained from 5–90 mins were analyzed using the Friedman test.

RSNA (Fig. 2A) and MAP (Fig. 2B) levels were observed following LCV injection of adiponectin or aCSF. Adiponectin (1 μg) caused a marked suppression of RSNA and MAP, which became significant 15–35 mins post-injection; the maximum suppressive response was observed 85–90 mins postinjection. The lowest levels attained were 51.9% ± 5.3% spikes/5 secs (RSNA) and 74.6% ± 4.5% mm Hg (MAP). In contrast, injection of aCSF did not affect RSNA or MAP levels significantly. RSNA and MAP decreased significantly by 90 mins following LCV injection of a lower dose of adiponectin (0.1 μg; Figs. 2A and B, respectively), whereas the lowest dose (0.01 μg adiponectin) was not suppressive (Figs. 2A and B, respectively). Neither aCSF nor adiponectin (0.01, 0.1, and 1 μg) significantly affected the HR after LCV injection (Fig. 2C). The significance of differences with 0 time in the groups for values obtained from 5–90 mins were analyzed using the Friedman test. Absolute basal (0 mins) RSNA, MAP, and HR values for the experiments shown in Figures 1 and 2 are summarized in Table 1. Differences between the basal values of the adiponectin and control groups were not significant (Mann-Whitney *U* test) for either route of injection.

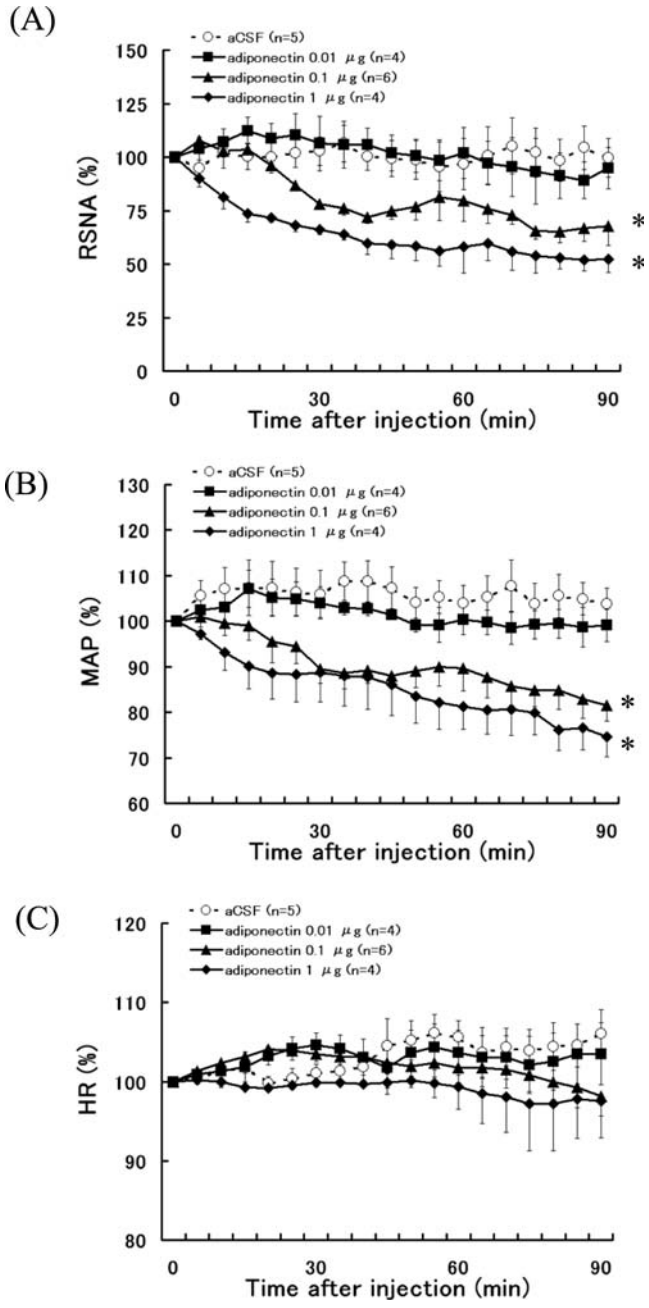


Figure 2. Effects of LCV injection of adiponectin on RSNA and MAP. (A) RSNA, (B) MAP, and (C) HR following LCV injection of aCSF (10 μl) or adiponectin (0.01, 0.1, or 1 μg) are expressed as mean ± SEM percentages of basal values (0 mins). The numbers of animals used in each group are indicated in parentheses. * $P < 0.05$ represents comparison with Time 0 (Friedman test).

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Figure 1. Effects of iv injection of adiponectin on RSNA and b/p. Representative trace data from recordings of (A) RSNA and b/p before (preinjection) and 90 mins following (postinjection) the iv injection of saline (0.1 ml) or adiponectin (10 μg). (B) RSNA, (C) MAP, and (D) HR following iv injection of saline (0.1 ml) or adiponectin (0.1, 1, or 10 μg) are expressed as mean ± SEM percentages of basal values (0 mins). The numbers of animals used in each group are indicated in parentheses. * $P < 0.05$ represents comparison with Time 0 (Friedman test).

Table 1. Basal Levels (0 mins) of RSNA, MAP, and HR in Experimental Group[a]

Groups	No. of RSNA (spikes/5 secs)	No. of MAP (mm Hg)	No. of HR (beats/min)
Experiment 1			
Saline	100.1 ± 23.4 (4)	66.6 ± 9.1 (4)	352.7 ± 15.7 (4)
Adiponectin (0.1 μg)	81.6 ± 9.7 (4)	67.5 ± 3.9 (4)	358.7 ± 5.5 (4)
Adiponectin (1 μg)	77.3 ± 6.1 (4)	66.4 ± 3.5 (4)	369.5 ± 24.6 (4)
Adiponectin (10 μg)	96.9 ± 15.8 (4)	76.7 ± 4.1 (4)	341.2 ± 22.8 (4)
Experiment 2			
Saline	84.0 ± 26.7 (5)	66.6 ± 9.1 (5)	375.4 ± 22.3 (5)
Adiponectin (0.01 μg)	75.7 ± 6.3 (4)	67.5 ± 3.9 (4)	329.2 ± 32.4 (4)
Adiponectin (0.1 μg)	62.3 ± 4.8 (6)	66.4 ± 3.5 (6)	308.0 ± 26.0 (6)
Adiponectin (1 μg)	87.1 ± 14.5 (4)	76.7 ± 4.1 (4)	379.5 ± 26.5 (4)
Experiment 3			
SCN-sham, saline	68.4 ± 16.5 (6)	70.3 ± 1.8 (6)	378.3 ± 32.5 (6)
SCN-sham, adiponectin	76.9 ± 20.3 (5)	77.3 ± 5.7 (5)	365.6 ± 39.9 (5)
SCN-lesion, saline	79.2 ± 16.8 (6)	78.2 ± 5.9 (6)	353.8 ± 33.1 (6)
SCN-lesion, adiponectin	67.4 ± 10.0 (7)	81.8 ± 4.6 (7)	345.1 ± 17.6 (7)

[a] Data are presented as means ± SEM. The numbers in parentheses indicate number of animals in each group. RSNA, renal sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate.

We investigated the effects of bilateral electrolytic lesions of the SCN on the autonomic and cardiovascular changes induced by iv injection of adiponectin (Fig. 3). Representative photomicrographs of the hypothalamus showing the SCN of a sham-operated rat and a bilaterally SCN-lesioned rat are presented in Figure 3A. Although part of the optic chiasm was damaged in some SCN-lesioned rats, the pupillary reflex induced by light stimulation of both eyes remained intact in all SCN-lesioned rats. In addition, the rhythmic locomotor activity was vanished in all SCN-lesioned rats (Fig. 3B). In sham-operated rats, RSNA and MAP were suppressed by iv injection of 10 μg of adiponectin, whereas SCN-lesioned rats remained unaffected (Figs. 3C and D), suggesting that bilateral lesions of the SCN eliminated adiponectin-induced changes in RSNA and MAP. In both SCN-lesioned and sham-operated rats, neither saline nor adiponectin significantly affected HR after iv injection (Fig. 3E). The significance of differences with 0 time in the groups for values obtained from 5–90 mins were analyzed using the Friedman test. Absolute basal (0 mins) RSNA, MAP, and HR values for sham-operated or SCN-lesioned rats are shown in Table 1; differences between the respective basal values of groups treated with saline or adiponectin were not statistically significant (Mann-Whitney *U* test).

In addition, we determined the changes in plasma glucose levels before and at 90 mins after iv injection of adiponectin or saline (Table 2). The basal values (before injection) were not significantly different between groups treated with saline or 0.1, 1, or 10 μg of adiponectin. However, the plasma glucose levels at 90 mins after iv injection of either 1 or 10 μg of adiponectin were significantly lower than the basal values ($P < 0.05$, paired *t* test). Moreover, pretreatment with hexamethonium clearly inhibited decrease in the plasma glucose levels induced by 10 μg of adiponectin. The plasma glucose changes (Δmg dl^{-1}) from 0 to 90 mins following treatment with iv injections of saline; 0.1, 1, or 10 μg of adiponectin; or 10 μg adiponectin and pretreatment with hexamethonium were –4.8 ± 5.6, 0.3 ± 15.7, –20 ± 4.3, –26.7 ± 7.7, and –6.1 ± 2.0, respectively. Therefore, it is suggested that iv injection of adiponectin decreases plasma glucose levels *via* action on autonomic nerves.

We determined the changes in plasma adiponectin levels before and after iv injection of adiponectin or saline (Table 3). The basal values (0 secs or 0 mins) were not significantly different between groups treated with saline or 10 μg of adiponectin. In addition, iv injection of saline or 10 μg of adiponectin did not change the plasma adiponectin levels 30 secs–90 mins after injection.

Discussion

In the present study, we observed that either iv or LCV injection of adiponectin suppressed RSNA and b/p in a dose-dependent manner and that this effect could be eliminated by bilateral electrolytic lesions of the SCN. These findings suggest that adiponectin affects autonomic neurotransmission and cardiovascular function through the SCN.

Generally, it is known that plasma adiponectin levels in rats are about 2–4 μg/ml (19), and we actually examined the plasma adiponectin levels, resulting in about 2.5–4.0 μg/ml (Table 3). Therefore, adiponectin doses (0.1, 1, and 10 μg) injected intravenously in the present study are lower. Unfortunately, we did not examine the effects of high doses of adiponectin, but Masaki *et al.* (20) confirmed that peripheral injections of high doses of adiponectin activated sympathetic nerve activity in brown adipose tissue. With respect to these effects, we previously found that L-carnosine also causes similar responses in RSNA and b/p

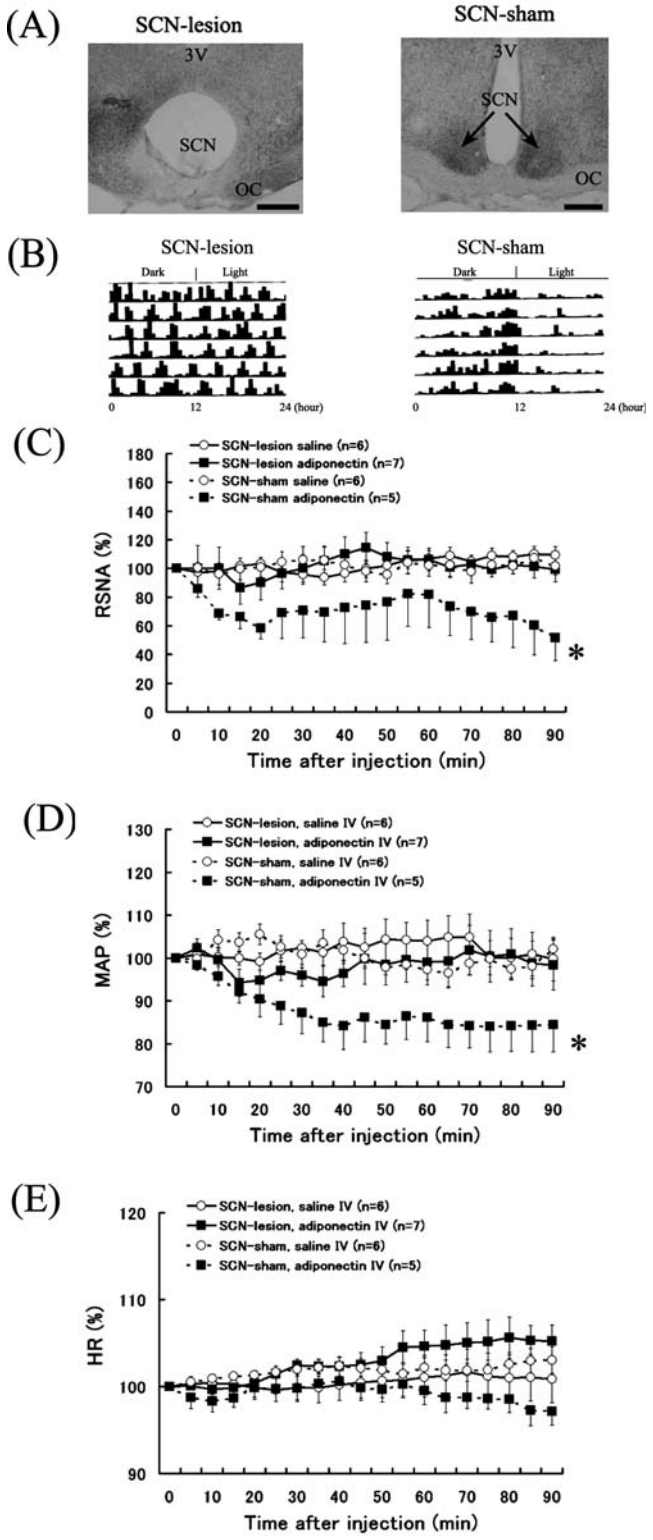

Figure 3. Effects of bilateral lesions of the SCN on changes in RSNA and MAP following iv injection of adiponectin. (A) Representative photomicrographs of coronal brain sections, including the SCN from sham-operated (SCN-sham) rats and from rats that received SCN lesions (SCN-lesion). Abbreviations: SCN, the suprachiasmatic nucleus (arrows show the intact bilateral SCN on the SCN-sham rats); 3V, the third ventricle; CO, the optic chiasm. Scale bars = 300 μm. (B) Representative actograms show locomotor activity of SCN-sham and SCN-lesion rats individually housed in 12:12 light and darkness conditions. (C) RSNA, (D) MAP, and (E) HR after iv injection of saline (0.1 ml) or adiponectin (10 μg) are expressed as mean ± SEM percentages of basal values (0 mins). Data from SCN-sham and SCN-lesion rats are shown. * $P < 0.05$ represents comparison with Time 0 (Friedman test).

Table 2. Effects of IV Injections of Adiponectin on Plasma Glucose Levels (mg dl^{-1})[a]

Group	Before injection	After injection
Saline	155.0 + 17.1	150.2 + 22.2
Adiponectin (0.1 μg)	143.0 + 22.6	143.3 + 15.2
Adiponectin (1 μg)	162.3 + 4.7	142.0 + 2.5*
Adiponectin (10 μg)	143.3 + 19.3	116.7 + 27.0*
HC + adiponectin (10 μg)	160.8 + 17.8	154.7 + 19.4

[a] Data are presented as means + SE for four rats. Statistical significance of differences was evaluated by paired *t* tests. HC, hexamethonium chloride.
* $P < 0.05$ compared between before and 90 mins after injection.

by inducing both suppression and elevation at lower and higher doses, respectively (10). We will, therefore, investigate the effects of high doses of adiponectin on RSNA and b/p in the future.

A recent study demonstrated that the concentration of adiponectin in the cerebrospinal fluid increased following iv injections of adiponectin, suggesting that peripheral adiponectin might cross the blood-brain barrier and function by direct action in the brain (21). In addition, it has been proposed that other peripheral peptides, such as leptin and ghrelin, act on the brain directly by crossing the blood-brain barrier (22, 23) because central injections of peripherally effective doses of these peptides do not affect either RSNA or b/p (24). In the present study, the minimum suppressive dose of both RSNA and b/p by peripherally and centrally administered adiponectin were 1 μg and 0.1 μg, respectively (Figs. 1 and 2). Thus, our data support the hypothesis that adiponectin affects RSNA and b/p by crossing the blood-brain barrier and acting directly on the brain. On the other hand, a recent study observed that it would be hard for adiponectin to pass through the blood-brain barrier (25). Therefore, we explored the mechanisms for adiponectin actions in the brain. First, we (26) and Niijima (27) demonstrated that leptin, produced in white adipose tissue (WAT), acts on afferent nerves, transmitting information from the WAT to the brain, and changes autonomic nerve activities, including RSNA (26, 27). We further confirmed that infradiaphragmatic denervation of all the vagal nerves surrounding the esophagus eliminated the responses of RSNA and b/p to iv injection of 1 μg adiponectin (data not shown). In addition, there was no change in the plasma adiponectin levels after iv injection of adiponectin (Table 3). Therefore, these data suggest that adiponectin might also send information to the brain through the afferent nerves,

Table 3. Effects of IV Injections of Adiponectin on Plasma Adiponectin Levels ($\mu g\ ml^{-1}$)[a]

Time after injection	Saline group	Adiponectin 10-μg group
(secs)		
0	3.00 ± 0.38	4.00 ± 0.36
30	3.15 ± 0.42	3.56 ± 0.50
60	3.00 ± 0.40	3.50 ± 0.32
300	2.83 ± 0.38	3.47 ± 0.28
(mins)		
0	3.20 ± 0.23	2.70 ± 0.44
30	2.89 ± 0.19	2.57 ± 0.41
60	2.97 ± 0.28	2.56 ± 0.40
90	2.77 ± 0.31	2.29 ± 0.47

[a] Data are presented as means ± SE for four rats.

but this hypothesis is not certain, and further research will be necessary in the future. Second, circulating adiponectin might act on the arcuate nucleus connecting the SCN reciprocally. Yi *et al.* (28) recently found that there is reciprocal connection between the arcuate nucleus and the circumventricular organs, playing an important role in the exchange of circulating information, suggesting that peripheral hormones, including adiponectin, might act on the brain without passing the blood-brain barrier. Therefore, it is possible that the arcuate nucleus might be responsible for sensing signals of peripheral adiponectin.

In the present experiment, we observed that bilateral lesions of the SCN eliminated adiponectin-induced suppression of RSNA and b/p (Fig. 3). Studies using pseudorabies virus, which exhibits retrograde and multisynaptic transport, suggest that the SCN (a master circadian oscillator) sends multisynaptic sympathetic and parasympathetic projections to the peripheral organs, including the kidneys (6, 29). Moreover, bilateral lesions of the SCN eliminate changes in RSNA and b/p that are induced by strong light (9), odors (11, 12), and L-carnosine (10), and these findings implicate the SCN in mediating the effects of adiponectin on RSNA and b/p. Although studies have indicated that adiponectin receptors 1 and 2 are expressed primarily in skeletal muscle and the liver, expression of these receptors has been identified in the brain (30). At present, we do not know whether these receptors localize to the SCN, and their distribution in the brain will be examined in future studies. However, we have observed evidence indicating that brain histaminergic neurons are involved in the mechanisms controlling the autonomic and cardiovascular effects of odors (11, 12, 31, 32) and L-carnosine (10). Therefore, the site of adiponectin activity in the brain might be the hypothalamic tuberomammillar nucleus, where histaminergic neurons localize.

Previously, we demonstrated that light stimulation (9), odor exposure (11, 12, 31, 32), leptin (27), and L-carnosine (33) affected both the sympathetic and parasympathetic nerves. In a preliminary experiment on anesthetized rats, we observed that iv injection of adiponectin inhibited adrenal sympathetic nerve activity and elevated gastric parasympathetic nerve activity (unpublished observation). Adrenaline is a one of factors related to cardiovascular functions and glucose metabolism. Because adrenaline secretion is controlled by the adrenal sympathetic nerve, suppression of this nerve might be involved in both the hypoglycemic and hypotensive effects of adiponectin. In fact, we previously showed that hyperglycemic response to 2-deoxy-D-glucose (2DG) and the adrenal sympathetic nerve activity were suppressed by lower-dose L-carnosine (33). In addition, we found that a ganglion blocker eliminated the response of blood glucose levels to iv injections of adiponectin (Table 2). Therefore, these results support the suggestion that adiponectin might affect the regulation of blood glucose through autonomic neurotransmissions. Because histamine H1- or H3-receptor blockers abolished the effects of odors (11, 12) and L-carnosine (10) on RSNA and b/p, a blocker, such as thioperamide, might also negate the suppressive effect of adiponectin, and this possibility should be investigated in future studies.

There are several potential limitations of this study that need to be addressed. We used anesthetized rats in the present study, and it could be that different results would have been obtained in conscious rats, especially because urethane anesthesia affects sympathetic nerve activity and blood glucose levels (34). With regard to L-carnosine affecting sympathetic nerve activity in urethane-anesthetized rats, our previous study has showed that L-carnosine inhibited 2DG-hyperglycemia in conscious rats (33). At present, it is not clear whether adiponectin has the suppressing effects on 2DG-hyperglycemia in conscious rats as well as on L-carnosine. Therefore, further investigation using conscious rats will be needed in the future.

In conclusion, the present findings suggest that adiponectin acts in the brain and suppresses RSNA and b/p in a dose-dependent manner. The SCN might be involved in this mechanism. However, further study is required to reveal the precise pathway responsible for the sympathetic and cardiovascular effects of adiponectin. A recent study showed that chronic administration of adiponectin inhibited obesity-related hypertension in mice, and b/p in the adiponectin-knockout mice was elevated, suggesting that adiponectin supplementation might be effective therapy for hypertension in patients with the metabolic syndrome (4). Therefore, our results, showing that acute administration of adiponectin lowered the b/p, support this idea and suggest the immediate effect of adiponectin on b/p.

1. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271:10697–10703, 1996.
2. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221:286–289, 1996.
3. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa

Y. Decreased plasma adiponectin concentration in patients with essential hypertension. Am J Hypertens 16:72–75, 2003.
4. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, Hibuse T, Ryo M, Nishizawa H, Maeda N, Maeda K, Shibata R, Walsh K, Funahashi T, Shimomura I. Adiponectin replenishment ameliorates obesity-related hypertension. Hypertension 47:1108–1116, 2006.
5. Guyenet PG, Koshiya N, Huangfu D, Baraban SC, Stornetta RL, Li YW. Role of medulla oblongata in generation of sympathetic and vagal outflows. Prog Brain Res 107:127–144, 1996.
6. Buijs RM, Chun SJ, Niijima A, Romijn HJ, Nagai K. Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. J Comp Neurol 431:405–423, 2001.
7. Nagai K, Nagai N, Shimizu K, Chun S, Nakagawa H, Niijima A. SCN output drives the autonomic nervous system: with special reference to the autonomic function related to the regulation of glucose metabolism. Prog Brain Res 111:253–272, 1996.
8. Nagai K, Niijima A, Nagai N, Hibino H, Chun SJ, Shimizu K, Nakagawa H. Bilateral lesions of the hypothalamic suprachiasmatic nucleus eliminated sympathetic response to intracranial injection of 2-deoxy-D-glucose and VIP rescued this response. Brain Res Bull 39: 293–297, 1996.
9. Niijima A, Nagai K, Nagai N, Nakagawa H. Light enhances sympathetic and suppresses vagal outflows and lesions including the suprachiasmatic nucleus eliminate these changes in rats. J Auton Nerv Syst 40:155–160, 1992.
10. Tanida M, Niijima A, Fukuda Y, Sawai H, Tsuruoka N, Shen J, Yamada S, Kiso Y, Nagai K. Dose-dependent effects of L-carnosine on the renal sympathetic nerve and blood pressure in urethane-anesthetized rats. Am J Physiol Regul Integr Comp Physiol 288:R447–455, 2005.
11. Tanida M, Niijima A, Shen J, Nakamura T, Nagai K. Olfactory stimulation with scent of essential oil of grapefruit affects autonomic neurotransmission and blood pressure. Brain Res 1058:44–55, 2005.
12. Tanida M, Niijima A, Shen J, Nakamura T, Nagai K. Olfactory stimulation with scent of lavender oil affects autonomic neurotransmission and blood pressure in rats. Neurosci Lett 398:155–160, 2006.
13. Tanida M, Yamano T, Maeda K, Okumura N, Fukushima Y, Nagai K. Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci Lett 389:109–114, 2005.
14. Nagai N, Nagai K, Chun SJ, Shimizu K, Takezawa K, Tsuji M, Sugahara K, Nakagawa H. Roles of the suprachiasmatic nucleus and vasoactive intestinal peptide in the response of plasma arginine vasopressin to osmotic challenge. Endocrinology 137:504–507, 1996.
15. Paxisons G, Watson C. The Rat Brain in Stereotaxic Coordinates (4th ed.). Sydney: Academic Press, 1998.
16. Chun SJ, Niijima A, Nagai N, Nagai K. Effect of bilateral lesions of the suprachiasmatic nucleus on hyperglycemia caused by 2-deoxy-D-glucose and vasoactive intestinal peptide in rats. Brain Res 809:165–174, 1998.
17. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83, 1999.
18. Yamamoto H, Nagai K, Nakagawa H. Time-dependent involvement of autonomic nervous system in hyperglycemia due to 2-deoxy-D-glucose. Am J Physiol 255:E928–933, 1988.
19. Ran J, Hirano T, Fukui T, Saito K, Kageyama H, Okada K, Adachi M. Angiotensin II infusion decreases plasma adiponectin level *via* its type 1 receptor in rats: an implication for hypertension-related insulin resistance. Metabolism 55:478–488, 2006.
20. Masaki T, Chiba S, Yasuda T, Tsubone T, Kakuma T, Shimomura I, Funahashi T, Matsuzawa Y, Yoshimatsu H. Peripheral, but not central, administration of adiponectin reduces visceral adiposity and upregulates the expression of uncoupling protein in agouti yellow (Ay/a) obese mice. Diabetes 52:2266–2273, 2003.
21. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS. Adiponectin acts in the brain to decrease body weight. Nat Med 10:524–529, 2004.
22. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides 17: 305–311, 1996.
23. Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. J Pharmacol Exp Ther 302:822–827, 2002.
24. Matsumura K, Tsuchihashi T, Fujii K, Iida M. Neural regulation of blood pressure by leptin and the related peptides. Regul Pept 114:79–86, 2003.
25. Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55:141–147, 2006.
26. Tanida M, Iwashita S, Ootsuka Y, Terui N, Suzuki M. Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. Neurosci Lett 293:107–110, 2000.
27. Niijima A. Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. J Auton Nerv Syst 73:19–25, 1998.
28. Yi CX, van der Vliet J, Dai J, Yin G, Ru L, Buijs RM. Ventromedial arcuate nucleus communicates peripheral metabolic information to the suprachiasmatic nucleus. Endocrinology 147:283–294, 2006.
29. Sly JD, Colvill L, McKinley JM, Oldfield JB. Identification of neural projections from the forebrain to the kidney, using the virus pseudorabies. J Auton Nerv Syst 77:73–82, 1999.
30. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769, 2003.
31. Shen J, Niijima A, Tanida M, Horii Y, Maeda K, Nagai K. Olfactory stimulation with scent of grapefruit oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 380:289–294, 2005.
32. Shen J, Niijima A, Tanida M, Horii Y, Maeda K, Nagai K. Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 383:188–193, 2005.
33. Yamano T, Niijima A, Iimori S, Tsuruoka N, Kiso Y, Nagai K. Effect of L-carnosine on the hyperglycemia caused by intracranial injection of 2-deoxy-D-glucose in rats. Neurosci Lett 313:78–82, 2001.
34. Sanchez-Pozo A, Alados JC, Sanchez-Medina F. Metabolic changes induced by urethane-anesthesia in rats. Gen Pharmacol 19:281–284, 1988.