Dose-Different Effects of Orexin-A on the Renal Sympathetic Nerve and Blood Pressure in Urethane-Anesthetized Rats

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Previous studies have demonstrated that central injection of orexin-A affects renal sympathetic nerve activity (RSNA) and blood pressure (BP) in both anesthetized and unanesthetized rats. In the present study, we examined, using urethane-anesthetized rats, the dose-dependent effects of intravenous (iv) or intralateral cerebral ventricular (LCV) injection of various doses of orexin-A on RSNA and BP. We found that injection of a low dose of orexin-A (10 ng iv or 0.01 ng LCV) suppressed RSNA and BP significantly. Conversely, a high dose (1000 ng iv or 10 ng LCV) of orexin-A elevated both RSNA and BP significantly. Pretreatment with either iv or LCV injection of thioperamide, a histaminergic H_3-receptor antagonist, eliminated the effects of a low dose of orexin-A on both RSNA and BP. Both iv and LCV injection of diphenhydramine, a histaminergic H_1-receptor antagonist, abolished the effects of a high dose of orexin-A on RSNA and BP. Furthermore, bilateral lesions of the hypothalamic suprachiasmatic nucleus (SCN) abolished the effects of both low and high doses of orexin-A on RSNA and BP. These findings suggest that orexin-A affects RSNA and BP in a dose-dependent manner and that the SCN and histaminergic nerve may be involved in the dose-different effects of orexin-A in rats.
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Key words: autonomic nerve; suprachiasmatic nucleus; histaminergic nerve; thioperamide; diphenhydramine

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Introduction

Orexin-A has been identified as a hypothalamic neuropeptide and is thought to be involved in the control mechanisms for food intake (1), sleep (1), thermoregulation (2), and autonomic and cardiovascular functions (3). With respect to autonomic and cardiovascular regulations, a previous study demonstrated that intracerebroventricular injection of orexin-A (0.3 and 3 nmol, or ~1 and 10 μg, respectively) increases renal sympathetic nerve activity (RSNA) and blood pressure (BP) in conscious rats (3). To our knowledge, the effects of intravenous (iv) injection of various amounts (0.1–1000 ng) of orexin-A on RSNA and BP have not yet been evaluated.

Orexin neurons in the lateral hypothalamic area project to the histaminergic tuberomammillary nucleus (TMN), a site of origin for histamine neurons (4). Central neural histamine is involved in the regulation of cardiovascular functions through the histaminergic H_1-receptor (5). Thus, the effects of orexin-A on RSNA and BP also may be mediated by histaminergic neurons. Furthermore, we observed evidence in rats that the hypothalamic suprachiasmatic nucleus (SCN) is involved in the control of BP through autonomic nerves (6) in addition to functioning as a master circadian oscillator (7). These facts suggest that the SCN may play an important role in changes of the RSNA and the BP by orexin-A. Therefore, in the present study, we first assessed the effects of peripheral and central administrations of various amounts of orexin-A on RSNA and BP. Next, we evaluated the effects of histaminergic blockers (H_3- and H_1-receptor antagonists) and bilateral lesions of the SCN on changes induced by in urethane-anesthetized rats.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 300–350 g were used. Rats were housed in a room maintained at 24°C ± 1°C and illuminated for 12 hrs (0800–2000 hrs) every day. Food and water were freely available. Rats were adapted to the 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. General preparation was performed as described previously (6). Briefly, on the day of the experiment, food was removed 4–6 hrs before surgery. Anesthesia was induced with an intraperitoneal (ip) injection of 1 g/kg of urethane. A polyethylene catheter was inserted into the left femoral vein for iv injections, and another catheter was inserted into the left femoral artery for BP determination. The rat was then fixed in a stereotaxic apparatus after a tracheal cannulation. The body temperature was maintained at 37.0°C–37.5°C using an infrared lamp and a thermometer inserted into the rectum. The rat was paralyzed with gallamine triethiodide (10 mg iv initially and 4 mg/hr iv thereafter) and then ventilated artificially by respiratory pump (Harvard Apparatus, Edenbridge, UK) with a gas mixture of O_2 and room air (20% O_2). A pneumothorax was induced to reduce respiratory movement. The end-expiratory P_{CO_2} concentration was maintained at 3.0%–4.0% by adjusting the ventilation volume. Before and after the rat was paralyzed with gallamine triethiodide, we evaluated the adequacy of the depth of anesthesia by checking every half-hour throughout the experimental procedure whether rapid variation of arterial BP (±5 mm Hg) and heart rate (HR; ±10%) would be caused by paw pinch (8). When any one of these responses was found, a supplemental urethane (0.1–0.2 g/kg) was given with an ip injection. The depth of anesthesia was maintained under certain conditions during experimental period. Using a dissecting microscope, the left renal nerve was exposed retroperitoneally through a left flank incision. The distal end of the nerve was ligated and then hooked up with a pair of silver-wire electrodes for recording the efferent RSNA. The recording electrodes were immersed in a pool of liquid paraffin oil to prevent dehydration and for electrical insulation. The rat was allowed to stabilize for 30–60 mins after being placed on the recording electrodes.

The RSNA was amplified, filtered, monitored by an oscilloscope, and stored on magnetic tape. The activity was sampled with the LEG-1000 system (Nihon Kohden, Tokyo, Japan). Data were obtained as described previously (6). The catheter in the left femoral artery was connected to a BP transducer, and the output signal of the transducer was amplified in a BP amplifier. The BP was averaged (mean arterial pressure [MAP]). Two needle electrodes were placed under the skin at the right arm and left leg to record an electrocardiogram (ECG) to monitor HR. The ECG signal was amplified with a bioelectric amplifier. The BP and ECG were monitored with an oscilloscope, sampled with the LEG-1000 system, and stored on a hard disk for off-line analysis.

Intracerebroventricular Cannulation. At least 1 week before the experiment, a brain cannula made of polyethylene tubing (PE-10; Clay Adams, Parsippany, NJ) was inserted into the left lateral cerebral ventricle (LCV; 1.5 mm caudal to the bregma; 2.0 mm lateral to the midline; 3.0 mm below the skull surface) under pentobarbital anesthesia (35 mg/kg ip) as described previously (9).

Experimental Protocol. Baseline measurements of RSNA, MAP, and HR were made for 5 mins just before iv injections of orexin-A (0.1, 1, 10, 100, and 1000 ng/0.1 ml saline) or saline (0.1 ml) alone and LCV injections of orexin-A (0.01, 0.1, 1, and 10 ng/10 µl artificial cerebrospinal fluid [aCSF]) or aCSF (10 µl) alone. After the injection, these parameters were recorded for 120 mins. Effects of thioperamide maleate (200 µg/0.1 ml saline iv or 20 µg/100 µl aCSF LCV), a histaminergic H_3-antagonist, or diphenhydramine hydrochloride (50 µg/0.1 ml saline iv or 5 µg/100 µl aCSF LCV), a histaminergic H_1-antagonist, on orexin-A–induced effects on RSNA and BP were examined. These antagonists were administered iv 30 mins before iv injection of orexin-A. We observed that thioperamide maleate or diphenhydramine hydrochloride alone did not affect RSNA, MAP, or HR (data not shown). At the end of the experiment, hexamethonium chloride (10 mg/kg iv) was administered to ensure that the recording was made from postganglionic efferent sympathetic nerve activity.

SCN Lesions. In some rats (n = 12), bilateral electrolytic lesions were made in the SCN 2 to 3 weeks before the iv or LCV injection of orexin-A using experimental methods described previously (9, 10). Briefly, under pentobarbital anesthesia (35 mg/kg ip), a stainless steel electrode was inserted into the SCN with coordinates (1.2 mm posterior to the bregma; 0 mm from the midline; 9.0 mm from the skull surface) from the atlas of Paxisons and Watson (11), and then a 1.0 mA anodal direct current was passed through the electrode for 20 secs. Control rats (n = 11) received a sham operation following the same procedure but without the current. At the end of the experiment, the brain was removed, and a histologic examination was performed to verify adequate placement of bilateral lesions in the SCN by cresyl violet staining. Only rats with accurately placed lesions were used as SCN-lesioned (SCNL) animals.

Data Analyses. The RSNA, MAP, and HR measured during each 5-min period after injections of orexin-A, saline, or aCSF were evaluated by digital signal processing and statistical analyses. All data were expressed as the mean ± SEM. Because of the interindividual variability in the preinjection state, the percentage change from baseline also was calculated for RSNA and MAP. Analysis of variance (ANOVA) with repeated measures was applied to compare group responses of RSNA and BP induced by orexin-A, saline, or aCSF. The Mann-Whitney U test was applied to compare basal levels of RSNA and BP in the groups. A level of $P < 0.05$ was considered to be statistically significant.

Results

Sample recordings of RSNA and BP before and throughout a 120-min period following iv injection of

saline or orexin-A are presented in Figure 1A. Saline injection did not affect either RSNA or MAP. Both, however, were suppressed by iv injection of 10 ng of orexin-A, and both were elevated by iv injection of 1000 ng of orexin-A. Following injection of 10 ng of orexin-A, both RSNA and MAP decreased gradually (Fig. 1B and D, respectively), with the greatest level of suppression occurring at 105 and 120 mins, respectively. The lowest levels attained were 67.4% ± 3.9% for RSNA and 82.0% ± 2.5% for MAP. Following injection of 1000 ng of orexin-A, both RSNA and MAP increased gradually (Fig. 1B and D, respectively), with maxima occurring at 40 and 115 mins, respectively. The highest levels attained were 149.7% ±18.0% for RSNA and 113.2% ± 1.5% for MAP. In contrast, injection of saline did not cause a significant alteration in the levels of either RSNA or MAP. At 120 mins following iv injection of lower doses of orexin-A (1 and 100 ng), the levels of both RSNA and MAP decreased (Fig. 1C and E, respectively). The maximum suppressive responses occurred following injection of 10 ng of orexin-A. In contrast, at 120 mins, the higher dose of orexin-A (1000 ng) significantly increased both RSNA and MAP. The significance of the differences between values from 5–120 mins as a group was analyzed by repeated-measures ANOVA. The following comparisons were made: for RSNA, saline versus 10 ng of orexin-A ($P < 0.0005$, $F = 20.6$) and saline versus 1000 ng of orexin-A ($P < 0.005$, $F = 12.7$); for MAP, saline versus 10 ng of orexin-A ($P < 0.0005$, $F = 15.6$) and saline versus 1000 ng of orexin-A ($P < 0.0005$, $F = 29.9$). Absolute basal (0-min) RSNA and MAP values for the experiments shown in Figure 1 are summarized in Table 1. Differences in respective basal values were not statistically significant (Mann-Whitney U test).

Figure 2 shows the change in RSNA (Fig. 2A) and MAP (Fig. 2C) after LCV injection of orexin-A or aCSF. The injection of a lower dose of orexin-A (0.01 ng) into the LCV significantly suppressed RSNA and MAP, and the maximum suppression was observed at 80–85 mins, with the lowest levels of 35.0% ± 7.3% for RSNA and 88.8% ± 3.0% for MAP. On the other hand, the LCV injection of 10 ng of orexin-A elevated RSNA and MAP, and the maximum values occurred at 15–85 mins, with the highest levels of 234.9% ± 65.5% for RSNA and 134.9% ± 21.1% for MAP. In contrast, aCSF injection did not significantly affect RSNA and MAP levels. As in the case of iv injection of orexin-A, LCV injection of a lower dose of orexin-A (0.01 ng) significantly decreased RSNA (Fig. 2B) and MAP (Fig. 2D), and a higher dose of orexin-A (10 ng) significantly increased RSNA (Fig. 2B) and MAP (Fig. 2D), at 90 mins. The significance of the differences between values from 5–90 mins as a group was analyzed by repeated-measure ANOVA. The following comparisons were made: for RSNA, aCSF versus 0.01 ng of orexin-A ($P < 0.0005$, $F = 26.4$) and aCSF versus 10 ng of orexin-A ($P < 0.0005$, $F = 44$); for MAP, aCSF versus 0.01 ng of orexin-A ($P < 0.0005$, $F = 48.5$)and aCSF versus 10 ng of orexin-A ($P < 0.0005$, $F = 23.9$). Absolute values of basal RSNA and MAP in LCV injection experiments are summarized in Table 1; values did not differ significantly among any of these groups.

In a previous study (6), we found that thioperamide inhibits the suppressive effects of a low dose of L-carnosine on RSNA and BP and that diphenhydramine eliminated the enhancement of the response caused by a high dose of L-carnosine on RSNA. In the present study, RSNA and MAP were compared between a saline-saline or aCSF-saline control group and groups given iv injections of orexin-A. Both RSNA and MAP were significantly reduced by 10 ng of orexin-A and elevated by iv injection of 1000 ng of orexin-A in saline-pretreated rats (Fig. 3A and B) and in aCSF-pretreated rats (Fig. 3C and D). Pretreatment with thioperamide or diphenhydramine eliminated the effects of injection of both lower and higher doses of orexin-A, respectively, for both iv (Fig. 3A and B) and LCV (Fig. 3C and D) experimental groups. In contrast, pretreatment with diphenhydramine did not affect suppression of the RSNA and MAP induced by iv injection of 10 ng of orexin-A, and pretreatment with thioperamine did not affect elevation of RSNA and MAP induced by iv injection of 1000 ng of orexin-A (data not shown). The significance of the differences between values from 5–120 mins as a group was analyzed by repeated-measures ANOVA. The following comparisons were made: for RSNA, saline-saline versus saline–orexin-A (10 ng: $P < 0.005$, $F = 12.1$; 1000 ng: $P < 0.005$, $F = 9.3$), saline–orexin-A (10 ng) versus thioperamine–orexin-A (10 ng; $P < 0.0005$, $F = 15.0$), saline–orexin-A (1000 ng) versus diphenhydramine–orexin-A (1000 ng; $P < 0.05$, $F = 7.4$; Fig. 3A), aCSF-saline versus aCSF–orexin-A (10 ng: $P < 0.05$, $F = 7.9$; 1000 ng: $P < 0.0005$, $F = 19.7$), aCSF–orexin-A (10 ng) versus thioperamine–orexin-A (10 ng; $P < 0.005$, $F = 13.2$), thioperamine-saline versus thioperamine–orexin-A (10 ng; NS, $F = 3.4$), aCSF-orexin-A (1000 ng) versus diphenhydramine–orexin-A (1000 ng; $P < 0.0005$, $F = 16.9$), and diphenhydramine–saline versus diphenhydramine–orexin-A (1000 ng; NS, $F = 1.9$; Fig. 3C); for MAP, saline-saline versus saline–orexin-A (10 ng: $P < 0.005$, $F = 11.4$; 1000 ng: $P < 0.05$, $F = 6.6$), saline–orexin-A (10 ng) versus thioperamine–

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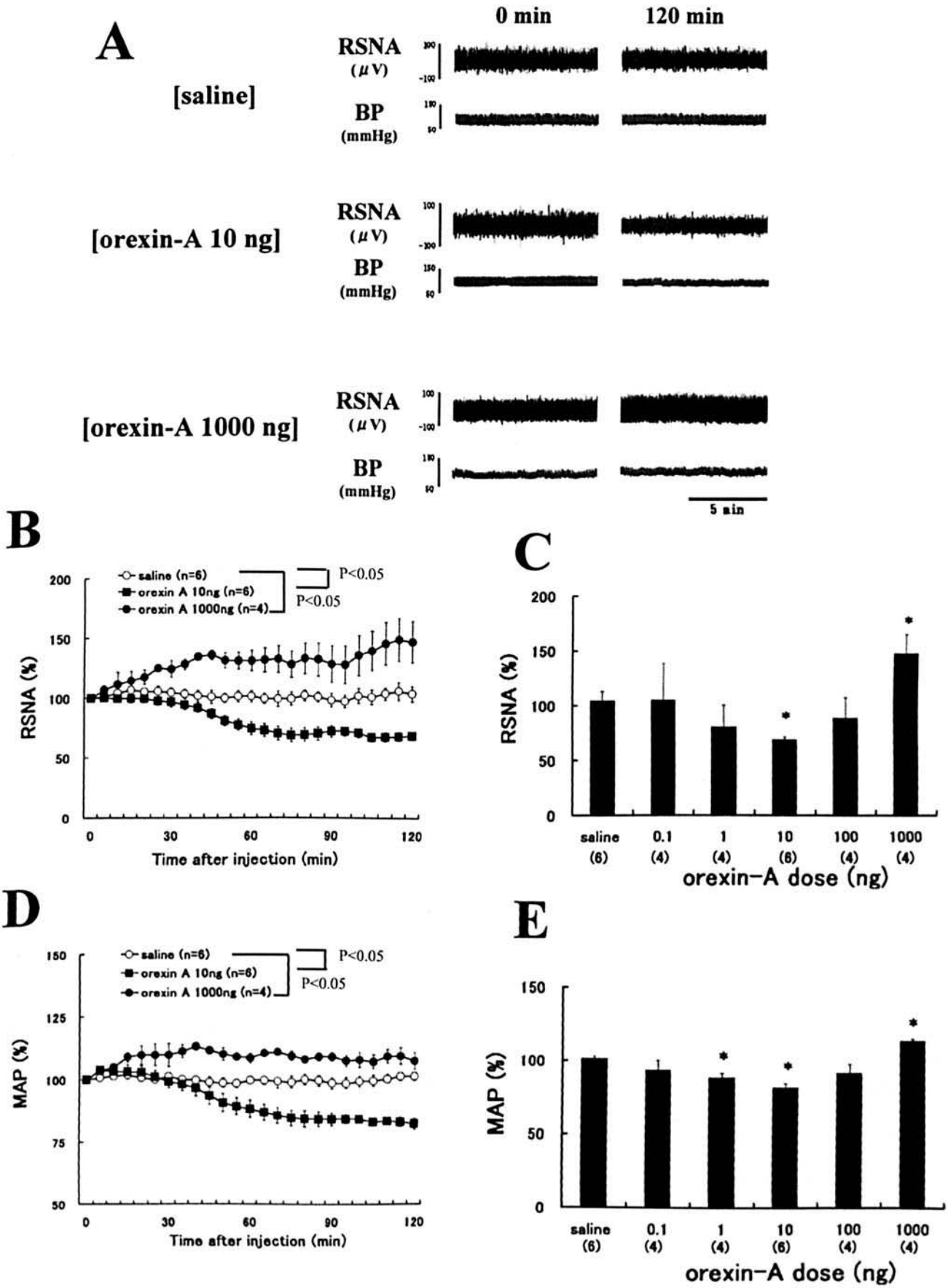
Figure 1. Effects of iv injection of orexin-A on RSNA and arterial BP. (A) Representative trace data from recordings of RSNA and BP before (0 min) and 120 mins after the iv injection of saline or orexin-A (10 and 1000 ng). (B–E) RSNA (B and C) and MAP (D and E) after iv injection of saline (0.1 ml) or orexin-A (10 or 1000 ng) are expressed as the mean ± SEM of the percentages of values at 0 min. Bars in C and E show RSNA and MAP values 120 mins after injection of five doses (0.1, 1, 10, 100, and 1000 ng) of orexin-A and saline. Numbers of animals used are shown in the parentheses. Asterisks indicate significant ($P < 0.05$) differences between RSNA and MAP after saline or orexin-A injections.


A
0 min
120 min
[saline]
RSNA (μV)
BP (mmHg)
[orexin-A 10 ng]
[orexin-A 1000 ng]
5 min
B
saline (n=6)
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orexin A 1000ng (n=4)
P<0.05
P<0.05
RSNA (%)
Time after injection (min)
C
RSNA (%)
saline (6)
0.1 (4)
1 (4)
10 (6)
100 (4)
1000 (4)
orexin-A dose (ng)
D
MAP (%)
Time after injection (min)
E
MAP (%)
orexin-A dose (ng)

Table 1. Basal Levels of RSNA and MAP in Experimental Groups[a]

Groups	No. of rats	RSNA (spikes/5 sec)	MAP (mm Hg)
Experiment 1			
Saline	6	59.5 ± 6.4	90.7 ± 3.3
0.1 ng	4	73.0 ± 18.1	92.4 ± 5.6
1 ng	4	79.3 ± 9.7	92.7 ± 7.2
10 ng	6	60.7 ± 6.1	87.7 ± 3.9
100 ng	4	59.4 ± 5.1	90.4 ± 3.9
1000 ng	4	75.0 ± 11.2	91.8 ± 4.4
Experiment 2			
aCSF	4	110.1 ± 32.4	92.6 ± 7.0
0.01 ng	4	128.5 ± 29.8	93.6 ± 9.3
0.1 ng	5	143.9 ± 20.2	88.0 ± 2.4
1 ng	4	176.2 ± 58.6	88.0 ± 9.0
10 ng	4	140.3 ± 17.7	92.9 ± 3..4
Experiment 3			
Saline iv ± saline	5	46.9 ± 8.2	103.0 ± 13.5
Saline iv ± orexin-A (10 ng)	5	77.4 ± 17.4	93.9 ± 10.2
Saline iv ± orexin-A (1000 ng)	6	60.3 ± 18.2	79.6 ± 12.7
Thiop iv ± orexin-A (10 ng)	6	61.7 ± 7.0	93.5 ± 3.0
Diphen iv ± orexin-A (1000 ng)	5	35.1 ± 12.8	88.2 ± 8.0
aCSF LCV ± saline	5	88.9 ± 16.6	64.7 ± 2.6
aCSF LCV ± orexin-A (10 ng)	5	114.0 ± 10.4	70.3 ± 1.8
aCSF LCV ± orexin-A (1000 ng)	5	101.2 ± 15.8	80.0 ± 6.8
Thiop LCV ± saline	4	112.1 ± 20.9	84.6 ± 6.6
Diphen LCV ± saline	4	89.7 ± 16.4	75.9 ± 9.7
Thiop LCV ± orexin-A 10 ng	6	124.2 ± 31.2	76.6 ± 4.4
Diphen LCV ± orexin-A 1000 ng	4	102.7 ± 21.5	67.4 ± 1.8
Experiment 4			
SCN-sham, saline	6	79.7 ± 8.0	80.4 ± 3.3
SCN-sham, orexin-A (10 ng)	6	71.3 ± 8.8	85.9 ± 7.0
SCN-sham, orexin-A (1000 ng)	5	73.9 ± 9.7	79.6 ± 5.5
SCN-lesion, saline	7	63.5 ± 10.2	81.9 ± 7.2
SCN-lesion, orexin-A (10 ng)	7	73.5 ± 5.6	79.3 ± 8.5
SCN-lesion, orexin-A (1000 ng)	5	67.1 ± 5.8	76.1 ± 6.6

[a] Data are shown as the mean ± SEM. thiop, thioperamide; diphen, diphenhydramine.

orexin-A (10 ng; $P < 0.05$, $F = 8.0$), saline–orexin-A (1000 ng) versus diphenhydramine–orexin-A (1000 ng; $P < 0.05$, $F = 7.5$; Fig. 3B), aCSF-saline versus aCSF-orexin-A (10 ng: $P < 0.005$, $F = 13.3$; 1000 ng: $P < 0.05$, $F = 12.1$), aCSF–orexin-A (10 ng) versus thioperamine–orexin-A (10 ng; $P < 0.05$, $F = 7.6$), thioperamine-saline versus thioperamine-orexin-A (10 ng; NS, $F = 2.5$), aCSF–orexin-A (1000 ng) versus diphenhydramine–orexin-A (1000 ng; $P < 0.005$, $F = 13.6$), and diphenhydramine-saline versus diphenhydramine–orexin-A (1000 ng; NS, $F = 1.4$; Fig. 3D). Absolute basal (0-min) RSNA and MAP values for the experiments are shown in Figure 3 and summarized in Table 1. Differences in respective basal values were not statistically significant (Mann-Whitney U test).

Previously, we observed that bilateral lesions of the SCN abolished not only suppressive actions of a low dose of L-carnosine but also the enhancing effects of a high dose on both RSNA and MAP (6). Therefore, in the present study, we also examined the effects of bilateral lesions of the SCN on changes induced by orexin-A on RSNA and MAP. Figure 4A presents representative photomicrographs of a sham-operated rat and a rat with bilateral SCN lesions. In some SCNL rats, a part of the optic chiasm was damaged as well; however, a pupillary reflex could be induced by bilateral light stimulation of the eyes in all SCNL rats used in the present study. Figure 4B and C summarizes the data from both sham-operated and SCNL rats. In sham-operated rats, the levels of RSNA and MAP, when compared with saline-treated groups, were significantly reduced by 10 ng of orexin-A and elevated by 1000 ng of orexin-A, respectively. In SCNL rats, however, neither injection of 10 ng nor 1000 ng of orexin-A caused any effect on level of either RSNA (Fig. 4B) or MAP (Fig. 4C). Thus, bilateral lesions of the SCN eliminated both the suppressive effects of the lower dose (10 ng) and the elevating effects of the higher dose (1000 ng) of orexin-A on RSNA and MAP. For the two SCN treatments (sham and lesion), the significance of the differences between values from 5–120 mins as a group were analyzed by repeated-measures ANOVA. The following comparisons were made: for RSNA, sham-saline versus

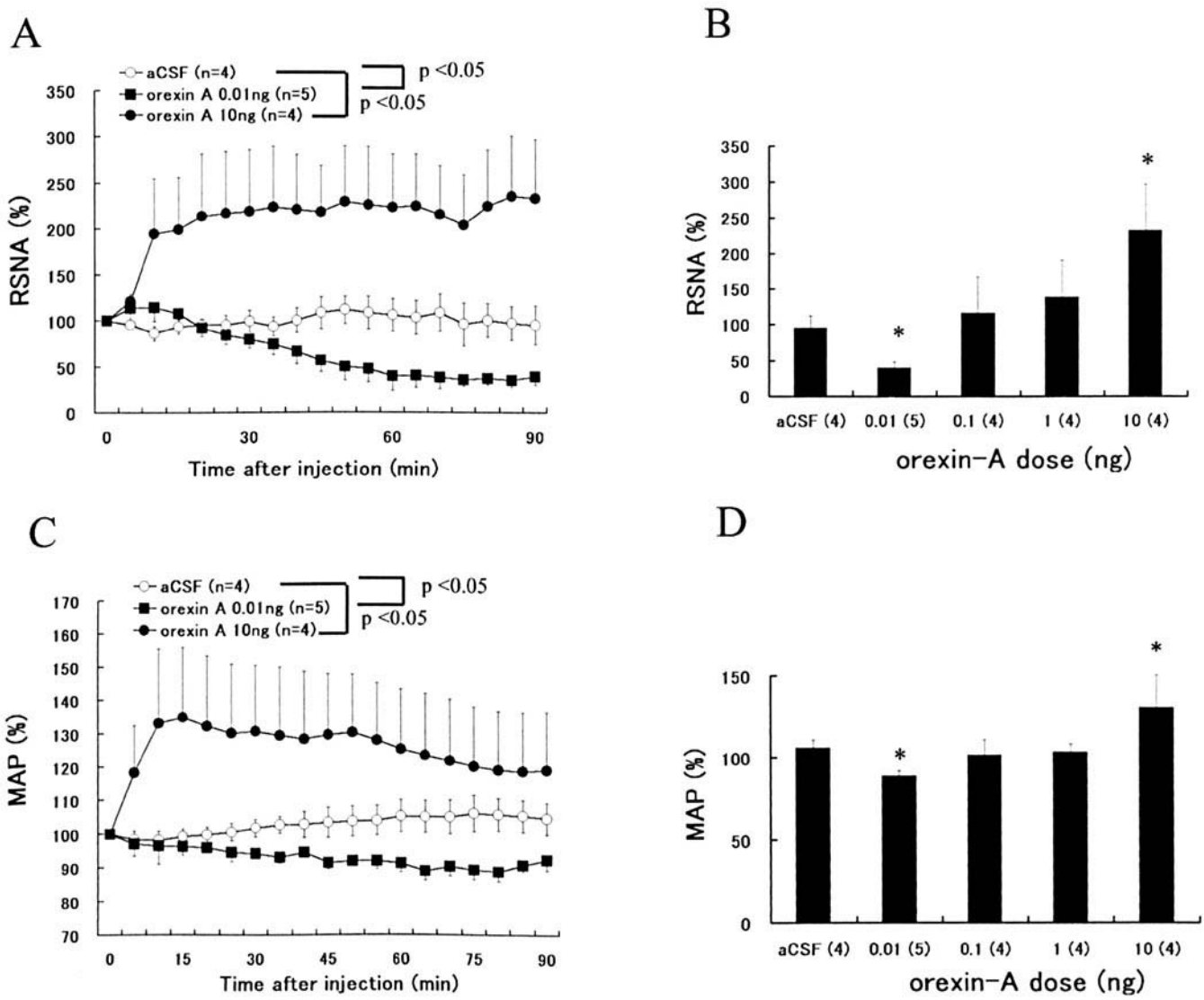

Figure 2. Effects of LCV injection of orexin-A on RSNA and arterial BP. RSNA (A and B) and MAP (C and D) after LCV injection of aCSF (10 μl) or orexin-A (0.01 or 10 ng) are expressed as the mean ± SEM of the percentages of values at 0 min. Bars in B and D show RSNA and MAP values 90 mins after injection of four doses (0.01, 0.1, 1 and 10 ng) of orexin-A and aCSF. Numbers of animals used are shown in the parentheses. Asterisks indicate significant ($P < 0.05$) differences between RSNA and MAP after aCSF or orexin-A injections.

sham–orexin-A (10 ng: $P < 0.005$, $F = 9.4$; 1000 ng: $P < 0.05$, $F = 6.6$), SCNL-saline versus SCNL–orexin-A (10 ng: NS, $F = 0.19$; 1000 ng: NS, $F = 1.05$), sham-saline versus SCNL-saline (NS, $F = 0.07$), sham–orexin-A (10 ng) versus SCNL–orexin-A (10 ng; $P < 0.0005$, $F = 16.6$), and sham–orexin-A (1000 ng) versus SCNL–orexin-A (1000 ng; $P < 0.05$, $F = 8.0$; Fig. 4B); for MAP, sham-saline versus sham–orexin-A (10 ng: $P < 0.005$, $F = 9.5$; 1000 ng: $P < 0.05$, $F = 5.8$), SCNL-saline versus SCNL–orexin-A (10 ng; NS, $F = 1.4$), SCNL-saline versus SCNL–orexin-A (1000 ng; NS, $F = 0.04$), sham-saline versus SCNL-saline (NS, $F = 0.07$), sham–orexin-A (10 ng) versus SCNL–orexin-A (10 ng; $P < 0.005$, $F = 12.0$), and sham–orexin-A (1000 ng) versus SCNL–orexin-A (1000 ng; $P < 0.05$, $F = 6.29$; Fig. 4C). Absolute basal (0-min) RSNA and MAP values for the experiments shown in Figure 4 are summarized in Table 1. Differences in respective basal values were not statistically significant (Mann-Whitney U test).

Discussion

A previous study (3) found that central injection of orexin-A (0.3 and 3 nmol, or ~1 and 10 μg, respectively) increases RSNA and BP in conscious rats. In the present study, we observed that peripheral and central injections of low doses of orexin-A (peripheral, 10 ng or ~0.003 nmol; central, 0.01 ng or ~0.003 pmol) suppressed RSNA and BP, but a high dose of orexin-A (peripheral, 1000 ng or ~0.3 nmol; central, 10 ng or ~0.003 nmol) elevated RSNA and BP (Fig. 1). These data indicate the biphasic effects of orexin-A on RSNA and BP. In the preliminary study, we observed that ip and LCV administrations of lower amounts (ip, 0.003 nmol; LCV, 0.03 pmol) of orexin-A suppressed 2-

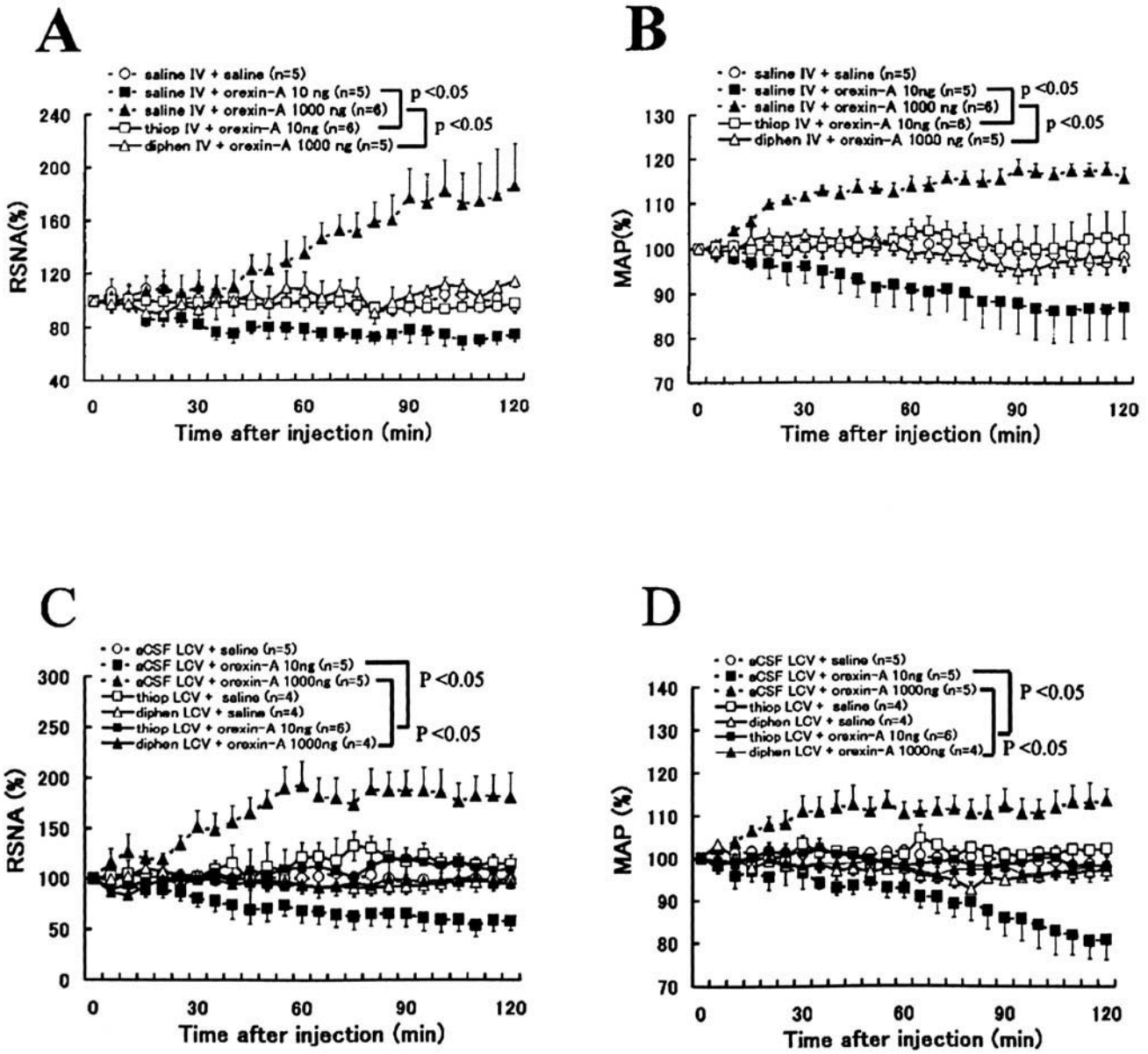

Figure 3. Effects of thioperamide (thiop) and diphenhydramine (diphen) on changes in RSNA and MAP following iv injection of 10 and 1000 ng of orexin-A. RSNA (A and C) and MAP (B and D) after iv injection of saline (0.1 ml) or orexin-A (10 or 1000 ng) are expressed as the mean ± SEM of the percentages of values at 0 min. The iv (A and B) or LCV (C and D) injections of saline, thiop, or diphen were given 30 mins before iv injection of either saline or orexin-A. Significant (P <0.05) differences between the groups from 5–120 mins were analyzed by ANOVA.

deoxy-D-glucose-hyperglycemia and that iv injection of 0.003 nmol of orexin-A inhibited adrenal sympathetic nerve activity and activated pancreatic vagal nerve activity (data not shown). Thus, the suppressive effect of lower doses of orexin-A on hyperglycemia and hypertension may be mediated via the autonomic nervous system.

With respect to the biphasic, dose-different effects of orexin-A on RSNA and BP, we previously proposed that L-carnosine, a dipeptide produced in skeletal muscle, also has the biphasic effects on RSNA and BP and that the effect of L-carnosine may be realized via histaminergic neural function in the brain (6). In the present study, IV and LCV preinjections of thioperamide abolished the suppressing effects of 10 ng of orexin-A on RSNA and BP;

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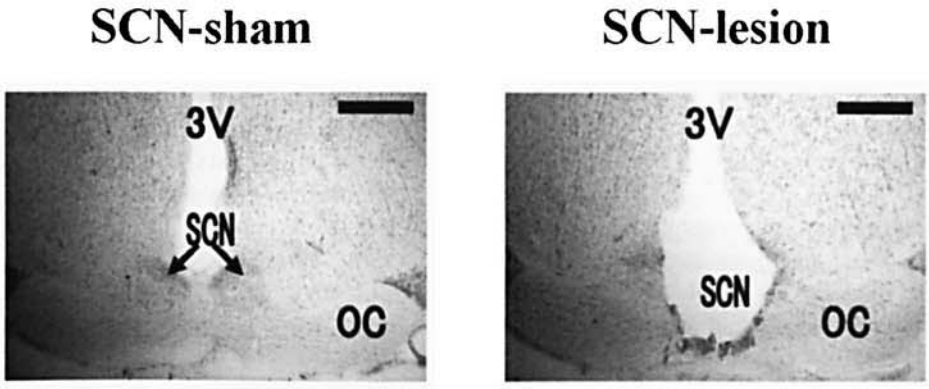
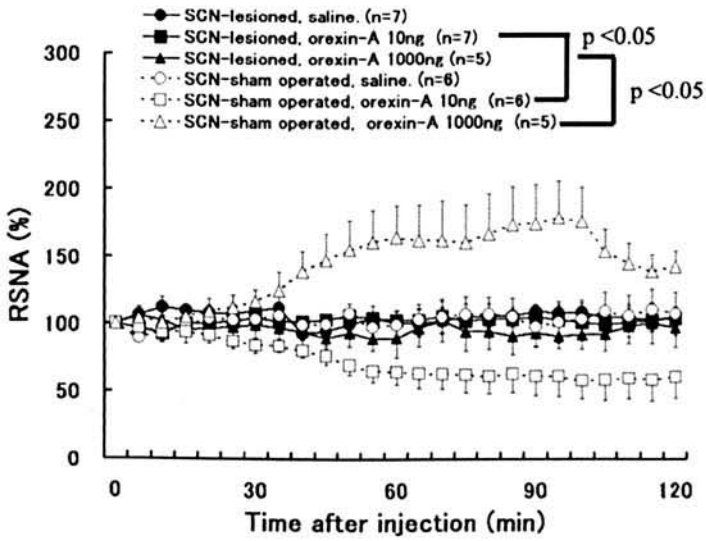
Figure 4. Effects of bilateral lesions of the SCN on changes in RSNA and MAP after iv injection of orexin-A. (A) Representative photomicrographs of coronal sections including the SCN from sham-operated (SCN-sham) rats and from rats the received SCN lesions (SCN-lesioned). Arrows show the intact bilateral SCN on the SCN-sham rats. (B and C) RSNA (B) and MAP (C) after iv injection of saline (0.1 ml) or orexin-A (10 or 1000 ng) are expressed as the mean ± SEM of the percentages of values at 0 min. Data from SCN-sham and SCN-lesioned rats are shown. Significant (P <0.05) differences between the groups from 5–120 mins were analyzed by ANOVA. 3V, third ventricle; OC, optic chiasm. Bars, 300 μm.

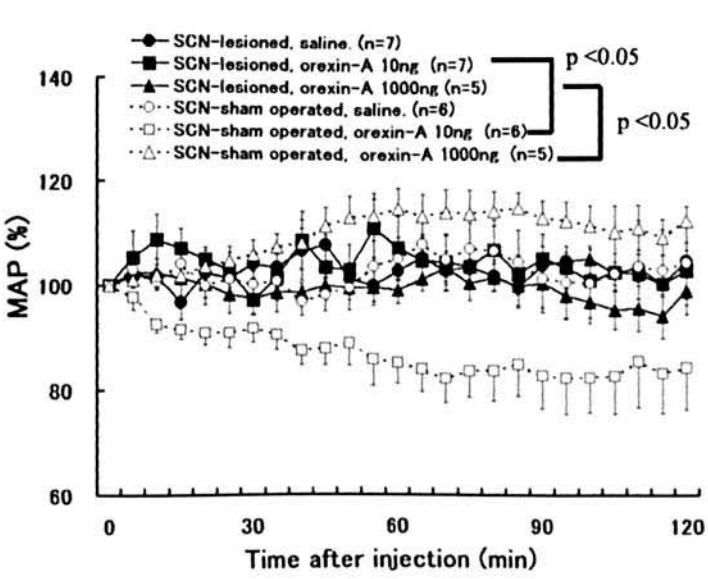
A


SCN-sham
SCN-lesion
3V
SCN
OC
3V
SCN
OC

B


SCN-lesioned, saline. (n=7)
SCN-lesioned, orexin-A 10ng (n=7)
SCN-lesioned, orexin-A 1000ng (n=5)
SCN-sham operated, saline. (n=6)
SCN-sham operated, orexin-A 10ng (n=6)
SCN-sham operated, orexin-A 1000ng (n=5)
p <0.05
p <0.05
RSNA (%)
300
250
200
150
100
50
0
0
30
60
90
120
Time after injection (min)

C


SCN-lesioned, saline. (n=7)
SCN-lesioned, orexin-A 10ng (n=7)
SCN-lesioned, orexin-A 1000ng (n=5)
SCN-sham operated, saline. (n=6)
SCN-sham operated, orexin-A 10ng (n=6)
SCN-sham operated, orexin-A 1000ng (n=5)
p <0.05
p <0.05
MAP (%)
140
120
100
80
60
0
30
60
90
120
Time after injection (min)

preinjection of diphenhydramine eliminated the elevating effects of 1000 ng of orexin-A (Fig. 3). In the histaminergic nervous system, the presynaptic H_3-receptor mediates autoinhibition of histamine release from the histaminergic neurons to the synaptic clefts. The affinity of the H_3-receptor for histamine is much higher than the affinities of the postsynaptic histaminergic H_1- and H_2-receptors (12). Therefore, a small amount of histamine suppresses histamine release from the presynaptic histaminergic neurons via the H_3-receptor. A large amount of histamine, however, functions by transmitting histaminergic neural signals via the H_1-receptor. Thus, it appears that 10 and 1000 ng of orexin-A elicit releases of small and large amounts, respectively, of histamine. In this respect, it was reported that 1 nmol of orexin-A accelerated histamine release in the hypothalamus (13) and that the histaminergic receptors were involved in the arousal effect of orexin-A (14). Therefore, these data let us suggest that the histaminergic neurons are involved in the effects of orexin-A on RSNA and BP. Indeed, the elevating effects of a high dose of orexin-A on RSNA and BP were blocked by a histaminergic H_1-receptor antagonist, and the suppressing effects of a low dose of orexin-A were abolished by a histaminergic H_3-receptor antagonist (Fig. 3). On the other hand, Yasuda *et al.* (15) recently confirmed that αFMH, an irreversible inhibitor of the histamine-synthesizing enzyme histidine decarboxylase, did not attenuate the orexin-A–induced response of the sympathetic nerve innervating brown adipose tissue. Thus, the role of histaminergic neurons in sympathetic effects of orexin-A is different from the RSNA with the brown adipose tissue sympathetic nerve, but it is not clear why the different response occurs. Further examination must be required in the future.

Orexin-A is synthesized primarily in the lateral hypothalamic nuclei. With respect to transportation from the peripheral tissue to the brain, as with other peptides, such as leptin (16) and ghrelin (17), which are released from peripheral tissues, orexin-A also enters the brain from blood through the blood-brain barrier to act on the central nervous system (CNS) (18). The peripherally effective doses of these peptides do not affect RSNA or BP following central injection (19, 20). In the present study, peripherally and centrally elevating doses of orexin-A on RSNA and BP were 1000 and 10 ng, respectively, whereas iv injection of 10 ng of orexin-A did not elevate RSNA or BP (Figs. 1 and 2). In addition to consideration of the peptides mentioned above, our data strongly support the idea that orexin-A released to blood acts on the CNS through the blood-brain barrier to affect RSNA and BP.

The actions of orexin-A are mediated via two G protein–coupled receptors, the orexin-1 and orexin-2 receptors, and they highly express in the some hypothalamic nuclei (21). The present study did not determine whether the effects of orexin-A on RSNA and BP are mediated by orexin-A receptors, but we noted the SCN, one of hypothalamic nuclei and a master circadian oscillator, playing an important role in the control of glucose metabolism and BP via the regulation of the autonomic nervous system (6, 22). Thus, we examined the role of SCN in the effects of orexin-A on RSNA and BP, and we found that bilateral lesions of the SCN completely eliminated the responses of both RSNA and BP to orexin-A (Fig. 4), suggesting that SCN might be one of the action regions of orexin-A in producing RSNA and BP changes. In addition to the present data, bilateral lesions of the SCN in urethane-anesthetized rats eliminated changes in autonomic nerve activities induced by illumination (23, 24), olfactory stimulation (25), L-carnosine (6), and lactobacillus (26). Therefore, the SCN might be involved not only in the above-mentioned mechanisms but also in those of orexin-A on RSNA and BP. Using pseudorabies virus to investigate the neural connection between the SCN and the peripheral tissues, we found evidence that the SCN sends multisynaptic neural signals to the peripheral tissues and that separate SCN neurons send signals to the peripheral sympathetic and parasympathetic neurons (27, 28). These multisynaptic efferent projections, identified from the SCN to the spinal cord containing group of neurons in the sympathetic pathway, modulate BP (27, 28). With respect to the kidney, Sly *et al.* (29) verified the existence of an efferent neural pathway from the SCN to the kidney using pseudorabies virus. Although multisynaptic efferent projections identified from the SCN to the spinal cord containing sympathetic preganglionic cells and to the medulla oblongata containing group of neurons in the sympathetic pathway modulate BP (27, 28), the exact descending pathway that is responsible for the cardiovascular effect of orexin-A is unclear at present. These pathways may all be associated with the suppression and elevation of RSNA and BP.

Because expression of the orexin-2 receptor has been identified in the TMN, which is the site of origin for histamine neurons (21), it is possible that the close relation of orexin-A with histamine neurons might be involved in the effects of orexin-A on RSNA and BP through the CNS. In fact, our present data that LCV pretreatment of histamine-receptor antagonists inhibited the sympathetic and cardiovascular effects of orexin-A (Fig. 3) strongly support this idea. Histaminergic H_1- and H_3-receptors, which are widely distributed within the CNS, including the SCN and histaminergic neurons in the TMN of the hypothalamus projecting to the SCN (30, 31), seem to be involved in the mechanistic actions of orexin-A on RSNA and BP. From the results of the present study, it is not clear whether histaminergic receptors in the TMN or SCN are involved in effects of orexin-A, but we suggest that the histamine derived from hypothalamic TMN might be involved in the biphasic effects of orexin-A on RSNA and BP through the SCN, as observed. The detailed mechanisms, however, must be defined in future.

In conclusion, the present findings suggest that orexin-A acts in a dose-dependent manner, affecting RSNA and

BP. The histaminergic nervous system in the brain and the SCN might be involved in this mechanism. Further study, however, is required to reveal the precise pathway responsible for the sympathetic and cardiovascular effects of orexin-A.

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