Dose-dependent effects of L-carnosine on the renal sympathetic nerve and blood pressure in urethane-anesthetized rats

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1Division of Protein Metabolism, Institute for Protein Research, Osaka University, Osaka; 2Niigata University School of Medicine, Niigata; 3Osaka University Graduate School of Medicine, Osaka; and 4Institute for Health Care Science, Suntory Limited, Shimamoto, Osaka, Japan

Submitted 30 April 2004; accepted in final form 6 October 2004

Tanida, Mamoru, Akira Niijima, Yutaka Fukuda, Hajime Sawai, Nobuo Tsuruoka, Jiao Shen, Shigeru Yamada, Yoshinobu Kiso, and Katsuya Nagai. 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–R455, 2005. First published October 21, 2004; doi:10.1152/ajpregu.00275.2004.—The physiological function of L-carnosine (β-alanyl-L-histidine) synthesized in mammalian muscles has been unclear. Previously, we observed that intravenous (IV) injection of L-carnosine suppressed renal sympathetic nerve activity (RSNA) in urethane-anesthetized rats, and L-carnosine administered via the diet inhibited the elevation of blood pressure (BP) in deoxycorticosterone acetate salt hypertensive rats. To identify the mechanism, we examined effects of IV or intralateral cerebral ventricular (LCV) injection of various doses of L-carnosine on RSNA and BP in urethane-anesthetized rats. Lower doses (1 μg IV; 0.01 μg LCV) of L-carnosine significantly suppressed RSNA and BP, whereas higher doses (100 μg IV; 10 μg LCV) elevated RSNA and BP. Furthermore, we examined effects of antagonists of histaminergic (H1 and H3) receptors on L-carnosine-induced effects. When peripherally and centrally given, thioperamide, an H3 receptor antagonist, blocked RSNA and BP decreases induced by the lower doses of peripheral L-carnosine, whereas diphenhydramine, an H1 receptor antagonist, inhibited increases induced by the higher doses of peripheral L-carnosine. Moreover, bilateral lesions of the hypothalamic suprachiasmatic nucleus eliminated both effects on RSNA and BP induced by the lower (1 μg) and higher (100 μg) doses of peripheral L-carnosine. These findings suggest that low-dose L-carnosine suppresses and high-dose L-carnosine stimulates RSNA and BP, that the suprachiasmatic nucleus and histaminergic nerve are involved in the activities, and that L-carnosine acts in the brain and possibly other organs.

L-CARNOSINE (β-ALANYL-L-HISTIDINE) is found in large amounts in skeletal muscles of vertebrates, where it is synthesized by carnosine synthetase (4). However, the physiological significance of L-carnosine has not been clear. A marked increase in the blood concentration of L-carnosine and hypothension was reported in a patient with a congenital deficiency of carnosinase, the L-carnosine-degrading enzyme (33). In addition, L-carnosine concentrations in skeletal muscles of hypertensive rats were markedly reduced (13). These findings suggest that L-carnosine has histotensive effects. We previously showed that intravenous (IV) injection of L-carnosine reduced the renal sympathetic nerve activity (RSNA) in urethane-anesthetized rats and that diets containing L-carnosine inhibited the hypertensive response in DOCA salt hypertensive rats (25).

The RSNA, or the postganglionic sympathetic fibers that innervate the renal vascular bed (9), is controlled by the medullary sympathetic premotoneurons in the central nervous system (CNS) and plays an important role in modulating blood pressure (BP) (10, 29). We previously showed that not only peripheral but also central injections of specific doses of L-carnosine inhibited the hyperglycemia induced by intracranial injection of 2-deoxy-D-glucose (2-DG) (2-DG hyperglycemia) (22, 36). Thus L-carnosine may act on the CNS and induce changes in renal sympathetic and cardiovascular responses in rats. However, effects of central administration of L-carnosine on RSNA and BP have not yet been evaluated.

L-Histidine, a component of L-carnosine, is converted to histamine in the brain. We previously demonstrated that L-carnosine-induced inhibition of 2-DG hyperglycemia was eliminated by pretreatment with thioperamide, an antagonist of the histaminergic H3 receptor (36). Thus the suppressive effects of L-carnosine on RSNA and BP may also be mediated by the histaminergic H3 receptor. Furthermore, we observed evidence in rats that the hypothalamic suprachiasmatic nucleus (SCN) is involved in the control of glucose metabolism through autonomic nerves (19, 20), in addition to functioning as a master circadian oscillator (17). These facts suggest that the SCN may modulate BP through the RSNA. Therefore, in this study, we first assessed the effects of peripheral and central administrations of various amounts of L-carnosine on RSNA and BP. Next, we evaluated the effects of histaminergic blockers (H3- and H1-receptor antagonists) and bilateral lesions of the SCN on changes induced by L-carnosine in urethane-anesthetized rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing ~350 g, were used. Rats were housed in a room maintained at 24 ± 1°C and illuminated for 12 h (0800 to 2000) everyday. Food and water were freely available. Rats were adapted to the environment for at least 1 wk 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. On the experimental day, food was removed 4–6 h before surgery. Anesthesia was induced with an...
intraperitoneal (IP) injection of 1 g/kg 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 37.0–37.5°C using an infrared lamp and a thermometer inserted into the rectum. The rat was paralyzed with gallamin triethiodide (initially 10 mg IV, thereafter 4 mg/h IV) and then ventilated artificially by respiratory pump (Harvard Apparatus) with a gas mixture of O2 and room air (20% O2). A pneumothorax was induced to reduce respiratory movement. The end-expiratory Pco2 concentration was maintained at 3.0–4.0% by adjusting the ventilation volume. Before and after the rat was paralyzed with gallamin triethiodide, we evaluated the adequacy of the depth of anesthesia by checking whether rapid eye movements occurred at 3.0–4.0% by adjusting the ventilation volume. Before and after the rat was paralyzed with gallamin triethiodide, we evaluated the adequacy of the depth of anesthesia by checking whether rapid eye movements occurred.

Briefly, under pentobarbital anesthesia (35 mg/kg IP), a stainless steel tracheal cannula was inserted into the trachea. The body temperature was maintained at 37.0–37.5°C using a heating pad. The heart 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 min after being placed on the recording electrodes.

The RSNA was amplified, filtered (VC11, Nihon Kohden), monitored by an oscilloscope, and stored on magnetic tape. The activity was sampled with the LEG-1000 system (Nihon Kohden). Data were obtained as described previously (11, 24, 25, 31). The catheter in the left femoral artery was connected to a BP transducer (DX-100, Nihon Kohden), and the output signal of the transducer was amplified in a BP amplifier (AP641G, Nihon Kohden). 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 ECG to monitor HR. The ECG signal was amplified with a bioelectric amplifier (AB-620G, Nihon Kohden). The BP was averaged, filtered, and stored on magnetic tape. The activity was sampled with the LEG-1000 system, and stored on a hard disk for offline analysis.

Intracerebroventricular cannulation. At least 1 wk before the experiment, a brain cannula made of polyethylene tubing (PE-10; Clay Adams, Parsippany, NJ) was inserted into the lateral anterior-posterior (AP), 1.5 mm caudal to the bregma; lateral (L), 2.0 mm lateral to the midline; ventral (V), 3.0 mm below the skull surface] under pentobarbital anesthesia (35 mg/kg IP) as previously described (23).

Experimental protocol. Baseline measurements of RSNA, MAP, and HR were made for 5 min just before IV injections of L-carnosine (0.01, 0.1, 1, 10, and 100 µg/0.1 ml saline) or saline (0.1 ml) and LCV injections of L-carnosine [0.001, 0.01, 0.1, and 10 µg/10 µl artificial cerebrospinal fluid (aCSF)] or aCSF (10 µl). After the injection, these parameters were recorded for 120 min. The effects of thiopental maleate (200 µg/0.1 ml saline IV; 20 µg/100 µl aCSF LCV), a histaminergic H3 antagonist, or diphenhydramine hydrochloride (50 µg/0.1 ml saline IV; 5 µg/100 µl aCSF LCV), a histaminergic H1 antagonist, on the l-carnosine-induced effects on RSNA and BP were examined. These antagonists were administered IV 30 min before IV injection of L-carnosine. We observed that thiopental or diphenhydramine alone did not affect RSNA, MAP, or HR (data are not shown). At the end of the experiment, hexamethonium chloride was administered (10 mg/kg IV) to ensure that the recording was made from postganglionic efferent sympathetic nerve activity.

SCN lesions. In some rats (n = 12), 2–3 wk before the IV or LCV injection of l-carnosine, bilateral electrolytic lesions were made in the SCN by the experimental methods described previously (23, 24). Briefly, under pentobarbital anesthesia (35 mg/kg IP), a stainless steel electrode was inserted into the SCN with coordinates (A-P, 1.2 mm posterior to the bregma; L, 0 mm from the midline; V, 9.0 mm from the skull surface) of the atlas of Paxinos and Watson (27) and then a 1.0-mA anodal direct current was passed through the electrode for 20 s. Control rats (n = 10) received a sham operation by the same procedure but without the current. At the end of the experiment, the brain was removed, and a histological 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 animals.

Blood sampling and measurement of the plasma concentration of l-carnosine. In some rats (n = 26), a catheter made of Silastic (Dow Corning, Midland, MI) and PE-50 (Clay Adams) was inserted into the right atrium of the heart 3 days before the experiment, and another catheter made of PE-50 was inserted into the left femoral artery for IV injections under the pentobarbital anesthesia (35 mg/kg IP). On the experimental day, food was removed 4–6 h before IV injections. IV injection of saline or L-carnosine (1 and 100 µg/0.1 ml saline) was done at 1300, and blood samples (0.2 ml each) were withdrawn from the heart using the cardiac catheter before (0 s) and 15, 30, 60, 120, 180, 300 s and 15, 30, and 60 min after the administration of these agents under urethane anesthesia. The measurement of the plasma l-carnosine was done as described previously (22). Briefly, the blood samples were mixed with EDTA (300 nmol) in a volume of 10 µl and centrifuged, and plasma samples obtained were stored at −80°C until analysis. To determine the concentration of plasma l-carnosine, trichloroacetic acid in a final concentration of 0.5 M was added to 100 µl of the plasma, and denatured proteins were removed by centrifugation (1,000 g for 10 min). The concentration of l-carnosine of the supernatant was analyzed with an amino acid analyzing system (Shim-Pack ISC-07/S1504 Li, Shimadzu) (22).

Data analyses. RSNA, MAP, and HR measured during each 5-min period after injections of l-carnosine, saline, or aCSF were evaluated by digital signal processing and statistical analyses. All data were expressed as means ± SE. Because of the interindividual variability in the preinjection state, percent change from baseline was also calculated for RSNA and MAP. Change from baseline of plasma l-carnosine levels in the saline or l-carnosine-treated rats was compared by Student’s paired t-test. ANOVA with repeated measures was applied to compare group responses of RSNA and BP induced by l-carnosine, saline, or aCSF. In addition, to determine the effect of l-carnosine on RSNA, BP, and plasma l-carnosine levels, Duncan’s multiple range test was done.

RESULTS

Typical recording data of the RSNA and arterial BP before and after 120 min after IV injection of saline or l-carnosine are shown in Fig. 1. l-Carnosine (1 µg) suppressed RSNA and BP 120 min after it was injected IV in urethane-anesthetized rats. However, IV injection of a 100-fold higher dose of l-carnosine (100 µg) elevated the RSNA and BP. Figure 2 shows changes in RSNA (Fig. 2A) and MAP (Fig. 2C) after IV injections of l-carnosine or saline. Suppressive effects of l-carnosine on RSNA and MAP became significant 25–30 min after the injection of 1 µg of l-carnosine, and the maximum suppression occurred at 105–110 min, respectively, with the lowest levels of 71.8 ± 5.3% (RSNA) and 80.7 ± 3.2% (MAP). Enhancing effects of l-carnosine on RSNA and MAP became apparent 30–45 min after the injection of 100 µg of l-carnosine, and the maximum elevation occurred at 100–105 min, respectively, with the highest levels of 163.8 ± 32.3% (RSNA) and 113.0 ± 25% (MAP). In contrast, saline did not significantly affect levels of RSNA and MAP until 120 min after the injection. IV injections of lower doses of l-carnosine (0.01 and 0.1 µg) dose
dependently decreased RSNA (Fig. 2B) and MAP (Fig. 2D), and maximum suppressive responses occurred 120 min after the injection of 1 μg. However, the higher dose of L-carnosine (100 μg) did not decrease but significantly increased levels of RSNA and MAP. The significance of the differences between values from 5 to 120 min analyzed as a group by ANOVA (repeated measures) is as follows: RSNA: saline vs. L-carnosine (1 μg), $P = 0.0005$ ($F = 236$); saline vs. L-carnosine (100 μg), $P < 0.0005$ ($F = 99$); MAP: saline vs. L-carnosine (1 μg), $P < 0.0005$ ($F = 152$); saline vs. L-carnosine (100 μg), $P < 0.0005$ ($F = 214$).

Table 1 shows changes in the plasma L-carnosine level after IV injection of saline or L-carnosine (1 and 100 μg). The IV injection of the higher dose of L-carnosine (100 μg) markedly and significantly elevated the plasma L-carnosine level 15 s after the injection, and the plasma concentration returned to the basal value within 15 min after the injection. On the other hand, the lower dose of L-carnosine (1 μg) slightly elevated the plasma L-carnosine levels 30 s after the injection, but the level returned to the basal level within 5 min after the injection. Neither the higher nor the lower dose of L-carnosine significantly affected the L-carnosine level 15–60 min after the injection.

Figure 3 shows change in RSNA (Fig. 3A) and MAP (Fig. 3C) after LCV injection of L-carnosine or aCSF. The injection of a lower dose of L-carnosine (10 ng) into the LCV significantly suppressed RSNA and MAP, which became significant 20–30 min after the injection; the maximum suppression was observed at 105–120 min, with the lowest levels of 71.8 ± 5.3% (RSNA) and 80.7 ± 3.2% (MAP), respectively. On the other hand, the LCV injection of 10 μg of L-carnosine elevated RSNA and MAP, which became significant 20–45 min after L-carnosine injection; the maximum values occurred at 105–120 min, with the highest levels of 142.6 ± 18.5% (RSNA) and 120.5 ± 6.2% (MAP), respectively. In contrast, saline injection did not significantly affect RSNA and MAP levels. As in the case of IV injection of L-carnosine, LCV injection of a lower dose of L-carnosine (0.01 μg) significantly decreased RSNA (Fig. 3B) and MAP (Fig. 3D), and a higher dose of L-carnosine (10 μg) significantly increased RSNA (Fig. 3B) and MAP (Fig. 3D) levels at 120 min. The significance of the differences between values from 5 to 120 min analyzed as a
EFFECTS OF CARNOSINE ON BLOOD PRESSURE

Table 1. Response of the plasma l-carnosine concentration to intravenous injection of saline or l-carnosine in rats

<table>
<thead>
<tr>
<th>Time After Injection</th>
<th>Saline</th>
<th>L-Carnosine, 1 µg</th>
<th>L-Carnosine, 100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seconds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.9±0.7</td>
<td>1.6±0.5</td>
<td>1.9±0.7</td>
</tr>
<tr>
<td>15</td>
<td>2.1±0.9</td>
<td>2.9±0.6</td>
<td>112.1±66.0*</td>
</tr>
<tr>
<td>30</td>
<td>2.2±0.8</td>
<td>11.5±9.3</td>
<td>36.1±13.6*</td>
</tr>
<tr>
<td>60</td>
<td>2.1±0.8</td>
<td>10.4±9.0</td>
<td>20.8±7.7*</td>
</tr>
<tr>
<td>300</td>
<td>2.3±0.9</td>
<td>1.7±0.6</td>
<td>17.6±12.3</td>
</tr>
<tr>
<td>Minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.0±0.5</td>
<td>4.8±1.7</td>
<td>2.6±0.4</td>
</tr>
<tr>
<td>15</td>
<td>4.2±9.5</td>
<td>3.2±0.5</td>
<td>2.7±0.5</td>
</tr>
<tr>
<td>30</td>
<td>4.0±6.1</td>
<td>4.0±0.6</td>
<td>4.0±0.5</td>
</tr>
<tr>
<td>60</td>
<td>4.7±6.3</td>
<td>2.7±0.5</td>
<td>3.3±0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4 except for 1-µg l-carnosine values in seconds, where n = 5. (Significant difference *P < 0.05) from baseline.

The graph by ANOVA (repeated measures) is as follows: RSNA: aCSF vs. l-carnosine (0.01 µg), P < 0.0005 (F = 90); aCSF vs. l-carnosine (10 µg), P < 0.0005 (F = 68); MAP: aCSF vs. l-carnosine (0.01 µg), P < 0.0005 (F = 113); aCSF vs. l-carnosine (10 µg), P < 0.0005 (F = 291). Absolute values of basal RSNA and MAP in IV and LCV injection experiments (with respect to the data in Figs. 2 and 4) are summarized in Table 2; values did not differ significantly among any of these groups.

Figure 4 shows changes in RSNA (Fig. 4, A and C) and MAP (Fig. 4, B and D) after IV injection of l-carnosine with and without pretreatment of peripheral and central thioperamide or diphenhydramine. RSNA and MAP were significantly reduced in saline- (Fig. 4, A and B) or aCSF- (Fig. 4, C and D) pretreated rats by IV injection of the lower dose (1 µg) of l-carnosine and elevated by IV injection of the higher dose (100 µg) of l-carnosine, compared with RSNA and MAP in saline-saline or aCSF-saline control groups. The suppression and elevation of RSNA and MAP by the lower and higher doses of l-carnosine were, however, eliminated by both IV (Fig. 4, A and B) and LCV (Fig. 4, C and D) pretreatment with thioperamide and diphenhydramine, respectively.

Figure 5, A and B, shows representative photomicrographs of the hypothalamus, including the SCN of a sham-operated rat and a bilaterally SCN-lesioned rat. As evident in this figure, a part of the optic chiasm was damaged as well in some SCN-lesioned rats. However, a pupillary reflex was induced in all SCN-lesioned rats by light stimulation to both eyes. As shown in Fig. 5, C and D, RSNA and MAP were significantly reduced in sham-operated rats by the lower dose (1 µg) of l-carnosine and elevated by the higher dose (100 µg) of l-carnosine, respectively, compared with levels in saline-treated groups. However, in SCN-lesioned rats, the lower (1 µg) dose of l-carnosine did not affect RSNA (Fig. 5C) and MAP (Fig. 5D). When the higher dose (100 µg) of l-carnosine was administered to SCN-lesioned rats, increases in RSNA and MAP were not observed, and a slight and significant (P < 0.0005 by ANOVA) decrease in RNSA was detected instead. Thus bilateral lesions in the SCN eliminated both the suppressive effects of the lower (1 µg) dose of l-carnosine and the elevating effects of the higher (100 µg) dose of l-carnosine on RSNA and MAP. In SCN-lesioned rats, preinjection values of RSNA were 48.6±6.2 pulses/s in the saline group and 46.4±5.5 and 63.7±7.1 pulses/s in the 1- and 100-µg l-carnosine groups,
respectively. In SCN-lesioned rats, preinjection values of MAP were 94.4 ± 3.3 mmHg in the saline group and 86.6 ± 4.8 and 88.0 ± 3.1 mmHg in the 1- and 100-µg L-carnosine groups, respectively. In the sham-operated rats, preinjection values of RSNA and MAP of the saline group and the 1- and 10-µg L-carnosine groups were 67.6 ± 9.2, 67.7 ± 11.0, and 77.8 ± 10.7 pulses/s and 81.8 ± 7.2, 96.3 ± 4.6, and 83.8 ± 5.5 mmHg, respectively. The respective preinjection values did not differ significantly among groups.

DISCUSSION

Previously, our laboratory reported that IV injection of L-carnosine inhibited RSNA in urethane-anesthetized rats and that a diet containing L-carnosine decreased BP in DOCA salt hypertensive rats (25). However, the central effect of L-carnosine on RSNA and BP and dose-related effects of L-carnosine were not evaluated. In the present study, we first demonstrated that peripheral and central injections of lower doses (peripheral, 1 µg; central, 10 ng) of L-carnosine suppressed RSNA and BP in urethane-anesthetized rats (Figs. 2 and 3). However, peripheral and central injections of higher doses (IV, 100 µg; LCV, 10 µg) of L-carnosine elevated RSNA and BP (Figs. 2 and 3). Since we previously observed that peripheral and central administrations of lower amounts (peripheral, 0.05–5 nmol; central, 0.05–0.5 nmol) but not higher amounts (peripheral, 5 µmol; central, 5 nmol) of L-carnosine suppressed 2-DG hyperglycemia and that the effective suppressive dose of L-carnosine inhibited sympathetic adrenal and hepatic nerves (36); the suppressive effect of lower doses of L-carnosine on the hyperglycemia and hypertension may be mediated via the autonomic nervous system. However, parallel changes in RSNA and BP were observed with respect to the effective doses of L-carnosine whether administered IV or via the intralateral cerebral ventricle (Figs. 2 and 3).

There are several potential limitations of this study that need to be addressed. First, we used anesthetized rats in the present study, and it could be discussed that different results would have been obtained in conscious rats. However, previously, we have shown evidence that the biphasic dose-dependent effects of L-carnosine on 2-DG hyperglycemia in conscious rats are mediated by the histaminergic receptors in the CNS (22, 36). Moreover, in the present study, we demonstrated evidence that
the biphasic dose-dependent effects of L-carnosine on RSNA and BP in anesthetized rats are mediated by the histaminergic receptors in the CNS (Fig. 4). Therefore, it is possible that the potential effect of anesthesia might be minimal in histaminergic receptor-mediated effects of L-carnosine on RSNA and BP.

Second, the peak response of the plasma L-carnosine level induced by IV injection of L-carnosine occurred within 30 s after injection, and maximum responses of RSNA and BP to IV injection of the higher and lower doses of L-carnosine were observed later than the peak concentration of the plasma L-carnosine after the injection. That is, although the higher-dose of L-carnosine caused long-lasting sympathetic and cardiovascular elevations, it elicited an instant increase in the plasma L-carnosine level to a supraphysiological range with short duration, the peak being observed 15 s after the injection (Table 1). On the other hand, the lower-dose L-carnosine induced long-lasting sympathetic and cardiovascular suppression and a slight increase in the plasma L-carnosine level with short duration, the peak being observed 30 s after the injection (Table 1). These findings suggest that the elevation of plasma L-carnosine level with short duration might contribute to cause changes in RSNA and BP. In this respect, the carnosine-degrading enzyme carnosinase exists in the blood (4), and previously we found a daily variation of the plasma carnosinase activity that was parallel with the daily variation of the plasma L-carnosine concentration (22). Therefore, it is possible that the plasma carnosinase might be responsible for the rapid clearance of the plasma L-carnosine. The reason is not clear why the effects of L-carnosine on RSNA and BP were so long lasting. In this respect, it might be possible that L-carnosine is degraded by carnosinase, the resultant L-histidine is converted to histamine by histidine decarboxylase, and this histamine affects RSNA and BP. The time required for these processes might explain why effects of L-carnosine were observed for such a long time in this study. Furthermore, it might also be possible that the effect of histamine on the autonomic and

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cardiovascular responses may last for a long time because of the delay of the clearance of histamine from the synaptic clefts. In supporting this, previous studies showed that sympathetic and cardiovascular responses to central injection of histamine lasted over 60 min after injection (12, 37). This may explain the long-lasting effect of l-carnosine, but more research is necessary to reveal whether this is the case. In regard to the brain carnosinase, in our laboratory, we recently identified and cloned a type of carnosinase and detected the existence of the carnosinase-like substance in the rat hypothalamus with an immunohistochemistry using an antibody against the carnosinase (unpublished observations).

With respect to this and the biphasic dose-dependent effects of l-carnosine on 2-DG hyperglycemia (22, 36), our laboratory previously proposed that the effect of l-carnosine may be realized via histaminergic neural function in the brain, because l-histidine, a component of l-carnosine, is converted to histamine by histidine decarboxylase in the brain (22, 36). In the histaminergic nervous system (32), the presynaptic H3 receptor mediates the autoinhibition of histamine release from the histaminergic neurons to synaptic clefts, and the affinity of the H3 receptor for histamine is much higher than affinities of postsynaptic histaminergic H1 and H2 receptors (1). Therefore, a small amount of histamine is thought to suppress histamine release from the presynaptic histaminergic neurons via the H3 receptor. However, a large amount of histamine functions to transmit histaminergic neural signals. Therefore, small amounts of histamine in the synaptic cleft may inhibit release of histamine and reduce histaminergic transmission, whereas larger amounts may enhance histaminergic transmission. In fact, a previous study demonstrated that LCV injection of a larger dose of histamine elevates the BP in normal animals (12). With regard to 2-DG hyperglycemia, larger amounts of histamine administered into the brain enhanced the 2-DG hyperglycemia, and smaller amounts of histamine administered into the brain inhibited 2-DG hyperglycemia (22, 36). Therefore, the above proposal is likely, although we are not sure whether histamine derived from l-carnosine interacts with the histaminergic H1 and H3 receptors or whether another mechanism mediates the responses.

In the present experiment, a lower dose of l-carnosine administered in both peripheral and central sites suppressed RSNA and BP (Figs. 2 and 3), central and peripheral thioperamide eliminated the suppressive effects of the lower dose of l-carnosine.

### Table 1: Comparison of RSNA and MAP

<table>
<thead>
<tr>
<th>Condition</th>
<th>RSNA (%)</th>
<th>MAP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-saline</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SCN-lesioned-saline</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>SCN-lesioned-L-carnosine 1 μg</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>SCN-lesioned-L-carnosine 100 μg</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>sham-operated-saline</td>
<td>140</td>
<td>140</td>
</tr>
</tbody>
</table>

### Figure 5

*Effects of bilateral lesions of the SCN on changes in RSNA (A) and MAP (B) after IV injection of l-carnosine.* Photomicrographs of representative coronal brain sections, including the SCN of a sham-operated rat (A) and an SCN-lesioned rat (B), are shown. SCN, suprachiasmatic nucleus; 3V, third cerebral ventricle; OC, optic chiasm. The length of the scale bars is 300 μm.

*Significant differences between RSNA and MAP after saline and L-carnosine injections detected by Duncan’s multiple range test (P < 0.05). The significance of the differences between values from 5–120 min analyzed as a group by ANOVA (repeated measures) is as follows. C: RSNA: sham-saline vs. sham-t-carnosine (1 μg), P < 0.0005 (F = 108); sham-saline vs. sham-t-carnosine (100 μg), P < 0.0005 (F = 72.5); SCN-L-saline vs. SCN-L-t-carnosine (1 μg), P = not significant (F = 2.42); SCN-L-saline vs. SCN-L-t-carnosine (100 μg), P < 0.0005 (F = 31); sham-saline vs. SCN-L-saline, P = not significant (F = 0.05); sham-t-carnosine (1 μg) vs. SCN-L-t-carnosine (1 μg), P < 0.0005 (F = 131); sham-t-carnosine (100 μg) vs. SCN-L-t-carnosine (100 μg), P < 0.0005 (F = 150). D: MAP: sham-saline vs. sham-t-carnosine (1 μg), P < 0.0005 (F = 80); sham-saline vs. sham-t-carnosine (100 μg), P < 0.0005 (F = 49); SCN-L-saline vs. SCN-L-t-carnosine (1 μg), P = not significant (F = 0.001); SCN-L-saline vs. SCN-L-t-carnosine (100 μg), P = not significant (F = 2.71); sham-saline vs. SCN-L-saline, P = not significant (F = 0.81); sham-t-carnosine (1 μg) vs. SCN-L-t-carnosine (1 μg), P < 0.0005 (F = 74); sham-t-carnosine (100 μg) vs. SCN-L-t-carnosine (100 μg), P < 0.0005 (F = 210).
l-carnosine on RSNA and BP, and central and peripheral diphenhydramine abolished the elevating effects of the higher dose of l-carnosine on RSNA and BP (Fig. 4). Because we focused on the effect of peripheral l-carnosine, which might be released during exercise (22), effects of histaminergic antagonists on the peripheral effects of l-carnosine were examined. In particular, the present findings that LCV pretreatment of these histamine receptor antagonists inhibited the sympathetic and cardiovascular effects of l-carnosine strongly support the idea that central histaminergic receptors might be involved in the effects of l-carnosine. Histaminergic H1 and H3 receptors, widely distributed within the CNS, including the SCN and histaminergic neurons in the preamammillary regions of the hypothalamus projecting to the SCN (26, 28), seem to be involved in the mechanism of l-carnosine actions on RSNA and BP. Furthermore, it is also possible that l-histidine, a product of carnosine degradation by the blood carnosinase, might be transferred to the brain, and hypothalamic preamammillary neurons uptake this l-histidine and convert it to histamine by histidine decarboxylase. Because we obtained the data that the SCN anatomically was identified as origins of the autonomic nerves (5, 6) and that bilateral lesions of the SCN eliminated peripheral effects of l-carnosine on RSNA and BP, these lines of evidences let us suggest that the resultant histamine derived from hypothalamic preamammillary neurons might be involved in the biphasic effects of l-carnosine on RSNA and BP through the SCN observed in this study. These possibilities must be examined in future.

On the other hand, we found that the SCN, a master circadian oscillator, plays an important role in the control of glucose metabolism via the regulation of the autonomic nervous system (7, 8, 19–21, 34). Moreover, bilateral lesions of the SCN eliminated changes in autonomic nerve activities induced by illumination (24). Therefore, to elucidate the role of the SCN in the RSNA and BP changes induced by l-carnosine, we examined effects of SCN bilateral lesions on changes in RSNA and BP after the IV injection of l-carnosine. Both the suppressing and elevating effects induced by l-carnosine on RSNA and BP disappeared in the SCN-lesioned rats (Fig. 5). These findings suggest that the SCN is involved in the effective mechanism of l-carnosine on RSNA and BP. Using pseudorabies virus to investigate the neural connection between the SCN and the tissues related to control of glucose metabolism, we found evidence that the SCN sends multisynaptic neural signals to the pancreas and liver and that separate SCN neurons send signals to the peripheral sympathetic and parasympathetic neurons (5, 6). These multisynaptic efferent projections, identified from the SCN to the spinal cord-containing group of neurons in the sympathetic pathway, modulate BP (5, 6). With respect to the kidney, Sly et al. (30) 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 (5, 6), the exact descending pathway responsible for the cardiovascular effect of l-carnosine is unclear at present. These pathways may all be associated with the suppression and elevation of RSNA and BP. In the present study, sometimes the SCN lesions affected a part of the area of the organum vasculosum of the lamina terminalis, which is known as an important area controlling cardiovascular functions. Therefore, in the case of SCN lesions, it might be possible that the lesioning of the organum vasculosum of the lamina terminalis might be responsible for the disappearances of the effects induced by l-carnosine. In this connection, we observed that the sympathetic excitation due to 2-DG, which was eliminated by bilateral lesions of the SCN, was restored by intracranial injection of VIP, which is one of the neuropeptides that existed in the SCN neurons (20). Therefore, it is likely that the SCN is implicated in the responses to l-carnosine observed in this study.

Other peptides such as leptin and ghrelin, which are released from peripheral tissues, enter the brain from blood through the blood-brain barrier to act on the CNS (2, 3). The peripherally effective doses of these peptides do not affect RSNA or BP following central injection (15, 16). In the present study, peripherally and centrally suppressive doses of l-carnosine on RSNA and BP were 1 and 0.01 μg, respectively, whereas IV injection of 0.01 μg of l-carnosine did not affect RSNA or BP (Figs. 2 and 3). In addition to consideration of the peptides mentioned above, our data strongly support the idea that l-carnosine is released from muscles to blood and acts on the CNS through the blood-brain barrier to affect RSNA and BP. However, we recently observed that denervation of the vagal afferent nerves from the hepatoporal areas inhibited the suppressive effect of l-carnosine on RSNA (data not shown). Therefore, the direct or indirect effect of l-carnosine in the brain as well as the hepatovagal pathway might be involved in the peripheral signaling cascade of l-carnosine on the CNS. However, leptin appears to elevate the RSNA through the afferent nerve pathway in rats (31). These data and previous findings suggest that the histaminergic H1 and H3 receptors are involved in the mechanism of l-carnosine-induced elevations and inhibitions of RSNA and BP, respectively. The detailed mechanism still must be defined. Previously, we found that running wheel exercise increased the blood concentration of l-carnosine from ~7 to 13 μM (22). The beneficial effect of exercise on hypertension might be explained by the release of l-carnosine from muscles during exercise.

In conclusion, we found that l-carnosine administrated not only peripherally but also centrally induced suppressive and augmenting responses in RSNA and BP and that these effects of l-carnosine were eliminated by bilateral lesions in the SCN. The present findings suggest that the SCN is involved in neural pathways responsible for the l-carnosine effects on RSNA and BP. Considering the present results and the fact that SCN lesions eliminated changes in activities of autonomic nerves due to 2-DG (21) as well as changes due to light exposure (24), the SCN might be essential for changes in autonomic nerve activities due to stressful stimulation and endogenous factors. These hypotheses need to be further tested, and the detailed mechanistic functions of l-carnosine on RSNA and BP must be examined in the future.

ACKNOWLEDGMENTS

We thank Drs. Shinya Suzuki and Hidekazu Kaneko (National Institute of Advanced Industrial Science and Technology) for assistance with the experimental instruments.
REFERENCES
