Possible Role of L-Carnosine in the Regulation of Blood Glucose through Controlling Autonomic Nerves

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Mammalian muscles synthesize L-carnosine, but its roles were unknown. Previously, we found in rats that the administration of a certain amount of L-carnosine elicited an inhibition of the hyperglycemia induced by the injection of 2-deoxy-D-glucose (2DG) into the lateral cerebral ventricle (LCV), and that intravenous injection of L-carnosine inhibited sympathetic nerves and facilitated the parasympathetic nerve. Moreover, the suppressive effect of L-carnosine on the hyperglycemia induced by 2DG was eliminated by thioperamide, a histaminergic H3 receptor. These findings suggested that L-carnosine might control the blood glucose level through regulating autonomic nerves via H3 receptor. To further clarify the function of L-carnosine, we examined its role in the control of the blood glucose. In this experiment, the following results were observed in rats: (i) A certain amount (0.01% or 0.001%) but not a larger amount (0.1%) of L-carnosine given as a diet suppressed the hyperglycemia induced by LCV-injection of 2DG (2DG-hyperglycemia); (ii) LCV-injection but not the injection into the intraperitoneal space (IP) of a certain amount of L-histidine suppressed the 2DG-hyperglycemia; (iii) treatments of diphenhydramine, an H1 antagonist, and α-fluoromethylhistidine, an inhibitor of histamine-synthesizing enzyme, reduced the 2DG-hyperglycemia; (iv) the plasma L-carnosine concentration and carnosinase activity showed daily changes; (v) the plasma L-carnosine concentration was significantly lower in the streptozotocin-diabetic rats; (vi) exercise by a running wheel tended to increase carnosine synthase activity in the gastrocnemius muscle and elevated the plasma L-carnosine concentration in the dark (active) period, and enhanced the plasma carnosinase activity in the light period; (vii) IP-injection of certain amount of L-carnosine stimulated the feeding response to IP-injection of 2DG. These findings suggest a possibility that L-carnosine released from muscles due to exercise functions to reduce the blood glucose level through the regulation of the autonomic nerves. Exp Biol Med 228:1138–1145, 2003

Key words: histamine; histidine; diabetes; exercise; food intake

Muscles of mammals synthesize a dipeptide, L-carnosine (β-alanyl-L-histidine) (1), and L-carnosine content in the diaphragm of streptozotocin (STZ)-diabetic rats was reported to be lower than that in intact rats (2). Thus, it was possible that L-carnosine might be implicated in the control mechanism of glucose metabolism. Therefore, we examined the effect of L-carnosine on the hyperglycemia induced by intracranial injection of 2-deoxy-D-glucose (2DG), and found that intracranial, intraperitoneal, and intragastric administrations of certain amounts of L-carnosine caused a suppression of the hyperglycemia due to the 2DG-injection probably through the regulation of autonomic nerves (3). However, a larger or smaller amount than the effective amount did not inhibit the 2DG-hyperglycemia (3). In this work, we further examined possible roles of L-carnosine in the regulation of the blood glucose.

Materials and Methods

Animals. Male Wistar strain rats, weighing initially 250 to 300 g, were used. They were housed in a room maintained at 24 ± 1°C and illuminated for 12 h (0700–1900 h) by fluorescent tubes (80 lx). Food (type MF; Oriental Yeast Co., Tokyo) and water were freely available. In the experiment examining the effect of L-carnosine-containing diet, 0.1%, 0.01%, or 0.001% L-carnosine diet [0.1 g, 0.01 g, or 0.001g of L-carnosine was mixed in 100 g of the control diet (the type MF)] was given as a sole food. These rats were adapted to the environmental conditions for at least 1 week prior to the experiment. To examine the effect of exercise, rats were housed individually in metallic cages [13.5 × 40 × 18 (height) cm], each attached with

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a running wheel (outer diameter = 1 m, Biomedica Kikai Co., Osaka). Those rats that showed more than 3000 rotations of the wheel per day were used as rats of wheel running exercised group. Control animals were housed individually in metallic cages [13.5 × 40 × 18 (height) cm] without the wheels. Rats were made diabetic by IP-injection of streptozotocin (STZ) [60 mg/kg, dissolved in citrate buffer (pH 4.5)] 3 days before the experiment. This animal experiment was approved by the Committee for Use of Laboratory Animals of Institute for Protein Research, Osaka University.

Blood Sampling and Measurement of the Concentration of the Plasma Glucose. Three days before the experiment, a brain cannula made of polyethylene tubing (PE-10, Clay Adams, Parsippany, NJ) was inserted into the right lateral cerebral ventricle (LCV), and another catheter made of SILASTIC (Dow Corning, Midland, MI) and polyethylene (PE-50, Clay Adams) tubes was inserted into the right atrium of the heart under pentobarbital anesthesia (35 mg/kg) as previously described (3). On the experimental day 2DG (40 μmol/10 μl of artificial cerebrospinal fluid, aCSF) was injected into LCV using the brain cannula without anesthesia at 1300 h. Blood samples (0.2 ml each) were withdrawn from the heart using the heart catheter before (0 min) and 30, 60, 90, and 120 min after the administration of 2DG. In this experiment, food was deprived of the rats from 2 h prior to the experiment until the end of blood sampling. The measurement of the plasma glucose was done as described previously (3, 4). Briefly, the blood samples (0.2 ml each) were mixed with EDTA (300 nmol) in a volume of 10 μl. Blood samples were centrifuged, and plasma samples obtained were stored at −60°C until the glucose assay. The plasma glucose concentration was measured by the glucose oxidase method with a Fuji Dri-chem system (Fuji Film, Co., Tokyo). When effects of L-histidine and diphenhydramine (diphenhydramine hydrochloride, Sigma Co.) were examined, these agents were given into the intraperitoneal space (IP) or LCV just before the LCV injection of 2DG. When the effect of α-fluoromethylhistidine hydrochloride (FMH) was examined, IP-injection of FMH (100 mg/kg; kindly given by Drs. Tatehiko Watanabe and Atsushi Yamatodani) was given 24 h and 2 h before the experiment.

To examine daily changes in the plasma L-carnosine concentration and plasma carnosinase activity, animals maintained under a 12:12-hr light:dark cycle for more than 2 weeks were killed by decapitation and trunk blood was obtained. EDTA (final concentration of 1.5 μmol/ml) was added to the blood samples, and the plasma obtained were stored at −60°C until the assays

Determinations of Blood Concentration of L-Carnosine and Activities of Plasma Carnosinase and Muscle Carnosine Synthase. To determine the plasma concentration of L-carnosine, trichloroacetic acid (TCA) in a final concentration of 0.5 M was added to 100 μl of the plasma and denatured proteins were removed by a centrifugation (1000 × *g* for 10 min). The supernatant was subjected to an HPLC column for amino acid analysis. The concentration of L-carnosine was analyzed with an amino acid analyzing system from Shimadzu Co., Kyoto, using a Shim-Pack ISC-07/S1504 Li column for separation described previously (5). Carnosinase activity was measured by the procedure of Bando *et al.* (6). The plasma sample (100 μl) was pre-incubated with 300 μl of 50 mM Tris-HCl (pH 8.5) and 50 μl of 20 mM $MnCl_2$ at 37°C for 5 min. Then 100 μl of 50 mM L-carnosine solution was added and the mixture was incubated for 1 h at 37°C. The reaction was terminated by adding 50 μl of 50% TCA solution, and the supernatant obtained after centrifugation was analyzed by the HPLC system described above. Carnosinase activity was expressed by β-alanine formed/10 μl of the plasma/h. For the assay of carnosine synthetase activity a partially purified enzyme by 30% saturation of ammonium sulfate was used as mentioned previously (7, 8). That is, bilateral gastrocnemius muscles were excised, minced, and homogenized with 2 vols of 15 mM potassium phosphate buffer (pH 7.4) containing 2.5 mM dithiothreitol, 1 mM EGTA, and 3% glycerol. After centrifugation (8,000 × *g*) for 15 min, the supernatant was fractionated with 30% saturated ammonium sulfate. The precipitate was suspended with the pre-fractionated volume of homogenizing buffer and the supernatant after the centrifugation was used as partially purified enzyme. Carnosine synthetase assay was done as reported previously (7, 8). That is, 100 μl of the enzyme extract was added to the above homogenizing buffer containing 3 mM L-histidine, 30 mM β-alanine, 4 mM Mg-ATP, and 1 μM gabaculin (3-amino-2,3-dihydrobenzoic acid, Sigma Co.), and incubated for 1 h at 37°C. The incubation was stopped by adding equal volume of 6% TCA, and the concentration of L-carnosine in the supernatant after the centrifugation was analyzed with an amino acid analyzing system mentioned above.

Determinations of Food Intake after IP-Injections of 2DG and L-Carnosine. On the experimental day, animals were deprived of food for 2 h and IP-injection of 500 mg/kg of 2DG was given at 1300 h and food intake for 1 h was determined. When the effect of L-carnosine was examined, various amounts of L-carnosine dissolved in 0.1 ml of saline were injected intraperitoneally just before the 2DG-injection.

Data and statistics. Data are expressed as means ± SEM. Statistical analyses were done by analysis of variance (repeated measurements ANOVA) and Mann-Whiney U-test.

Results

Effects of Diet Containing L-Carnosine on 2DG Hyperglycemia. At first, we examined effects of diets containing 0.1%, 0.01%, and 0.001% L-carnosine given as a sole food for 7 days on the hyperglycemia induced by LCV-injection of 2DG (2DG-hyperglycemia) (Fig. 1). Consequently, it was found that 0.01% and 0.001% L-carnosine diets but not 0.1% L-carnosine diet significantly inhibited

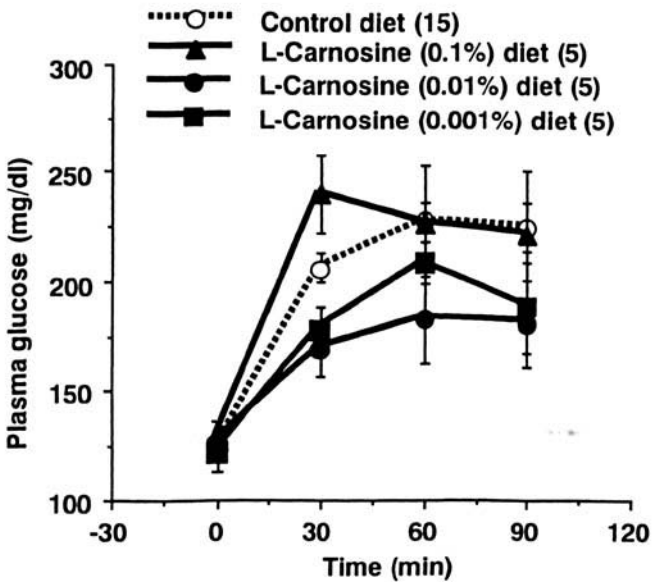

Figure 1. Effects of 0.1%, 0.01%, and 0.001% L-carnosine diets on the 2DG-hyperglycemia; 0.1%, 0.01%, or 0.001% L-carnosine diet was given as a sole food for 7 days. For control rats, control diet (type MF, Oriental Yeast Co, Ltd., Tokyo) was given. 2DG (40 μmol/10 μl of aCSF, LCV) was injected at 0 min. Data are shown as means ± SEM. Numbers of animal used are shown in parentheses. Significances of differences between post-injection values (30–90 min) were analyzed by ANOVA (repeated measures). Comparison: 0.1% vs control, NS ($F = 0.65$); 0.01% vs control, $P < 0.0005$ ($F = 16.7$); 0.001% vs control, $P < 0.05$ ($F = 7.0$). Other explanations are in the text.

the 2DG-hyperglycemia. This fact supports the previous finding that a certain amount of L-carnosine is able to suppress the 2DG-hyperglycemia.

Comparative Effects of L-Carnosine, Histamine, and L-Histidine on the 2DG-Hyperglycemia. Since L-carnosine is composed of β-alanine and L-histidine, we examined the effect of L-histidine on the 2DG-hyperglycemia. As seen in Figure 2, IP-injection of L-histidine did not cause a significant suppression of the 2DG-hyperglycemia, though a borderline ($0.05 < P < 0.1$ by ANOVA) suppression was observed when 5 nmol of L-histidine was injected (Fig. 2A). In contrast, LCV-injection of L-histidine elicited significant ($P < 0.0005$ by ANOVA) suppression on the 2DG-hyperglycemia when 0.005 or 0.05 nmol of L-histidine was given (Fig. 2B).

Previously, we observed a significant inhibition of the 2DG-hyperglycemia by certain amounts of L-carnosine (IP, 0.005–5 nmol; LCV, 0.05–5 nmol) and those of histamine (LCV: 1-100 nmol) but not by IP-injection of 1 to 100 nmol of histamine (3). To summarize actions of IP- and LCV-injections of L-carnosine, histamine, and L-histidine on the 2DG-hyperglycemia, the plasma glucose levels 60 min after LCV-injection of 2DG with either IP- or LCV injections of these agents are plotted as percentages of the glucose concentrations in which the glucose level at 0 min is expressed as 100%. Figure 3 shows the results. Values of experiments examined effects of L-carnosine and histamine are the results reported previously (3) and those of L-histidine are

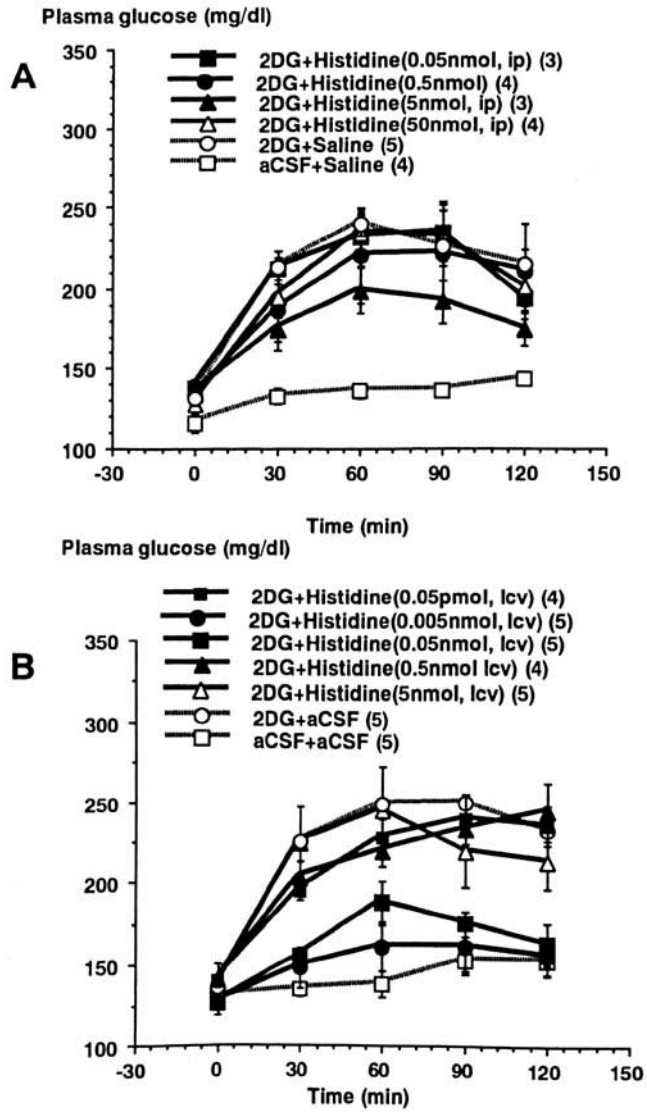

Figure 2. Effects of intraperitoneal and lateral cerebral ventricular injection of L-histidine on the 2DG-hyperglycemia. Rats were given intraperitoneal (IP) or lateral cerebral ventricular (LCV) injection of L-histidine. For control rats, saline (IP) or aCSF (LCV) was injected instead of L-carnosine. (A) IP-injection of L-carnosine was given. The significances of the differences between postinjection values (30–120 min) were analyzed by ANOVA. Comparison: 2DG + saline vs aCSF + saline, $P < 0.0005$ ($F = 270$); 2DG + saline vs 2DG + histidine (0.05), NS ($F = 0.52$); 2DG + saline vs 2DG + histidine (0.5), NS ($F = 1.62$); 2DG + saline vs 2DG + histidine (5), $0.5 < P < 0.1$ ($F = 3.24$); 2DG + saline vs 2DG + histidine (50), NS ($F = 1.03$). (B) LCV-injection of L-histidine was given. The significances of the differences between postinjection values (30–120 min) were analyzed by ANOVA. Comparison: 2DG + aCSF vs aCSF + aCSF, $P < 0.0005$ ($F = 166$); 2DG + aCSF vs 2DG + histidine (0.0005), $0.05 < P < 0.1$ ($F = 3.29$); 2DG + aCSF vs 2DG + histidine (0.005), $P < 0.0005$ ($F = 92$); 2DG + aCSF vs 2DG + histidine (0.05), $P < 0.0005$ ($F = 94$); 2DG + saline vs 2DG + histidine (0.5), NS ($F = 2.46$); 2DG + saline vs 2DG + histidine (5), NS ($F = 1.28$). Other explanations are in the text and the legend to Figure 1.

those in Figure 2. As seen in Figure 3, LCV-injections of certain doses of L-carnosine, histamine, and L-histidine significantly suppressed the increase in glucose levels 60 min after the 2DG-injection, but only IP-injections of certain doses of L-carnosine among them could significantly inhibit

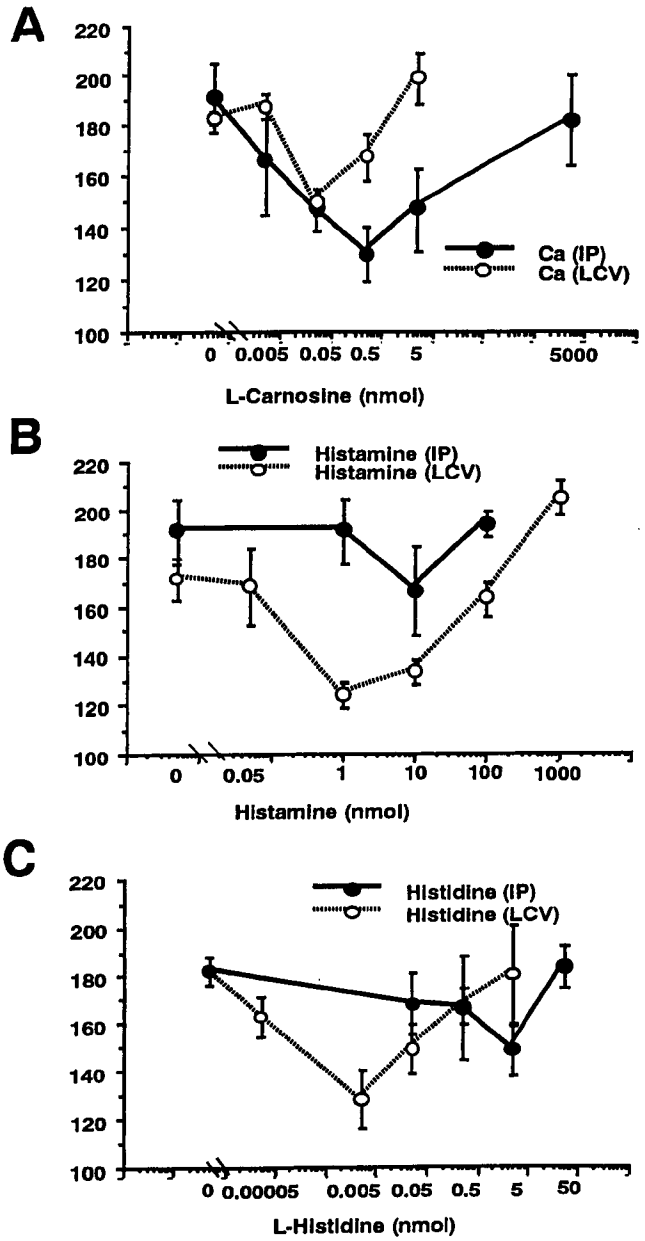

Figure 3. Plasma glucose levels 60 min after the injection of 2DG in rats injected with L-carnosine, histamine, and histidine. Glucose values were expressed as percentages of values at 0 min (100%) when LCV-injection of 2DG was done in rats that received LCV- or IP-injection of L-carnosine (Ca), histamine, or L-histidine. Data of effects of L-carnosine and histamine shown here are plotted from the results reported previously (3). Data are shown as means ± SEM. Logarithmic values in doses of agents are used as X-axis.

the increase in the glucose level after the 2DG-injection. These findings indicate that histamine and L-histidine show their inhibitory action on the 2DG-hyperglycemia only when these agents injected into LCV and these facts suggest a possibility that certain amount of histamine synthesized from L-histidine in the brain might have an inhibitory action on the 2DG-hyperglycemia.

Effects of Diphenhydramine and α-fluoromethylhistidine on the 2DG-Hyperglycemia. To examine the possibility that histamine might be implicated in the mechanism of 2DG-hyperglycemia, we examined effects of an antagonist of histaminergic H1-receptor, diphenhydramine hydrochloride (diphenhydramine), and an inhibitor of histamine synthesis, α-fluoromethylhistidine hydrochloride (FMH), on the 2DG-hyperglycemia. Consequently, either IP-injection of diphenhydramine or IP-injections of FMH significantly suppressed the 2DG-hyperglycemia (Fig. 4). These findings suggest that histamine functions to potentiate the 2DG-hyperglycemia through H1-receptor.

Daily Changes in the Plasma L-Carnosine Concentration and Carnosinase Activity. Since L-carnosine is synthesized in muscles (1) and suggested to function to reduce the blood glucose level (3), it was possible that L-carnosine released from muscles might be an endogenous factor to control the blood glucose concentration. In such

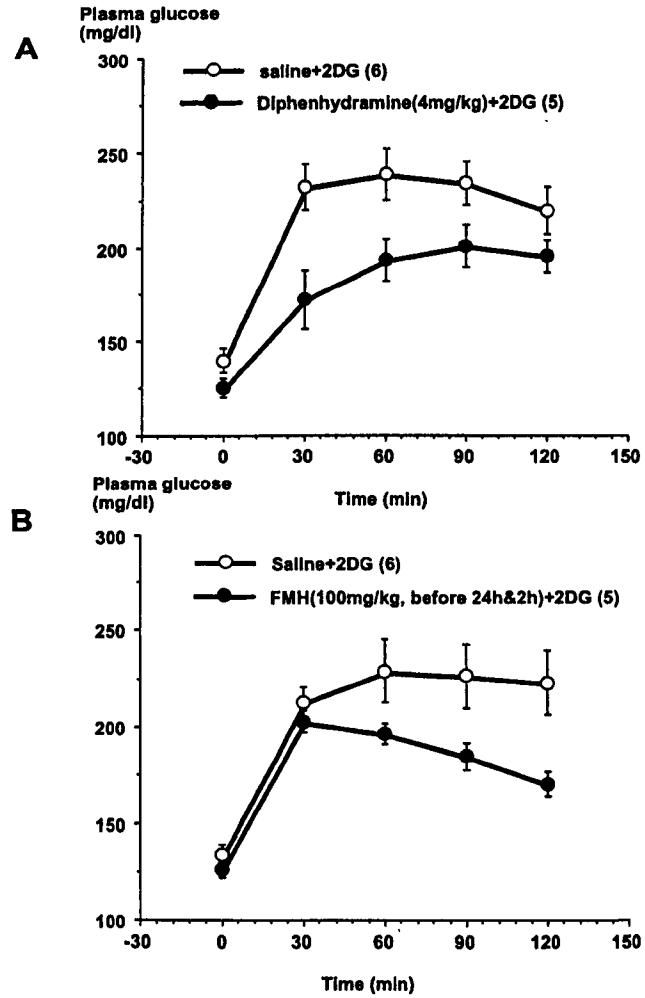

Figure 4. Effects of diphenhydramine, a histaminergic H1-antagonist, and α-fluoromethylhistidine, an inhibitor of histidine decarboxylase, on the 2DG-hyperglycemia. (A) IP-injection of diphenhydramine hydrochloride (4 mg/kg) was given just before LCV-injection of 2DG. Significance of the difference between postinjection values (30–120 min) was analyzed by ANOVA. Comparison: 2DG + saline vs 2DG + diphenhydramine, $P < 0.0005$ ($F = 27.2$). (B) IP-injections of α-fluoromethylhistidine hydrochloride (FMH, 100 mg/kg) at 24 h and 2 h before the LCV-injection of 2DG were given. Significance of the difference between postinjection values (30–120 min) was analyzed by ANOVA. Comparison: 2DG + saline vs 2DG + FMH, $P < 0.05$ ($F = 6.06$).

case, it is possible that the blood concentration of L-carnosine might show a daily change. Therefore, the daily changes in the plasma L-carnosine and plasma activity of an L-carnosine degrading enzyme, carnosinase, were examined in rats maintained under a 12:12-hr light:dark condition. As seen in Figure 5A, a significant daily change in the plasma concentration of L-carnosine was detected. The concentration was the highest at the end of the 12-h dark period, an active period of nocturnal animals like rats, and the lowest at the later half of the 12-h light period, a resting period for them. A significant daily change was also observed in the plasma carnosinase activity with higher values being detected in the later part of the 12-h dark period, and lower values in the later part of the 12-h light period (Fig. 5B).

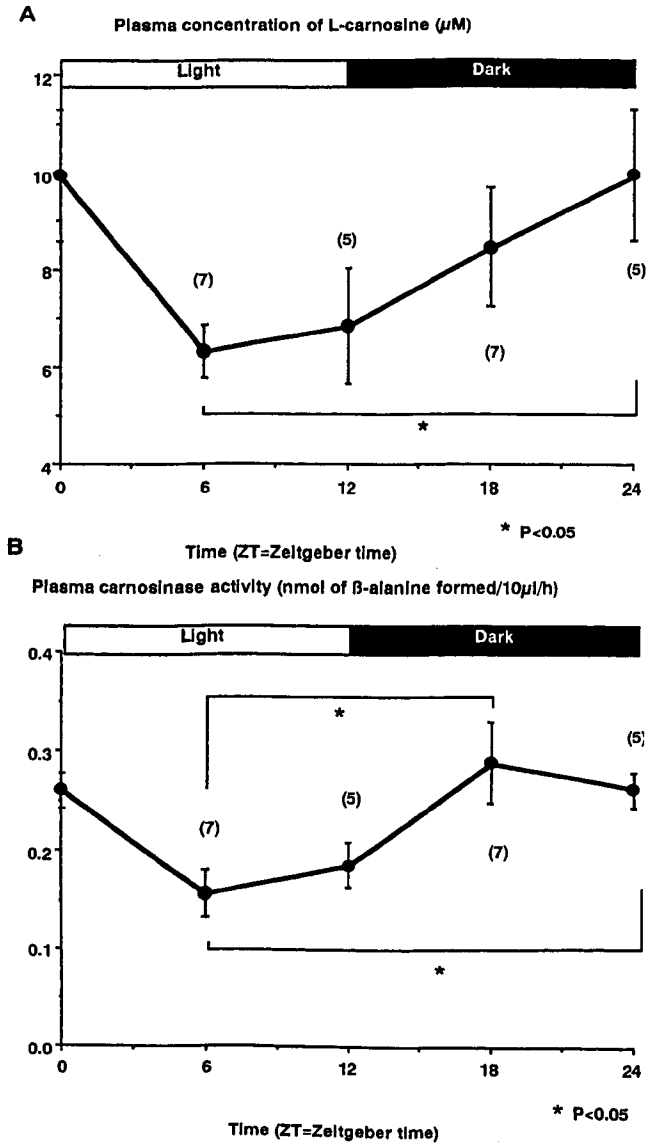

Figure 5. Daily changes in the plasma concentration of L-carnosine and the plasma carnosinase activity. Animals were killed at indicated times. Statistical significance of the difference between values at different time points were indicated by * ($P < 0.05$ by Mann-Whitney U-test). Other explanations are in the text and the legend to Figure 1.

Changes in the Plasma L-Carnosine Concentration and Related Enzyme Activities under Conditions of Diabetes and Exercise. Previously it was reported that L-carnosine content in the diaphragm reduced in STZ-diabetic rats (2). Thus it was possible that the plasma L-carnosine concentration in the diabetic rats might be lower than in intact rats. Therefore, changes in the plasma concentration were examined in STZ-diabetic rats. As seen in Figure 6, the plasma glucose concentration dramatically increased in the STZ-diabetic rats, and the plasma L-carnosine concentration was significantly lower in the STZ-diabetic rats than intact rats.

Moreover, it was examined whether the plasma concentration of L-carnosine changed when animals did exercise. For the sake of this, a running wheel is attached to each animal cage and changes in the plasma L-carnosine level and carnosinase activity and carnosine synthase activity in gastrocnemius muscles were examined in rats that showed more than 3000 rotations per day. The carnosine synthase activity in the muscle was similar in rats with and without the running wheel at the middle of the light period [Zeit-

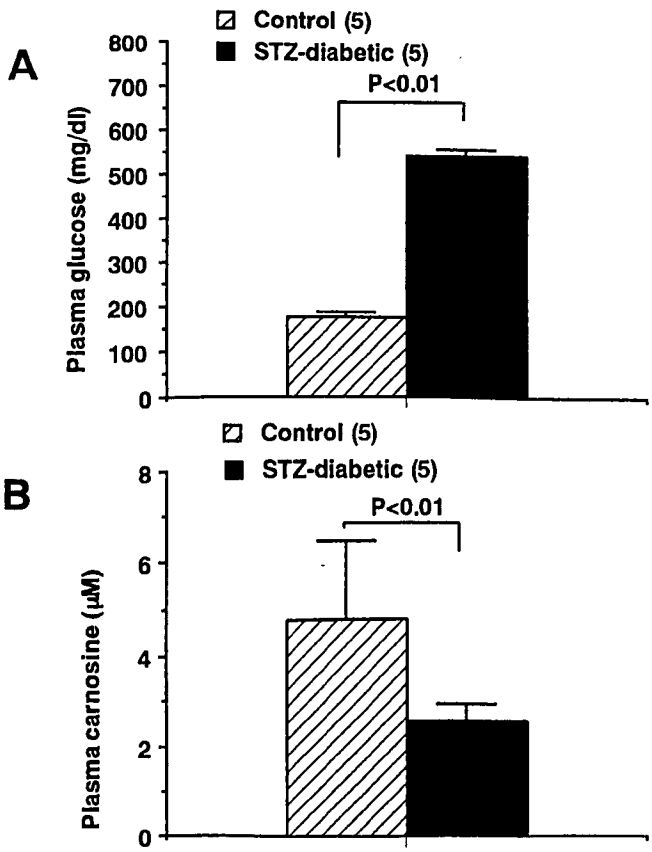

Figure 6. The plasma concentrations of glucose and L-carnosine in STZ-diabetic and intact control rats. Rats were made diabetic by the injection of streptozotocin (60 mg/kg, i.p.) 3 days prior to the experiment. On the experimental day food was deprived for 2 h and rats were killed at 1300 h. The significances of the differences between values of control and STZ-diabetic rats were significant ($P < 0.01$) by Mann-Whitney U-test. Other explanations are in the text and the legend to Figure 1.

geber time (ZT) 6] (Fig. 7A). However, at the middle of the dark period (ZT18) control rats without the wheel showed lower activity than ZT6, and exercised rats with the wheel showed a borderline ($0.05 < P < 0.1$) increase in the muscle carnosine synthase activity when compared with that of control rats without the wheel (Fig. 7A).

In respect to the plasma carnosinase activity, a similar activity was observed at ZT6 and ZT18 (Fig. 7B). However, exercised rats exhibited a significant ($P < 0.05$) increase in its activity compared with control rats without the running wheel at ZT6 (Fig. 7B). The plasma L-carnosine concentration was similar in rats with and without the running wheel at ZT6, but it was significantly ($P < 0.05$) higher in the exercised rats than control rats without the running wheel at ZT18 (Fig. 7C). These findings suggest that exercise can enhance the plasma L-carnosine concentration possibly through the increase in the activity of carnosine synthase in skeletal muscles.

Effect of L-Carnosine on Food Intake after LCV-Injection of 2DG. It was known that IP-injection of 2DG elicited food intake in rats (9). To examine whether L-carnosine affects the appetite in rats, the effect of IP-injection of various doses of L-carnosine on the food intake for 1 h after IP-injection of 2DG (500 mg/kg) was examined. As seen in Figure 8, only 100 ng of L-carnosine significantly ($P < 0.05$ by Mann-Whitney U-test) enhanced the food intake.

Discussion

In this experiment, the following results were observed in rats: (i) a certain amount (0.01% or 0.001%) but not a larger amount (0.1%) of L-carnosine given as a diet suppressed the 2DG-hyperglycemia (Fig. 1); (ii) LCV-injection but not IP-injection of certain amount of L-histidine suppressed the 2DG-hyperglycemia (Fig. 3); (iii) treatments of diphenhydramine, an H1 antagonist, and FMH, an inhibitor of histamine-synthesizing enzyme, reduced the 2DG-hyperglycemia (Fig. 4); (iv) the plasma L-carnosine concentration and plasma carnosinase activity showed daily changes (Fig. 5); (v) the plasma L-carnosine concentration was significantly lower in the STZ-diabetic rats than in intact rats (Fig. 6); (vi) exercise by a running wheel tended to increase carnosine synthase activity in the gastrocnemius muscle and significantly elevated the plasma L-carnosine concentration at the middle of the dark (active) period and enhanced the plasma carnosinase activity at the middle of the light (resting) period (Fig. 7); (vii) IP-injection of certain amount of L-carnosine stimulated the feeding response to IP-injection of 2DG (Fig. 8).

Previously, we found evidence that LCV-injection of 2DG causes hyperglycemia through the control of autonomic nerves (10–13). That is, LCV-injection of 2DG elicits excitations of sympathetic nerves and inhibits of excitations of parasympathetic nerves, and the resultant suppression of insulin secretion and enhancements of secretions of adrenaline and glucagon causes hyperglycemia. In respect

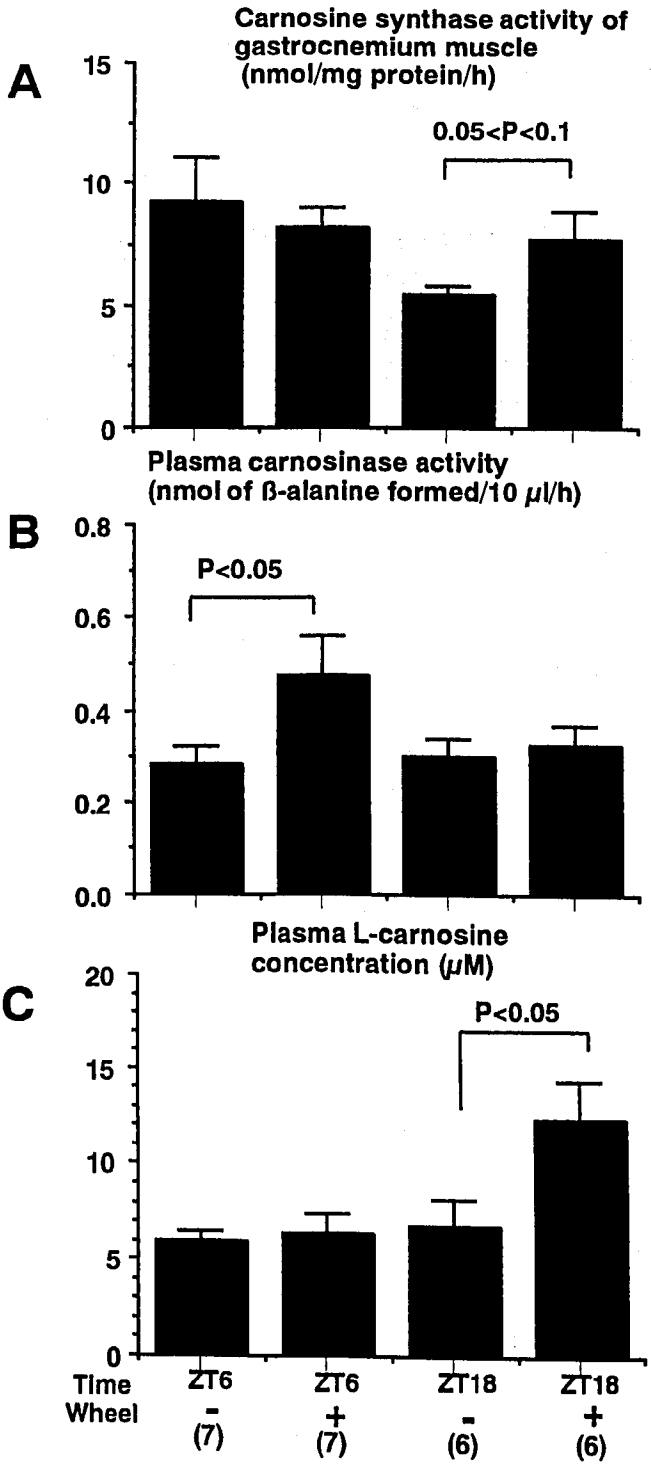

Figure 7. The activity of carnosine synthase activity in the gastrocnemius muscle, the plasma activity of carnosinase, and the plasma L-carnosine concentration in exercised rats with a running wheel and control rats at ZT6 and ZT18. The statistical significances by Mann-Whitney U-test of the differences between values are indicated. Other explanations are in the text and the legend to Figure 1.

to the mechanism of the lowering effect of L-carnosine on the 2DG-hyperglycemia, previously we observed evidence that a certain amount of L-carnosine lowers neural activities of sympathetic nerves and facilitates those of parasympathetic nerves (3, 14). It is indicated that the inhibition of the

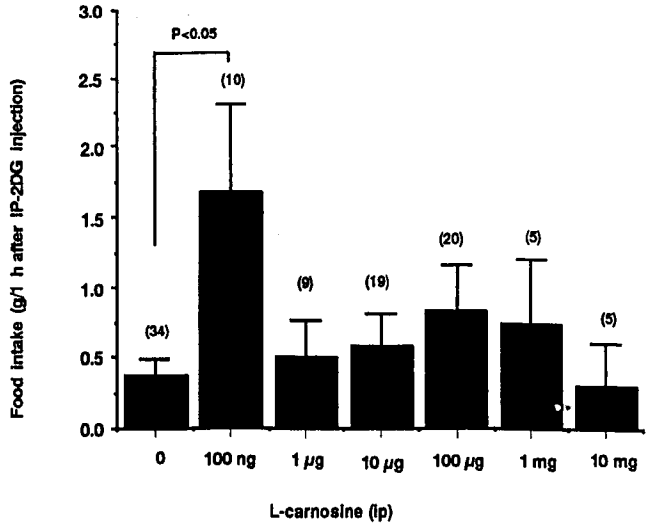

Figure 8. Effects of IP-injection of various doses of L-carnosine on the food intake after IP-injection of 2DG. Food intake for 1 h after IP-injection of 2DG was measured. The statistical significance of the difference between food intakes of rats injected with saline and 100 ng of L-carnosine by Mann-Whitney U-test is indicated. Other explanations are in the text and the legend to Figure 1.

pancreatic sympathetic nerve and the excitation of the pancreatic parasympathetic nerve cause both an increase in the insulin secretion and a suppression in the glucagon secretion from the pancreas (15). Therefore, the suppressive effect of L-carnosine on the 2DG-hyperglycemia seems to be realized by the above changes due to L-carnosine.

In this experiment, it was found that L-histidine, a component of L-carnosine, caused an inhibition of 2DG-hyperglycemia when it was injected into LCV (Fig. 2). Since L-histidine is converted to histamine in the brain and LCV-injection of certain amount of histamine also caused a suppression of the 2DG-hyperglycemia (3, Fig. 3), it is possible that histamine might be implicated in the mechanism of suppressive actions of L-histidine and L-carnosine on the 2DG-hyperglycemia. To examine this possibility, effects of diphenhydramine, an H1-blocker, and FMH, a specific suicide inhibitor of histidine decarboxylase, on the 2DG-hyperglycemia were examined. Consequently, both treatments significantly inhibited the hyperglycemia (Fig. 4). This suggests that the histaminergic H1-receptor is involved in the mechanism of the 2DG-hyperglycemia by elevating the blood glucose level. Comparing the effects of L-carnosine, histamine, and L-histidine, certain amounts of these agents could suppress the 2DG-hyperglycemia (2, Fig. 3). In respect to histaminergic neurons, previously it was suggested that histamine stimulated lipolysis in rat white adipose tissue through the facilitation of the sympathetic nervous system (16). Consistent with this, it was observed that LCV-injection of a higher amount of histamine enhanced the 2DG-hyperglycemia (3, Fig. 3B). In contrast, it was reported that the histaminergic H3 receptor has a higher affinity for histamine than H1 and H2 receptors and is implicated in inhibitions on histaminergic nerves as an inhibitory presynaptic receptor for the endogenous histamine release in the central and peripheral nerves (17). Therefore, it is possible that LCV-injections of certain amounts of L-carnosine, histamine, or L-histidine suppressed the 2DG-hyperglycemia through the stimulation of the histaminergic H3 receptor. In accordance with this possibility, we found that thioperamide, an antagonist for histaminergic H3 receptor, could inhibit both suppressive actions of L-carnosine and L-histamine on the 2DG-hyperglycemia (3). Considering these facts and previous findings, it seems that in the brain a higher amount of histamine facilitates and a lower amount of histamine inhibits sympathetic nerves, thus stimulates and suppresses the 2DG-hyperglycemia, respectively. Whether smaller amounts of L-carnosine and L-histidine suppress the 2DG-hyperglycemia by their direct effects on H3-receptor or not must be examined in future.

Regarding food intake, it was suggested that L-histamine functions to suppress appetite in rats and mice (18, 19). However, doses used in the experiments (18, 19) were much higher than doses used in the previous experiment. Previously, it was observed that LCV-injection of 1 μmol of histamine did not suppress the 2DG-hyperglycemia but enhanced it (3, Fig. 3). Therefore, it was possible that smaller doses of L-carnosine might enhance appetite via H3 receptor in rats. Thus, we examined the effect of IP-injection of smaller amount of L-carnosine on feeding response to IP-injection of 2DG. As seen in Figure 8, IP-injection of 100 ng (about 0.44 nmol) of L-carnosine could enhance the food intake for 1 h after the 2DG injection. Whether thioperamide is able to block this enhancement or not must be examined in future.

In respect to L-carnosine content, it was reported that L-carnosine content in the diaphragm of STZ-diabetic rats was lower than that of control rats (2). Consistent with this, it was observed that the plasma L-carnosine concentration of the STZ-diabetic rats was lower than that of control rats (Fig. 6). In this experiment significant daily changes were observed in the plasma concentration of L-carnosine and carnosinase (Fig. 5). This raised a possibility that L-carnosine might be an endogenous factor controlling the blood glucose. Since the highest L-carnosine concentration was observed at the end of the active period (Fig. 5), it was possible that L-carnosine might be released from muscles during and after exercise. Therefore, we examined effects of exercise using running wheels on the plasma concentration of L-carnosine, the plasma carnosinase activity, and the activity of muscle carnosine synthase in the middle of the light (ZT 6) and dark (ZT 18) periods. Consequently, it was found that the plasma L-carnosine concentration was higher in the exercised rats that showed more than 3000 rotations of the running wheel per day than in the control rats at ZT 18 (Fig. 7). The muscle carnosine synthase activity tended to be higher in the exercised rats than in the control rats at ZT 18 (Fig. 7). This tendency of the elevation of the synthase activity may explain the increase in the plasma concentration of L-carnosine. In contrast, the plasma carnosi-

nase activity was higher in the exercised rats than in the control rats at ZT 6 (Fig. 7). In spite of this, the plasma L-carnosine concentration and the activity of muscle carnosine synthase did not change at ZT 6. This might indicate that the release of L-carnosine from muscles might be changed at ZT 6. These possibilities must be examined in future.

In conclusion, the present findings presented a possibility that L-carnosine synthesized in muscles functions to control the blood glucose level through the regulation of the autonomic nervous system. Whether these are the cases and why the L-carnosine content decreases in STZ-diabetes are now under the investigation.

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