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REVIEW ARTICLE

Role of L-carnosine in the control of blood glucose, blood pressure, thermogenesis, and lipolysis by autonomic nerves in rats: involvement of the circadian clock and histamine

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Abstract L-Carnosine (β-alanyl-L-histidine; CAR) is synthesized in mammalian skeletal muscle. Although the physiological roles of CAR have not yet been clarified, there is evidence that the release of CAR from skeletal muscle during physical exercise affects autonomic neurotransmission and physiological functions. In particular, CAR affects the activity of sympathetic and parasympathetic nerves innervating the adrenal glands, liver, kidney, pancreas, stomach, and white and brown adipose tissues, thereby causing changes in blood pressure, blood glucose, appetite, lipolysis, and thermogenesis. CAR-mediated changes in neurotransmission and physiological functions were eliminated by histamine H1 or H3 receptor antagonists (diphenhydramine or thioperamide) and bilateral lesions of the hypothalamic suprachiasmatic nucleus (SCN), a master circadian clock. Moreover, a carnosine-degrading enzyme (carnosinase 2) was shown to be localized to histamine neurons in the hypothalamic tuberomammillary nucleus (TMN). Thus, CAR released from skeletal muscle during exercise may be transported into TMN-histamine neurons and hydrolyzed. The resulting L-histidine may subsequently be converted into histamine, which could be responsible for the effects of CAR on neurotransmission and physiological function. Thus, CAR appears to influence hypoglycemic, hypotensive, and lipolytic activity through regulation of autonomic nerves and with the involvement of the SCN and histamine. These findings are reviewed and discussed in the context of other recent reports, including those on carnosine synthetases, carnosinases, and carnosine transport.

Keywords Sympathetic nerve · Parasympathetic nerve · Suprachiasmatic nerve · Adrenal gland · Pancreas · Carnosinase

Abbreviations

BAT	Brown adipose tissue
BP	Blood pressure
BW	Body weight
CAR	L-Carnosine
CN	Carnosinase
2DG	2-Deoxy-D-glucose
DKO	Double knockout
FFA	Free fatty acids
IP	Intraperitoneal
IV	Intravenous
LCV	Lateral cerebral ventricular
MAP	Mean arterial pressure
NK	Natural killer
PEPT1	H^+/peptide cotransporter 1
PEPT2	H^+/peptide cotransporter 2
PHT1	Peptide/histidine transporter 1
RSNA	Renal sympathetic nerve activity
SCN	Suprachiasmatic nucleus
SGFO	Scent of grapefruit oil
SLVO	Scent of lavender oil

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STZ	Streptozotocin
TMN	Tuberomammillary nucleus
UCP	Uncoupling protein
WAT	White adipose tissue

Introduction

The mammalian autonomic nervous system, in collaboration with the endocrine system, maintains homeostasis of essential physiological functions, including body temperature, blood glucose, and blood pressure (BP). In order to elucidate the function or mechanism of action of drugs, foods, scents, music, and massage oils, we examined the activities of autonomic nerves innervating tissues/organs by electrophysiological methods in rats and mice (Niijima 1989; Niijima et al. 1998, 2002; Niijima and Nagai 2003; Tanida et al. 2005a; Nakamura et al. 2007; Shen et al. 2009; Horii et al. 2011). In particular, rats and mice were anesthetized with urethane, and sympathetic or parasympathetic nerve fibers innervating tissues or organs were exposed. The proximal ends of nerves were cut and placed on a pair of silver electrodes, and the changes in activity of nerve fibers were monitored by an electrophysiological method (Fig. 1). The relationship between changes in autonomic nerve activity and the corresponding changes in physiological functions is summarized in Table 1. Briefly, excitation of the sympathetic nerves innervating the white adipose tissue, brown adipose tissue, pancreas, adrenal gland, gut, kidney, cutaneous artery, and spleen produced breakdown of triglycerides (lipolysis) and thermogenesis, suppression of insulin secretion, stimulation of adrenaline secretion, elevations in BP and blood glucose, suppression of digestion, lowering of blood flow to the skin, and a decrease in the activity of natural killer (NK) lymphocytes, respectively. Excitation of the parasympathetic nerves or suppression of the sympathetic nerves innervating the aforementioned tissues and organs elicited the opposite effect on physiological functions to those induced by sympathetic excitation.

Physicians generally recommend physical exercise for patients with diabetes and hypertension associated with metabolic syndrome. Indeed, we employed the electrophysiological technique mentioned above and standard animal experimental techniques to provide evidence that L-carnosine (β-alanyl-L-histidine; CAR) is released from skeletal muscle in rats during physical exercise and that CAR may be involved, through changes in autonomic neurotransmission, in mediating the beneficial effects of physical exercise for diabetes and hypertension. We found that the plasma concentration of CAR in rats was highest at the end of a dark period (an active period for rats), and physical exercise on a running wheel increased the plasma concentration of CAR during a nocturnal active period (Nagai et al. 2003). In addition, small amounts of CAR lowered either the BP or blood glucose levels by suppressing the activity of sympathetic nerves innervating the adrenal glands, liver, and pancreas (Yamano et al. 2001; Niijima et al. 2002). Furthermore, we provided evidence that the suprachiasmatic nucleus (SCN) of the hypothalamus—a master circadian clock—and histaminergic neurons were involved in the aforementioned functions of CAR. The details of our findings and other recent reports concerning CAR are discussed in the current article.

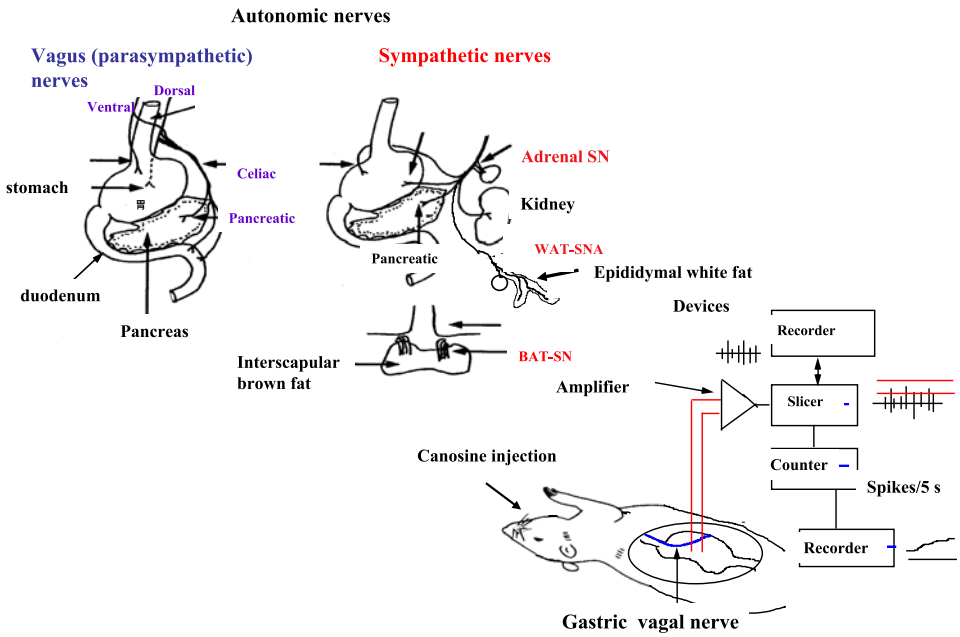

Fig. 1 Assay method of autonomic nerve activities in urethane-anesthetized rats. Proximal ends of autonomic nerve fibers are cut and their central ends are hooked up on silver-wire electrode and electrical activity determined with an electrophysiological method. Effects of oral, intravenous, intracranial, and intestinal administrations of agents, skin applications of creams and solutions, olfactory stimulations with scents of essential oils and music on autonomic nerve activities were determined

Table 1 Relationship between changes in autonomic neurotransmission and physiological functions

Tissue/organ	Sympathetic nerve excitation	Parasympathetic nerve excitation (sympathetic nerve suppression)
White adipose tissue	Lipolysis	Lipid accumulation
Brown adipose tissue	Thermogenesis (elevation of body temperature)	Decrease in thermogenesis (lowering of body temperature)
Pancreas	Suppression of insulin secretion	Increase in insulin secretion
	Increase in glucagon secretion	
Adrenal gland	Increase in adrenaline secretion (increase in BP and BG)	Decrease in adrenaline secretion (decrease in BP and BG, relaxation)
Liver	Glycogenolysis	Glycogen synthesis
	Gluconeogenesis	Decrease in gluconeogenesis
Kidney	Increase in BP	Decrease in BP
Gut	Suppression of digestion and absorption (decrease in appetite)	Facilitation of digestion and absorption (increase in appetite)
Skin artery	Vasoconstriction (decrease in cutaneous blood flow)	Vasodilatation (increase in cutaneous blood flow)
Skeletal muscle	Vasodilatation (increase in muscle blood flow)	
Spleen	Decrease in NK activity	Increase in NK activity

BP blood pressure, *BG* blood glucose, *NK* natural killer

Effect of CAR on blood glucose and BP in rats

CAR is found in large amounts in mammalian skeletal muscle, where it is synthesized by carnosine synthetase (Boldyrev and Severin 1990). Since the CAR content in the diaphragm of streptozotocin (STZ)-diabetic rats was lower than that in intact rats (Buse et al. 1980), it was hypothesized that CAR is involved in the control of glucose metabolism. Furthermore, a marked decrease in the BP and an increase in the blood concentration of CAR were observed in a patient with a congenital deficiency of carnosinase (CN), a CAR-degrading enzyme (Willi et al. 1997). Moreover, CAR content in the skeletal muscle of hypertensive rats was markedly reduced (Johnson and Hammer 1992). Therefore, these findings suggest that CAR may be involved in the control of BP. We previously observed that CAR was a major component of a particular chicken essence (Brand's Essence of Chicken; Cerebos Pacific, Ltd., Singapore), which causes a hypoglycemic action (Yamano et al. 2001). Indeed, chicken essence is believed to be a beneficial remedy for health in occidental countries. Therefore, the effects of CAR on blood glucose and BP in rats are discussed below in the context of other recently published reports.

Effect of CAR on blood glucose

Intracranial injection of 2-deoxy-D-glucose (2DG) causes a hypoglycemic state in mammals by inhibition of glucose transport and glucose phosphate isomerase (a glycolytic enzyme), leading to inhibition of cellular glucose uptake and glycolysis. In this crisis, the activity of sympathetic nerves innervating the adrenal gland, pancreas, and liver are elevated, resulting in enhanced adrenaline and glucagon secretion and impaired insulin secretion (Nagai et al. 1996a). Moreover, such changes result in increased glycogenolysis and gluconeogenesis in the liver, leading to hyperglycemia (Nagai et al. 1996a). Thus, to examine the influence of CAR on glucose metabolism, the effect of CAR on hyperglycemia induced by intracranial (lateral cerebral ventricular; LCV) injection of 2DG (2DG-hyperglycemia) was determined in rats. Peripheral administration of a small amount of CAR (0.005 to 5 nmol/300 g body weight [BW] by intraperitoneal injection [IP]) or administration of a food diet containing 0.001% CAR reduced 2DG-hyperglycemia, producing an increase in plasma insulin levels, a decrease in plasma glucagon levels, and suppression of the activity of sympathetic nerves innervating the adrenal gland and liver (Yamano et al. 2001; Nagai et al. 2003). Thus, these findings suggest that administration of CAR lowered the blood glucose level by influencing the autonomic nervous system. Since CAR

contains L-histidine, which produces histamine, the effect of L-histidine and histamine on 2DG-hyperglycemia was further examined. In this study, it was observed that L-histidine and histamine caused bell-shaped suppression of 2DG-hyperglycemia (Nagai et al. 2003), indicating the involvement of a histaminergic mechanism in the inhibitory effect of CAR on 2DG-hyperglycemia. In this regard, it was observed that the inhibition of 2DG-hyperglycemia due to CAR was eliminated by the administration of a histamine H3 receptor antagonist (thioperamide) (Yamano et al. 2001). Furthermore, administration of either a histamine H1 receptor antagonist (diphenhydramine) or an inhibitor of histamine synthesis (α-fluoromethylhistidine) inhibited 2DG-hyperglycemia (Nagai et al. 2003). Therefore, a histaminergic mechanism through the H1 receptor may be involved in 2DG-hyperglycemia, and a histaminergic mechanism through the H3 receptor may mediate the suppressive effect of CAR on blood glucose levels. Moreover, it was observed that either peripheral administration of D-carnosine (0.5 nmol, IP) or central administrations of D-carnosine (0.05 nmol, LCV) was unable to suppress 2DG-hyperglycemia (Yamano et al. 2001). However, administration of 0.1 or 1 mg (IP) of anserine suppressed 2DG-hyperglycemia, which was associated with the suppression of adrenal sympathetic nerve activity and plasma glucagon levels in rats and was inhibited by thioperamide (Kubomura et al. 2010). Thus, anserine, but not D-carnosine, elicited effects similar to those of CAR in rats.

The anti-diabetic effect of CAR in diabetic mice was investigated by adding 1 g/L CAR to drinking water for 4 weeks, which resulted in increased plasma insulin levels and decreased plasma glucagon and glucose levels (Lee et al. 2005). Furthermore, while the expression of human carnosinase 1 (CN1) in transgenic d*b*/d*b* mice produced elevations in the blood glucose and hemoglobin A_{1c} levels and a reduction in the blood insulin level, administration of CAR increased serum fasting insulin levels in d*b*/d*b* mice, and a significant correlation was observed between serum CAR levels and β-cell mass in the pancreas (Sauerhöfer et al. 2007). These results support the hypothesis that the hypoglycemic action of CAR may be caused by elevation of the blood insulin level and are in good agreement with our findings mentioned above.

Both the antioxidant activity of CAR (Boldyrev et al. 1988; Kohen et al. 1988; Aruoma et al. 1989) and the carnosinylation of protein carbonyl groups by CAR (Hipkiss et al. 2001) might be implicated in the function of CAR. Such mechanisms may mediate the hypoglycemic action of CAR through upstream or downstream changes in autonomic neurotransmission or in parallel with autonomic changes and require further investigation.

In thie regard, recently, it was reported that longterm (24 weeks) administration of 30 mg/kg/day of L-carnosine (CAR) and D-carnosine (D-CAR) reduced body weight and the plasma insulin level without affecting the plasma glucose level in Zucker obese rats (Aldini et al. 2011), suggesting the improvement of insulin resistance. Thus, the authors suggested that these pharmacological actions of CAR are not due to pro-histaminic effects, because D-CAR is not a precursor of L-histidine. Therefore, these long-term effects of the improvement of insulin tolerance observed in Zucker obese rats with pharmacological dose of L-CAR might not be realized from the pro-histamine action of CAR.

However, in our study the hypoglycemic effect was observed within 30 min after the administration of small amount of CAR in rats (Yamano et al. 2001; Nagai et al. 2003) without changing the body fat content. Furthermore, CAR suppresses not only the pancreatic sympathetic nerve activity but also the activities of sympathetic nerves innervating the adrenal gland and liver in rats (Yamano et al. 2001), and thus increases the plasma insulin level and decreases the plasma adrenaline and glucagon levels. Therefore, the hypoglycemic effect of CAR might be caused by the change in not only the blood concentration of insulin but also changes in the blood levels of other hormones such as adrenaline and glucagon. Furthermore, since it was suggested that the reduction in the hepatic sympathetic nerve activity may lower glycogenolysis in the liver through the inactivation of glycogen phosphorylase, a glycogenolytic enzyme (Shimazu and Amakawa 1968), this enzymatic change due to direct autonomic innervation seems to be involved in the hypoglycemic action of CAR. Moreover, we observed that hypoglycemic effects of CAR, L-histidine, and L-histamine were bell-shaped in rats (Nagai et al. 2003). That is, optimal doses of CAR, L-histidine, and L-histamine existed in the suppression of the plasma glucose level. This means that higher or lower doses of CAR, L-histidine, and L-histamine than those optimal doses could not suppress the plasma glucose level more effectively than the optimal doses of them in rats (Yamano et al. 2001; Nagai et al. 2003). Considering these facts and the finding that the hypoglycemic effect of CAR was eliminated by histamine H3 antagonist (Yamano et al. 2001), the short-term (acute) and physiological hypoglycemic effect of CAR might be realized by pro-histamine action of CAR.

Effect of CAR on blood pressure

As mentioned above, a marked decrease in the BP and an increase in the blood concentration of CAR were observed in a patient with a congenital deficiency of CN, a CAR-degrading enzyme (Willi et al. 1997). In our previous work, it was also found that a diet containing either 0.001% (w/w) or 0.0001% (w/w) CAR, given ad libitum lowered the BP of

DOCA-salt hypertensive rats (Niijima et al. 2002). Furthermore, small amounts of CAR (1 mg/300 g BW, IV) suppressed the activity of sympathetic nerve innervating the kidney (Niijima et al. 2002; Tanida et al. 2005a). These results suggest that CAR lowers BP by suppressing the sympathetic nerve. Thus, further experiments were carried out to investigate the mechanism by which CAR reduces BP. Administration of a small amount of CAR (1 mg/300 g BW, IV, or 0.01 mg/300 g BW, LCV) reduced renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP); however, a large amount of CAR (100 mg/300 g BW, IV, or 10 mg/300 g BW, LCV) elevated both RSNA and MAP (Tanida et al. 2005a). This suggests that CAR may influence BP through its effects on the autonomic nervous system, which are dependent on the dose administered. To determine whether a histaminergic mechanism is involved, we examined the effect of histamine H1 and H3 antagonists on CAR-mediated changes in RSNA and MAP. While an H1 antagonist (diphenhydramine) inhibited the elevation of RSNA and MAP by administration of a higher amount of CAR, an H3 antagonist (thioperamide) blocked suppression of RSNA and MAP by administration of a lower amount of CAR, via either IV or LCV routes (Tanida et al. 2005a). These findings suggest that the histamine H1 receptor plays a role in the mechanism by which CAR elevates RSNA and MAP and that the histamine H3 receptor is involved in the mechanism by which CAR suppresses RSNA and MAP. Moreover, intracranial (LCV) administration of lower amount of histamine (0.0001 nmol/300 g BW) suppressed RSNA and MAP, and that of higher amount of histamine (100 nmol/300 g BW) elevated RSNA and MAP (Tanida et al. 2007b).

The levels of CAR and anserine in the skeletal muscles of hypertensive rats were shown to be 35–45% lower compared with those in normotensive rats and were discussed with regard to the antioxidant properties of these dipeptides (Johnson and Hammer 1992). Moreover, the interaction of protein carbonyl groups with CAR was suggested to prevent the increase in BP associated with diabetes mellitus (Hipkiss et al. 2001). A number of studies have indicated that the antioxidant activity of CAR may also be responsible for the effect of CAR on BP (Boldyrev et al. 1988; Kohen et al. 1988; Aruoma et al. 1989).

Recently, it was reported that long-term (24 weeks) administration of 30 mg/kg/day of L-CAR and D-CAR lowered body weight and BP in Zucker obese rats (Aldini et al. 2011). The authors suggested that the pharmacological action of L-CAR on BP is not due to pro-histaminic effect, because D-CAR is not a precursor of L-histidine. Therefore, these long-term effects with pharmacological dose of CAR on BP might not require the mediation due to pro-histamine effect of L-CAR.

However, small amout of L-CAR rapidly (within 30 min) reduced BP and this effect was eliminated with an H3 blocker (Tanida et al. 2005a) without affecting body fat content. Moreover, it was observed that lower amount of histamine reduced RSNA and BP and higher amount of histamine increased RSNA and BT within 30 min after the administration in rats (Tanida et al. 2007b). Thus, these findings suggest that these acute effects of CAR on BP might be mediated by pro-histamine action of CAR.

Future studies are required to examine whether the action of pro-histamine, carnosinylation, antioxidation or buffering of CAR is/are involved in the mechanism of the effects of CAR on BP.

Involvement of the hypothalamic SCN in the effect of CAR on BP

There is evidence that the hypothalamic SCN, a master circadian clock, plays an important role in the control of glucose metabolism via the autonomic nervous system. In particular, bilateral electrolytic lesions of the SCN (SCN lesions) eliminated hyperglycemia, elevation of blood glucagon levels, suppression of blood insulin levels, and excitation of the sympathetic nerve innervating the adrenal gland, which resulted from intracranial 2DG injection (Yamamoto et al. 1984; Nagai et al. 1996a, b; Chun et al. 1998). Therefore, to investigate the role of the SCN in the CAR-mediated changes in RSNA and MAP, we examined the effect of SCN lesions on changes in RSNA and MAP after IV injection of CAR. We found that SCN lesions inhibit both the suppressive and elevating effects of CAR on either RSNA or MAP in rats (Tanida et al. 2005a). Thus, SCN may be implicated in the mechanism by which CAR suppresses or elevates RSNA and MAP.

Effect of CAR on lipolysis

We also examined the effect of CAR on lipolysis by determining the effect of a small (100 ng/300 g BW, IP) or higher amount (10 mg/300 g BW, IP) of CAR on the epididymal white adipose tissue sympathetic nerve activity (WAT-SNA) in urethane-anesthetized rats. While administration of a small amount of CAR enhanced WAT-SNA, a higher amount of CAR reduced WAT-SNA, and these enhancing and reducing effects of CAR were inhibited by histamine H1 (diphenhydramine) and histamine H3 (thioperamide) antagonists, respectively (Shen et al. 2008). Thus, the effect of CAR on WAT-SNA was the opposite of its effect on RSNA and MAP (Tanida et al. 2005a). However, increased sympathetic nerve activity (RSNA and WAT-SNA) by CAR was inhibited by the H1 antagonist,

whereas decreased sympathetic nerve activity (RSNA and WAT-SNA) by CAR was inhibited by the H3 antagonist (Tanida et al. 2005a; Shen et al. 2008). Similar to the response of WAT-SNA, a small amount of CAR (100 ng/300 g BW, IP) increased the concentration of plasma-free fatty acids (FFA), whereas a higher amount of CAR (10 mg/300 g BW, IP) decreased the plasma FFA concentration in rats (Shen et al. 2008). Since sympathetic nerve excitation of WAT via the release of its primary postganglionic neurotransmitter (noradrenaline) is the main mechanism of lipolysis initiation (Bartness and Bamshad 1998; Dodt et al. 2003), the above findings suggest that a lower amount of CAR may increase lipolysis by enhancing WAT-SNA, whereas a higher amount of CAR decreases lipolysis by suppressing WAT-SNA. Moreover, histaminergic mechanisms are likely to be involved in both responses to CAR.

Lipid metabolism was investigated by the addition of L-histidine and CAR (1 g/L) to the drinking water of mice fed on a high-fat diet for 8 weeks. As a result, the activity and mRNA expression of lipogenic enzymes and sterol regulatory element-binding protein in the liver and adipose tissue of mice diminished, and hyperinsulinemia was attenuated, leading to reductions in body weight and adipose tissue weight (Mong et al. 2011). Therefore, L-histidine and CAR may reduce lipogenesis and improve obesity in mice fed on a high-fat diet.

Effect of CAR on thermogenesis

Even though CAR enhances lipolysis, if the resultant FFA is not utilized in muscles, brown adipose tissue (a thermogenic, energy-expending tissue that mainly uses FFA as energy), or in other tissues and organs, the fat content (or body weight) will not change. Thus, we also examined the effect of CAR on brown adipose tissue sympathetic nerve activity (BAT-SNA) in urethane-anesthetized rats. Although the site of administration was different (IP vs. LCV), the effect of a small or large amount of CAR on interscapular BAT-SNA (Tanida et al. 2007a) was the opposite of its effect on WAT-SNA (Shen et al. 2008); a small amount of CAR (0.01 mg/300 g BW, LCV) suppressed BAT-SNA, while a high amount of CAR (100 mg/300 g BW, LCV) elevated BAT-SNA in urethane-anesthetized rats (Tanida et al. 2007a). Since excitation of BAT-SNA produces an increase in thermogenesis—mainly by using FFA—via activation of the adrenergic β-3 receptor and uncoupling protein 1 (UCP1) (Ganong 2005), we examined the effect of CAR on body temperature in unanesthetized rats. The body temperature of rats was lowered by a small amount of CAR (0.01 mg/300 g BW, LCV) and elevated by a high amount of CAR (100 mg/300 g BW, LCV) (Tanida et al. 2007a). However, changes in the body temperature of rats due to CAR were only observed in the light (resting) period and not in the dark (active) period (Tanida et al. 2007a). This suggests that a smaller amount of CAR may decrease heat production in the brown adipose tissue (BAT) by suppressing BAT-SNA, whereas a higher amount of CAR increases heat production in BAT by elevating BAT-SNA. Since these responses were time dependent (Tanida et al. 2007a), it is possible that they were mediated by the master circadian clock SCN. Indeed, we found that SCN lesions abolished both changes in autonomic neurotransmission (BAT-SNA) and body temperature (Tanida et al. 2007a), suggesting that the SCN is involved in the effect of CAR on BAT-SNA and thermogenesis. However, further studies are required to establish whether the antioxidant and carnosinylation actions of CAR are implicated in the influence of CAR on lipid metabolism and thermogenesis.

Changes in blood CAR levels

To investigate the mechanism by which blood CAR concentration is controlled, we examined daily variation in plasma CAR concentration in rats housed under conditions of 12 h of light and 12 h of darkness and fed ad libitum. We found that the plasma concentration of CAR followed a daily rhythm, in which the plasma CAR level was lowest towards the latter half of the light (resting) period and highest at the end of the dark (active) period (Nagai et al. 2003). Therefore, we hypothesized that CAR is released from its site of synthesis in skeletal muscles during the physically active period. To examine this hypothesis, the plasma CAR level was determined in rats housed in cages supplied with or without a running-wheel device. We observed that the plasma CAR concentration in rats completing more than 3,000 rotations was elevated during the middle of the dark (active) period (Zeitgeber time 18; 18 h after light onset under conditions of 12 h of light and 12 h of darkness), which was almost twice that in rats housed in cages without a running wheel (Nagai et al. 2003). These findings suggest that physical exercise may cause release of CAR from skeletal muscle.

In humans, since the plasma CAR concentration is approximately 100 times less than that in rats, it is difficult to accurately detect the plasma CAR concentration (Nagai et al., unpublished data). Indeed, following consumption of CAR-containing food, human plasma CAR levels were detectable in one study (Park et al. 2005) but undetectable in another study (Yeum et al. 2010). However, in the latter study, the urine CAR level increased by approximately 15-fold following CAR consumption (Yeum et al. 2010). The authors suggested that CAR from food sources is rapidly hydrolyzed by CN in the blood and excreted in the

urine and that CAR may function as a reactive carbonyl species sequestering agent. However, further studies are required to test this hypothesis.

Carnosine synthetase and carnosinase

CAR is synthesized from β-alanine and L-histidine by an ATP-dependent carnosine synthase enzyme, which has been detected in the extracts of chicken muscle and mouse brain (Kalyankar and Meister 1959; Horinishi et al. 1978). Moreover, CAR synthase activity was detected in glia cells—particularly in oligodendrocytes—derived from the rat brain (Hoffmann et al. 1996). Recently, it was reported that a mammalian gene with unknown function, *ATPGD1*, encodes an enzyme capable of synthesizing CAR and homocarnosine that has 15- to 25-fold higher catalytic efficiency with β-alanine than with γ-aminobutyrate (Drozak et al. 2010). However, the pattern and control of *ATPGD1* expression in the mammalian skeletal muscles and brain are currently unknown.

Recently, two types of CAR-degrading enzymes (CN1 and CN2) have been identified and characterized in humans and mice (Teufel et al. 2003; Otani et al. 2005). These enzymes are termed human CN1 (also known as carnosine dipeptidase 1; CNDP1), human CN2 (carnosine dipeptidase 2; CNDP2) (Teufel et al. 2003), and mouse CN2 (Otani et al. 2005). Human CN1 was identified as a dipeptidase that hydrolyzes Xaa-His dipeptides, including those with β-Ala (carnosine), γ-aminobutyric acid (homocarnosine), *N*-methyl-β-Ala, Ala, and Gly as the first residue. While CN2 has broader specificity than CN1, CN2 does not hydrolyze homocarnosine. CN1 is a secretory protein that is insensitive to inhibition by bestatin (Lenney et al. 1982), indicating that this enzyme represents the previously described serum carnosinase. In contrast, CN2 is a cytosolic enzyme that is sensitive to bestatin inhibition requires Mn^{2+} and dithiothreitol for enzymatic activity and is inhibited by Zn^{2+} (Teufel et al. 2003; Otani et al. 2005). Thus, it is likely that CN2 represents the previously described tissue carnosinase or non-specific dipeptidase enzyme. Although it has been reported that CN2 is inactive at neutral pH (Teufel et al. 2003), human CN2 was shown to degrade CAR at neutral pH (Pandya et al. 2011). Moreover, we also detected CAR-degrading activity of mouse CN2 at neutral pH (Okumura et al., unpublished data). At pH 8.0, human CN1 is suggested to be sixfold more efficient than human CN2, as calculated from turnover rates (Vmax/[E][t]) of CN1 and CN2 (Pandya et al. 2011). Since the intracellular Mn^{2+} level is not so high, whether CAR is really degraded in cells must be examined in future. The expression pattern of CN1 differs in humans, mice, and rats: human CN1 is expressed mainly in the brain (particularly in pyramidal cells of the hypocampus) and liver, while CN1 is mainly expressed in the kidney and is not expressed in the brain of mice and rats (Pandya et al. 2011). Therefore, although further experiments are required, CAR degradation in the brain may be carried out by CN2 in mice and rats and by both CN1 and CN2 in humans.

We expressed the CN2 protein in *Escherichia coli* and produced an antibody against CN2, which was used to examine the distribution of CN2 immunoreactivity in the brain (Otani et al. 2005). We observed that a few neuronal populations expressed CN2 at very high levels. In addition, CN2 was highly expressed in the parafascicular nucleus of the thalamus, the tuberomammillary nucleus (TMN) of the hypothalamus, and the mitral cell layer of the olfactory bulb (Otani et al. 2005). Furthermore, CN2 colocalized with histidine decarboxylase (a histamine-synthesizing enzyme) in neurons of the hypothalamic TMN (Otani et al. 2005, 2008), suggesting that CN2 is found in histamine neurons in the hypothalamic TMN. CN2 immunoreactivity in the neural fibers of the parafascicular nucleus was observed in the striatum. Since the striatum is one of the major targets of the glutamatergic projection from the parafascicular nucleus, CN2 may be involved in this glutamatergic pathway. Moreover, CAR is known to be present in the olfactory epithelium and olfactory bulb (Margolis 1973) and has been implicated in the neuronal transmission of odor stimulation to the brain. Therefore, one possible function of CN2 may be to eliminate CAR from the nerve terminals of olfactory neurons.

In addition, we also determined the crystal structure of CN2 in complex with bestatin and Zn^{2+} or Mn^{2+} at a resolution of 1.7 or 2.3 Å, respectively, by X-ray crystallography (Unno et al. 2008). We observed that CN2 contains 2 Zn^{2+} ions or 2 Mn^{2+} ions at the catalytic center and is active in the dimeric form (Unno et al. 2008).

Supplementation with β-alanine has been shown to increase the CAR content in the muscles of horses (Dunnett and Harris 1999). The increase in muscle CAR content was expected to enhance intramuscular hydrogen ion (H^+) buffering capacity (Vaughan-Jones et al. 2006). Indeed, exercise-induced acidosis was attenuated by chronic treatment of β-alanine, which increased muscle CAR content (Baguet et al. 2010). This suggests that the increase in muscle CAR content that results from supplementation with β-alanine may enhance pH buffering in acidotic blood associated with severe exercise.

Carnosine transporters and carnosine transport

We previously showed that the most effective dose of CAR affecting the blood glucose was 0.05 nmol for intracranial

(LCV) administration and 0.5 nmol for IP administration (Nagai et al. 2003); the most effective dose of CAR reducing the blood pressure was 0.01 mg for LCV administration and 1 μg for IV administration, and the most effective dose of CAR elevating the blood pressure was 10 μg for LCV administration and 100 μg for IV administration (Tanida et al. 2005a). These facts suggest that the action site of CAR may exist in the brain. Moreover, doses of 1 nmol (LCV) and 10 nmol (IP) histamine and 0.005 nmol (LCV) and 5 nmol (IP) L-histidine were the most effective doses suppressing the blood glucose (Nagai et al. 2003). Therefore, CAR, histamine, or histidine administration may affect blood glucose levels by acting on the brain, and CAR may also alter BP via its action on the brain. Moreover, elucidation of the transport of CAR, histamine, and histidine may provide further information on their functions.

Following ingestion, CAR is taken up by intestinal cells in humans (Asatoor et al. 1970) and rats (Tamaki et al. 1985). A study using purified brush-border membrane vesicles from the mouse small intestine suggested that CAR is transported intact (Rajendran et al. 1984). Furthermore, while H^+/peptide cotransporter 1 (PEPT1) (Son et al. 2004; Geissler et al. 2010) and human peptide/histidine transporter 1 (hPHT1) (Bhardwai et al. 2006) have been suggested to be involved in the intestinal absorption of CAR and anserine in humans, the precise details of these intestinal transporters remain to be clarified. The high-affinity type H^+/peptide cotransporter 2 (PEPT2) may be involved in the transport of CAR in the rat cerebellum (Fujita et al. 1999), choroid plexus epithelial cells (Teuscher et al. 2004; Hu et al. 2005), astrocytes (Bauer 2005; Xiang et al. 2006), cardiomyocytes (Lin and King 2007), and kidney (Jappar et al. 2009; Kamal et al. 2009). Moreover, PHT1, which may also mediate CAR transport, is observed throughout the whole brain (Yamashita et al. 1997). PEPT2 has been implicated in modulation of the disposition of exogenous CAR, but not in the homeostatic control of endogenous CAR levels in skeletal muscle (Kamal et al. 2009).

Involvement of histamine neurons and the hypothalamic SCN in CAR functions

The results described above suggest that the histamine receptors H1 and H3 are involved in the mechanism by which CAR regulates blood glucose, BP, lipolysis, and thermogenesis. In particular, it is thought that the H1 receptor is involved in sympathetic stimulation or parasympathetic suppression, whereas the H3 receptor is involved in parasympathetic stimulation or sympathetic suppression.

Furthermore, it has been suggested that the H3 receptor plays a role in the mechanism of auto-inhibition of histamine release from histamine neurons (Arrang et al. 1983). When histamine neurons form synaptic connections with the target neurons, the histamine H3 receptor normally localizes in the presynaptic membrane, while the histamine H1 receptor localizes in the postsynaptic membrane (Watanabe et al. 1984). Since the presynaptic H3 receptor has a much higher affinity for histamine than the postsynaptic H1 receptor, a small amount of histamine released from the histamine neurons into the synaptic cleft stimulates the presynaptic H3 receptors and inhibits synthesis and release of histamine from the histamine neurons to the synaptic cleft (Watanabe et al. 1984). In contrast, larger amounts of histamine released from the histamine neurons into the synaptic cleft stimulate the postsynaptic H1 receptors, resulting in neurotransmission from the histamine neurons to target neurons (Watanabe et al. 1984). Thus, it is likely that sympathetic suppression or parasympathetic stimulation is produced by stimulation of the H3 receptor with a small amount of histamine, whereas sympathetic stimulation or parasympathetic suppression is elicited by stimulation of the H1 receptor with a larger amount of histamine. This explanation also reflects the mechanism by which a smaller amount of histamine and CAR suppresses both RSNA and BP, while a larger amount of histamine and CAR elevates both RSNA and BP (Tanida et al. 2005a, 2007b). Similarly, this mechanism would also explain why a small amount of CAR lowered BAT-SNA and body temperature, whereas a higher amount of CAR elevated both BAT-SNA and body temperature (Tanida et al. 2007a). However, it remains to be determined how a smaller amount of CAR increased WAT-SNA and the plasma FFA concentration or how a larger amount of CAR lowered WAT-SNA and plasma FFA levels (Shen et al. 2008).

With respect to the involvement of the hypothalamic SCN master circadian clock in CAR function, we previously observed that bilateral electrolytic SCN lesions inhibited changes in autonomic nerve activity and hyperglycemia induced by intracranial injection of 2DG (Yamamoto et al. 1984; Nagai et al. 1996b). SCN lesions abolished changes in RSNA, BAT-SNA, BP, and body temperature caused by the administration of CAR (Tanida et al. 2005a, b, 2007a). This may be due to the fact that nerve fibers that pass through the lesioned areas are cut by electrolytic SCN lesions. In this regard, we previously observed in mice and rats that olfactory stimulation with scent of grapefruit oil (SGFO) elevated RSNA and BP (Tanida et al. 2005b, 2007c), that olfactory stimulation with scent of lavender oil (SLVO) increased gastric vagal nerve activity (GVNA) (Shen et al. 2005; Tanida et al. 2006, 2007c), and that electrolytic SCN lesions eliminated

changes in RSNA, BP, and GVNA due to SGFO and LVO (Tanida et al. 2006, 2007c). Furthermore, we also failed to observe either elevation of RSNA or BP due to olfactory stimulation with SGFO or an increase in GVNA due to olfactory stimulation with SLVO in double knockout (DKO) mice for circadian clock-related genes, cryptochrome 1 (*cry1*) and cryptochrome 2 (*cry2*), in which the circadian rhythm of locomotive activity was absent (Tanida et al. 2007c). Since bilateral SCN lesions in wild-type mice eliminated the response of RSNA and BP to SGFO and also the response of GVNA to SLVO (Tanida et al. 2007c), the results for *cry1/cry2* DKO mice suggest that the circadian clock mechanism in the SCN is involved in changes in autonomic neurotransmissions due to SGFO and SLVO. Thus, the circadian clock mechanism is also likely to be involved in the effects mediated by CAR, as mentioned above.

Using pseudorabies virus, which is retrogradely and multisynaptically transported in the nerves, we (Buijs et al. 2001, 2003) and others (Bamshad et al. 1998, 1999; Sly et al. 1999; Bartness et al. 2001) showed evidence that the SCN sends sympathetic and parasympathetic neuronal projections to peripheral organs and tissues including the pancreas, liver, kidney, adrenal glands, and white and brown adipose tissues. This presents the neural transmission route from the SCN to peripheral organs. With regard to the relationship between histamine neurons in the hypothalamic TMN and the SCN, it was suggested that histaminergic neurons in the TMN project to the SCN neurons (Michelsen et al. 2005). Since circulating peptides may rapidly circulate into brain interstitial space of the circumventricular organs of the brain lacking a blood–brain barrier, including the hypothalamus (Pardridge 1981), or circulate throughout the whole brain via PHT1 (Yamashita et al. 1997), it seems likely that CAR is transported into the histamine neurons in the TMN. Hydrolysis of CAR by CN2 yields L-histidine, which may be converted into histamine by histidine decarboxylase in the histamine neurons of the TMN. Therefore, further studies are required to confirm whether the resulting histamine from CAR affects autonomic nerve activity and alters blood glucose levels, BP, lipolysis, and thermogenesis.

Among the possible histidine donors such as anserine and homocarnosine, CN2 does not hydrolyze homocarnosine. Therefore, homocarnosine is unlikely to yield L-histidine via CN2 hydrolysis in the histamine neurons of the TMN. However, we found that 0.1 mg of anserine (IP) suppressed 2DG-hyperglycemia and 2DG-hyperglucagonemia and that the effects of anserine were inhibited by thioperamide (an H3 receptor antagonist) (Kubomura et al. 2010). Intravenous (IV) injection of a low dose (1 μg) of anserine suppressed RSNA and BP, whereas a high dose (1,000 μg) of anserine elevated RSNA and BP (Tanida et al. 2010). Furthermore, thioperamide eliminated the low-dose effect of anserine on RSNA and BP and diphenhydramine (an H1 receptor antagonist) abolished the high-dose effect of anserine on RSNA and BP (Tanida et al. 2010). Thus, these results suggest that anserine may exert similar effects as CAR through a comparable mechanism. L-histidine is classified as one of the essential amino aids (Laidlaw and Kopple 1987), and de novo synthesis of L-histidine in mammalian tissues only slightly, if at all, supports the tissue L-histidine demand. Since skeletal muscles contain a large amount of CAR and anserine may be a histidine reservoir for the whole body metabolism. Therefore, it seems reasonable to think that there is a L-histidine reservoir in the brain, and CAR as one of the reservoirs for L-histidine supply, particularly in L-histidine-consuming cells such as histaminergic neurons in the TMN. CN1 can hydrolyze CAR, anserine, and homocarnosine, and CN2 can hydrolyze CAR and anserine, but not homocarnosine. Thus, in the histaminergic neurons in the TMN, CAR, and anserine, but not homocarnosine, may be hydrolyzed by CN2 and the resultant L-histidine may be converted to histamine. In the peripheral part, homocarnosine may also function as L-histidine donor through the function of CN1. In this regard, whether anserine and homocarnosine are released during physical exercise or not must be examined in future.

Concluding remarks

In this review, we discuss the possible functions and mechanisms of action of CAR based both on our results and recent reports. The plasma CAR concentration is approximately 100 times lower in humans than in rats (Nagai et al. unpublished observations), which is probably due to the fact that CAR hydrolytic enzymes have a higher activity in human blood than in rat blood. While CN1 is expressed mainly in the brain and liver in humans, it is expressed mainly in the kidney, not in the brain, of rats and mice (Teufel et al. 2003). Therefore, CN1 may be involved in the hydrolysis of CAR in the human brain, which should be examined in future studies.

Our findings suggest that in rats, CAR may be released from skeletal muscles into the bloodstream during physical exercise (Nagai et al. 2003) and rapidly hydrolyzed so that only smaller amounts of CAR reach the histamine neurons in the hypothalamic TMN via regions lacking the blood–brain barrier or via PHT1. Moreover, a small amount of L-histidine derived from the low amount of CAR by CN2 may be converted to histamine by histidine decarboxylase in the histamine neurons of the hypothalamic TMN. The low amount of histamine may be released from the histamine neurons into the synaptic cleft, which is formed

between the histamine neurons and their target neurons, resulting in a reduction in the activity of sympathetic nerves innervating the adrenal, liver, pancreas, kidney, and brown adipose tissue and an enhancement of the white adipose tissue sympathetic nerve activity. Changes in sympathetic nerve activity may produce alterations in physiological functions, which would explain the observation that the effects of a small amount of CAR, including hypoglycemic, hypotensive, and lipolytic actions, are only produced when CAR is released from skeletal muscle. Table 2 summarizes the effects of a small amount of CAR on autonomic transmission and function.

The delivery of L-histidine to the brain across the blood–brain barrier is mediated by the L-type neutral amino acid transporter present in the membrane of brain capillary endothelial cells. Under normal conditions, the L-system transporter is saturated; therefore, an elevation in the plasma concentration of one of the amino acids transported by this transporter will reduce the uptake of others by the brain (Shulkin et al. 1995). As a result, even if CAR-containing foods such as beef and chicken meat are ingested, the plasma concentration of neutral amino acids increases, and the delivery of plasma L-histidine derived from CAR to the brain across the blood–brain barrier is restricted by inhibition of the L-system transporter by other amino acids present in the meat. However, if CAR is released from skeletal muscles during physical exercise, the specific increase in plasma L-histidine concentration through the degradation of CAR by CN1 may produce elevation of L-histidine in the interstitial space of the brain via the L-system amino acid transporter. In addition, L-histidine may also be taken up by histamine neurons, converted to histamine, and produce alterations in blood glucose, BP, lipolysis, and thermogenesis. We also observed that anserine produced a similar effect on blood glucose and BP as CAR (Kubomura et al. 2010; Tanida et al. 2010). Thus, if anserine is released from skeletal muscles during physical exercise, it may also produce an increase in the plasma L-histidine concentration via CN1-mediated hydrolysis, resulting in changes in blood glucose and BP. Which route of CAR hydrolysis, CN1- or CN2-route, might be implicated in the mechanism of pro-histamine action of CAR in humans, rats, and mice must be examined in future.

The important issue is that effects of CAR on the blood glucose, BP, lipolysis, and thermogenesis were dose-dependent or bell-shaped. That is, optimal concentrations of CAR in the blood or brain might exist for the functions of CAR on these parameters. Therefore, physiologically there is no need to attain the highest CAR concentration in the blood or brain to realize its actions of CAR on these parameters.

Investigation of the control mechanisms for the synthesis and release of CAR in the skeletal muscle and brain

Table 2 Effects of a small amount of L-carnosine on autonomic neurotransmission and physiological functions

Nerve	Changes in autonomic neurotransmission	Changes in physiological functions	References
Adrenal sympathetic nerve	Suppression	Decrease in adrenaline secretion (decreases in BP and BG)	Yamano et al. (2001)
Hepatic sympathetic nerve	Suppression	Decreases in glycogenolysis and gluconeogenesis (decrease in BG)	Yamano et al. (2001)
Pancreatic sympathetic nerve	Suppression	Increase in insulin secretion (decrease in BG)	Niijima et al. (unpublished data)
Renal sympathetic nerve	Suppression	Decrease in BP	Tanida et al. (2005a, b)
Brow adipose tissue sympathetic nerve	Suppression	Decrease in heat production (lowering of BT)	Tanida et al. (2007a)
White adipose tissue sympathetic nerve	Facilitation	Increase in lipolysis	Shen et al. (2008)
Splenic sympathetic nerve	Suppression	Increase in NK activity	Horii et al. (2012)

BP blood pressure, *BG* blood glucose, *BT* body temperature, *NK* natural killer

and the precise functions of CAR are important issues to be clarified in the near future. In parallel, we are interested in the effect of CAR on skeletal muscle sympathetic nerve activity, as sympathetic excitation in muscles causes dilatation of the muscle arterial vessels and increases the blood supply, and thus oxygen and nutrient supplies to muscles through the adrenergic β-2 receptor (Ganong 2005). If CAR elevates skeletal muscle sympathetic nerve activity, this might contribute for recovering from skeletal muscle fatigue, elevating free fatty acid supply for its energy or increasing protein content of the skeletal muscle. Especially, it is very interesting whether this is the case, because even though the low amount of CAR elevates lipolysis, it reduces brown adipose tissue sympathetic nerve activity, resulting in a decrease in the consumption of FFA derived from lipolysis. Therefore, if low amount of CAR increases the consumption of FFA in skeletal muscles, it fits teleologically. This must be examined in future.

It is possible that anti-oxidant action, carnosinylation, and buffering action of CAR might be involved in the mechanism of CAR actions. However, so far it is not clear what must be protected from peroxidations, carnosinylated or buffered by CAR to realize the actions of CAR. These issues and the mechanisms must be revealed in future.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Aldini G, Orioli M, Rossoni G, Savi F, Braidotti P, Vistoli G, Yeum K, Negrisoli G, Carini M (2011) The carbonyl scanvenger carnosine ameliorates dyslipidaemia and renal function in Aucker obese rats. J Cell Mol Med 15:1339–1354

Arrang JM, Garbarg M, Schwartz JC (1983) Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 302:832–837

Aruoma OI, Laughton MJ, Halliwell B (1989) Carnosine, homocarnosine and anserine: could they act as antioxidants in vivo? Biochem J 264:863–869

Asatoor AM, Bandoh JK, Lant AF, Milne MD, Navab F (1970) Intestinal absorption of carnosine and its constituent amino acids in man. Gut 11:250–254

Baguet A, Koppo K, Pottier A, Derave W (2010) Beta-alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. Eur J Appl Physiol 108:495–503

Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ (1998) Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. Am J Physiol 275:R291–R299

Bamshad M, Song CK, Bartness TJ (1999) CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am J Physiol 276:R1569–R1578

Bartness TJ, Bamshad M (1998) Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. Am J Physiol 275:R1399–R1411

Bartness TJ, Song CK, Demas GE (2001) SCN efferents to peripheral tissues: implications for biological rhythms. J Biol Rhythm 16:196–204

Bauer K (2005) Carnosine and homocarnosine, the forgotten, enigmatic peptides of the brain. Neurochem Res 30:1339–1345

Bhardwai RK, Herrera-Ruiz D, Eltoukhy N, Saad M, Knipp GT (2006) The functional evaluation of human peptide/histidine transporter 1 (hPHT1) in transiently transfected COS-7 cells. Eur J Pharm Sci 27:533–542

Boldyrev AA, Severin SE (1990) The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. Adv Enzyme Regul 30:175–194

Boldyrev AA, Dupin AM, Pindel EV, Severin SE (1988) Antioxidative properties of histidine-containing dipeptides from skeletal muscles of vertebrates. Comp Biochem Physiol B 89:245–250

Buijs RM, Chun S, Niijima A, Romijin HJ, Nagai K (2001) Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. J Comp Neurol 431:405–423

Buijs RM, Fleur SI, Wortel J, Heynigen CV, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A (2003) The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol 464:36–48

Buse MG, Weigand DA, Peeler D, Hedden MP (1980) The effect of diabetes and the redox potential on amino acid content and release by isolated rat hemidiaphragms. Metabolism 29:605–616

Chun S, Niijima A, Nagai N, Nagai K (1998) Effect of bilateral lesions of the suprachiasmatic nucleus on hyperglycemia caused by 2-deoxy-D-glucose and vasoactive intestinal peptide in rats. Brain Res 809:165–174

Dodt C, Lönnroth P, Wellhöner JP, Fehm HL, Elam M (2003) Sympathetic control of white adipose tissue in lean and obese humans. Acta Physiol Scand 177:351–357

Dunnett M, Harris RC (1999) Influence of oral beta-alanine and L-histidine supplementation on the carnosine content of the gluteus medius. Equine Vet J Suppl 30:499–504

Fujita T, Kishida T, Okada N, Ganapathy V, Leibach FH, Yamamoto A (1999) Interaction of kyotorphin and brain peptide transporter in synaptosomes prepared from rat cerebellum: implication of high affinity type H+/peptide transporter PEPT2 mediated transport system. Neurosci Lett 271:117–120

Ganong WF (2005) Review of medical physiology, 22nd edn. Lange Medical Books/McGraw-Hill, New York, pp 301–302

Geissler S, Zwarg M, Knütter I, Markwardt F, Brandsch M (2010) The bioactive dipeptide anserine is transported by human proton-coupled peptide transporters. FEBS J 277:790–795

Hipkiss AR, Brownson C, Carrier MJ (2001) Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. Mech Ageing Dev 122:1431–1445

Hoffmann AM, Bakardjiev A, Bauer K (1996) Carnosine-synthesis in cultures of rat glial cells is restricted to oligodendrocytes and carnosine uptake to astrocytes. Neurosci Lett 215:29–32

Horii Y, Tanida M, Shen J, Fujisaki Y, Fuyuki R, Hashimoto K, Niijima A, Nakashima T, Nagai K (2011) Effects of urea-containing cream on cutaneous arterial sympathetic nerve activity, blood flow and water evaporation. Skin Technol 17:75–81

Horii Y, Shen J, Fujisaki Y, Yoshida K, Nagai K (2012) Effects of L-carnosine on splenic sympathetic nerve activity and tumor proliferation. Neurosci Lett 510:1–5

Horinishi H, Grillo M, Margolis FL (1978) Purification and characterization of carnosine synthetase from mouse olfactory bulbs. J Neurochem 31:909–919

Hu Y, Ocheltree SM, Xiang J, Keep RF, Smith DE (2005) Glycyl-L-glutamine disposition in rat choroid plexus epithelial cells in primary culture: role of PEPT2. Pharm Res 22:1281–1286

Jappar D, Hu Y, Keep RF, Smith DE (2009) Transport mechanisms of carnosine in SKPT cells: contribution of apical and basolateral membrane transporters. Pharm Res 26:172–181

Johnson P, Hammer JL (1992) Histidine dipeptide levels in ageing and hypertensive rat skeletal and cardiac muscles. Comp Biochem Physiol B 103:981–984

Kalyankar GD, Meister A (1959) Enzymatic synthesis of carnosine and related beta-alanyl and gamma-aminobutyryl peptides. J Biol Chem 234:3210–3218

Kamal MA, Jiang H, Hu Y, Keep RF, Smith DE (2009) Influence of genetic knockout of Pept2 on the in vivo disposition of endogenous and exogenous carnosine in wild-type and Pept2 null mice. Am J Physiol Regul Integr Comp Physiol 296:R986–R991

Kohen R, Yamamoto Y, Cundy KC, Ames BN (1988) Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. Proc Natl Acad Sci USA 85:3175–3179

Kubomura D, Matahira Y, Nagai K, Niijima A (2010) Effect of anserine ingestion on hyperglycemia and the autonomic nerves in rats and humans. Nutr Neurosci 13:183–188

Laidlaw SA, Kopple JD (1987) Newer concepts of the indispensable amino acids. Am J Clin Nutr 46:593–605

Lee YT, Hsu CC, Lin MH, Liu KS, Yin MC (2005) Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. Eur J Pharmacol 513:145–150

Lenney JF, George RP, Weiss AM, Kucera CM, Chan PW, Rinzler GS (1982) Human serum carnosinase: characterization, distinction from cellular carnosinase, and activation by cadmium. Clin Chim Acta 123:221–231

Lin H, King N (2007) Demonstration of functional dipeptide transport with expression of PEPT2 in guinea pig cardiomyocytes. Pflug Arch 453:915–922

Margolis FL (1973) L-Carnosine in the primary olfactory pathway. Science 184:909–911

Michelsen KA, Lozada A, Kaslin J, Karlstedt K, Kukko-Lukjanov TK, Holopainen I, Ohtsu H, Panula P (2005) Histamine-immunoreactive neurons in the mouse and rat suprachiasmatic nucleus. Eur J Neurosci 22:1997–2004

Mong MC, Chao CY, Yin MC (2011) Histidine and carnosine alleviated hepatic steatosis in mice consumed high saturated fat diet. Eur J Pharmacol 653:82–88

Nagai K, Nagai N, Shimizu K, Chun S, Nakagawa H, Niijima A (1996a) SCN output drives the autonomic nervous system: with special reference to the autonomic function related to the regulation of glucose metabolism. Progr Brain Res 111:253–272

Nagai K, Niijima A, Nagai N, Hibino H, Chun SJ, Shimizu K, Nakagawa H (1996b) Bilateral lesions of the hypothalamic suprachiasmatic nucleus eliminated sympathetic response to intracranial injection of 2-deoxy-D-glucose and VIP rescued this response. Brain Res Bull 39:293–297

Nagai K, Niijima A, Yamano T, Otani H, Okumura N, Tsuruoka N, Nakai M, Kiso Y (2003) Possible role of L-carnosine in the regulation of blood glucose through controlling autonomic nerves. Exp Biol Med (Maywood) 228:1138–1145

Nakamura T, Tanida M, Niijima A, Hibino H, Shen J, Nagai K (2007) Auditory stimulation affects renal sympathetic nerve activity and blood pressure in rats. Neurosci Lett 416:107–112

Niijima A (1989) The effect of rANP on the efferent activity of the autonomic nerves. Arch Histol Cytol 52(Suppl):325–329

Niijima A, Nagai K (2003) Effect of olfactory stimulation with flavor of grapefruit oil and lemon oil on the activity of sympathetic branch in the white adipose tissue of the epididymis. Exp Biol Med (Maywood) 228:1190–1192

Niijima A, Chun SJ, Shima T, Bizot-Espiard JG, Guardiola-Lemaitre B, Nagai K (1998) Effect of intravenous administration of melatonin on the efferent activity of the adrenal nerve. J Auton Nerv Syst 71:134–138

Niijima A, Okui T, Matsumura Y, Yamano T, Tsuruoka N, Kiso Y, Nagai K (2002) Effects of L-carnosine on renal sympathetic nerve activity and DOCA-salt hypertension in rats. Auton Neurosci 97:99–102

Otani H, Okumura N, Hashida-Okumura A, Nagai K (2005) Identification and characterization of a mouse dipeptidase that hydrolyzes L-carnosine. J Biochem 137:167–175

Otani H, Okumura A, Nagai K, Okumura N (2008) Colocalization of carnosine-degrading enzyme CN2 with histidine decarboxylase in the tuberomammillary nucleus of the hypothalamus. Neurosci Lett 445:166–169

Pandya V, Ekka MK, Dutta RJ, Kumaran S (2011) Mass spectrometry assay for studying kinetic properties of dipeptidases: Characterization of human and yeast dipeptidases. Anal Biochem 418:132–142

Pardridge WM (1981) Transport of nutrients and hormones through the blood-brain barrier. Diabetologia 20(Suppl):246–254

Park YJ, Volpe SL, Decker EA (2005) Quantitation of carnosine in human plasma after dietary consumption of beef. J Agric Food Chem 53:4736–4739

Rajendran VM, Berteloot A, Ishikawa Y, Khan AH, Ramaswamy K (1984) Transport of carnosine by mouse intestinal brush-border membrane vesicles. Biochim Biophys Acta 778:443–448

Sauerhöfer S, Yuan G, Braun GS, Deinzer M, Neumaier M, Gretz N, Floege J, Kriz W, van der Woude F, Moeller MJ (2007) L-Carnosine, a substrate of carnosinse-1, influences glucose metabolism. Diabetes 56:2425–2432

Shen J, Niijima A, Tanida M, Horri Y, Maeda K, Nagai K (2005) Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 383:188–193

Shen J, Yao JF, Tanida M, Nagai K (2008) Regulation of sympathetic nerve activity by L-carnosine in mammalian white adipose tissue. Neurosci Lett 441:100–104

Shen J, Horii Y, Fujisaki Y, Nagai K (2009) Effects of L-arginine and L-lysine mixtures on splenic sympathetic nerve activity and tumor proliferation. Auton Neurosci 147:86–90

Shimazu T, Amakawa A (1968) Regulation of glycogen metabolism in liver by the autonomic nervous system. II. Neural control of glycogenolytic anzymes. Biochim Biophys Acta 165:335–348

Shulkin BL, Betz AL, Koeppe RA, Agranoff BW (1995) Inhibition of neutral amino acid transport across the human blood-brain barrier by phenylalanine. J Neurochem 64:1252–1257

Sly JD, Colvill L, McKinley JM, Oldfield JB (1999) Identification of neural projections from the forebrain to the kidney using the virus pseudorabies. J Auton Nerv Syst 77:73–82

Son DO, Satsu H, Kiso Y, Shimizu M (2004) Characterization of carnosine uptake and its physiological function in human intestinal epithelial Caco-2 cells. BioFactors 21:395–398

Tamaki N, Ikeda T, Fujimoto S, Mizutani N (1985) Carnosine as a histidine source: transport and hydrolysis of exogenous carnosine by rat intestine. J Nutr Sci Vitaminol (Tokyo) 31:607–618

Tanida M, Niijima A, Fukuda Y, Sawai H, Tsuruoka N, Shen J, Yamada S, Kiso Y, Nagai K (2005a) 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

Tanida M, Niijima A, Shen J, Nakamura T, Nagai K (2005b) Olfactory stimulation with scent of essential oil of grapefruit affects autonomic neurotransmission and blood pressure. Brain Res 1058:44–55

Tanida M, Niijima A, Shen J, Nakamura T, Nagai K (2006) Olfactory stimulation with scent of lavender oil affects autonomic neurotransmission and blood pressure in rats. Neurosci Lett 398:155–160

Tanida M, Gotoh H, Taniguchi H, Otani H, Shen J, Nakamura T, Tsuruoka N, Kiso Y, Okumura N, Nagai K (2007a) Effects of central injection of L-carnosine on sympathetic nerve activity innervating brown adipose tissue and body temperature in rats. Regul Pept 144:62–71

Tanida M, Kaneko H, Shen J, Nagai K (2007b) Involvement of histaminergic system in renal sympathetic and cardiovascular responses to leptin and ghrelin. Neurosci Lett 413:88–92

Tanida M, Yamatodani A, Niijima A, Shen J, Todo T, Nagai K (2007c) Autonomic and cardiovascular responses to scent stimulation are altered in *cry* KO mice. Neurosci Lett 413:177–182

Tanida M, Shen J, Kubomura D, Nagai K (2010) Effects of anserine on the renal sympathetic nerve and blood pressure in urethane-anesthetized rats. Physiol Res 59:177–185

Teufel M, Saudek V, Ledig JP, Bernhardt A, Boularand S, Carreau A, Cairns NJ, Carter C, Cowley DJ, Duverger D, Ganzhorn AJ, Guenet C, Heintzelmann B, Laucher V, Sauvage C, Smirnova T (2003) Sequence identification and characterization of human carnosinase and closely related non-specific dipeptidase. J Biol Chem 278:6521–6531

Teuscher NS, Shen H, Shu C, Xiang J, Keep RF, Smith DE (2004) Carnosine uptake in rat choroid plexus primary cell cultures and choroid plexus whole tissue from PEPT2 null mice. J Neurochem 89:375–382

Unno H, Yamashita T, Ujita S, Okumura N, Otani H, Okumura A, Nagai K, Kusunoki M (2008) Structural basis for substrate recognition and hydrolysis by mouse carnosinase CN2. J Biol Chem 283:27289–27299

Vaughan-Jones RD, Spitzer KW, Swietach P (2006) Spatial aspects of intracellular pH regulation in heart muscle. Prog Biophys Mol Biol 90:207–224

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, Toyama M, Wada H (1984) Distribution of the histaminergic neuron system in the central nervous system of rats: a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res 295:13–25

Willi SM, Zhang Y, Hill JB, Phelan MC, Michaelis RC, Holden KR (1997) A depletion in the long arm of chromosome 18 in a child with serum carnosinase deficiency. Pediatr Res 41:210–213

Xiang J, Hu Y, Smith DE, Keep RF (2006) PEPT2-mediated transport of 5-aminolevulinic acid and carnosine in astrocytes. Brain Res 1122:18–23

Yamamoto H, Nagai K, Nakagawa H (1984) Role of the suprachiasmatic nucleus in glucose homeostasis. Biomed Res 5:55–60

Yamano T, Niijima A, Iimori S, Tsuruoka N, Kiso Y, Nagai K (2001) Effect of L-carnosine on the hyperglycemia caused by intracranial injection of 2-deoxy-D-glucose in rats. Neurosci Lett 313:78–82

Yamashita T, Shimada S, Guo W, Sato K, Kohmura E, Hayakawa T, Takagi T, Tohyama M (1997) Cloning and functional expression of a brain peptide/histidine transporter. J Biol Chem 272:10205–10211

Yeum KJ, Orioli M, Regazzoni L, Carini M, Rasmussen H, Russel RM, Aldini G (2010) Profiling histidine dipeptides in plasma and urine after ingesting beef, chicken or chicken broth in humans. Amino Acids 38:847–858