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ORIGINAL ARTICLE

Pressor response to L-cysteine injected into the cisterna magna of conscious rats involves recruitment of hypothalamic vasopressinergic neurons

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Abstract The sulfur-containing non-essential amino acid L-cysteine injected into the cisterna magna of adult conscious rats produces an increase in blood pressure. The present study examined if the pressor response to L-cysteine is stereospecific and involves recruitment of hypothalamic vasopressinergic neurons and medullary noradrenergic A1 neurons. Intracisternally injected D-cysteine produced no cardiovascular changes, while L-cysteine produced hypertension and tachycardia in freely moving rats, indicating the stereospecific hemodynamic actions of L-cysteine via the brain. The double labeling immunohistochemistry combined with c-Fos detection as a marker of neuronal activation revealed significantly higher numbers of c-Fos-positive vasopressinergic neurons both in the supraoptic and paraventricular nuclei and tyrosine hydroxylase containing medullary A1 neurons, of L-cysteine-injected rats than those injected with D-cysteine as iso-osmotic control. The results indicate that the cardiovascular responses to intracisternal injection of L-cysteine in the conscious rat are stereospecific and include recruitment of hypothalamic vasopressinergic neurons both in the supraoptic and paraventricular nuclei, as well as of medullary A1 neurons. The findings may suggest a potential function of L-cysteine as an extracellular signal such as neuromodulators in central regulation of blood pressure.

Keywords L-Cysteine · Blood pressure · Vasopressinergic neurons · Hypothalamus · A1 neurons · c-Fos

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Introduction

L-Cysteine is a sulfur-containing non-essential amino acid and a basic brick for protein synthesis. Although it has neurotoxic actions when applied with overdoses especially to immature mammals, physiological and/or tissue-protective roles have also been suggested (Olney et al. 1990; Janáky et al. 2000).

With respect to a possible role in central cardiovascular regulation, pressor and tachycardiac responses to intracisternally injected L-cysteine in the conscious rat were revealed in a dose-dependent manner, equivalent to the pressor response to a neurotransmitter of L-glutamate (Takemoto 1990, 1992b, 1995a, 2012). The pressor response to L-cysteine was completely abolished by autonomic ganglionic blocking followed by intravenous injection of a vasopressin V1 receptor antagonist (Takemoto 1995a). Namely, intracisternally applied L-cysteine appears to stimulate some brain entities relating to hypothalamic vasopressinergic neurons together with autonomic activation, resulting in strong pressor modulation in the freely moving rat. There are many possible neuronal networks across the brain where L-cysteine stimulation results in the pressor response, as detailed in recent reviews (Guyenet 2006; Takemoto 2012). With respect to vasopressin release, noradrenergic A1 neurons in the caudal ventral medulla surface connect via their terminals to hypothalamic vasopressinergic neurons both in the supraoptic (SON) and paraventricular nuclei (PVN) (Armstrong 2004; Cunningham and Sawchenko 1988; Rinaman 2007). The present investigation focused on whether vasopressinergic neurons and/or A1 neurons are recruited in cardiovascular responses to intracisternal L-cysteine stimulation. Using the double immunohistochemistry that detects c-Fos protein as an indicator for neuronal activation combined with labeling

of vasopressin or tyrosine hydroxylase (TH), the responses of neurons to a hypertensive dose of L-cysteine stimulation were assessed in the hypothalamus and medulla.

Some other amino acids with the pressor response to intracisternal injections in conscious rats had different blood pressure responses with their stereoisomer: D-proline produced a weak depressor response against the pressor response to L-proline, and D-arginine produced a pressor response the same as L-arginine (Takemoto 1990, 1993, 1995b, c, 2012). The reactive thiol residue in the case of L-cysteine molecule may be involved in producing the cardiovascular responses. To better understand the process responsible for producing the action at the molecular level, hemodynamic responses to the stereoisomer D-cysteine were also examined. The results provided no hemodynamic response to D-cysteine, providing an iso-osmotic control for c-Fos experiments.

Methods

All protocols and surgical procedures used in this study were performed in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan and the guidelines of the Committee of Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

Animal preparation

Twelve male Wistar rats (300–350 g), which had been cared for and handled by an experimenter for at least 3 weeks, were anesthetized with sodium pentobarbital (i.p., 50 mg/kg). Seven rats received L-cysteine treatment and five received D-cysteine. Intracisternal tubing was made by fusing tubing sp (single lumen polyethylene tube) 10 (0.28 and 0.6 mm of the inner and outer diameters and 7 mm in final length) to sp 19 (0.35 and 1.05 mm of the diameters and 30 mm in length) (Natsume, Japan), then the narrow side of sp 10 was slightly bent with a hot air stream (Takemoto 1991, 1992a). The tubing was inserted into the cisterna magna through a hole drilled in the interparietal bone (Takemoto 1991). The tubing tip was oriented rostrally following verification by withdrawal of cerebrospinal fluid by a syringe, differently from the previous method for taking cerebrospinal fluid (Takemoto 1991, 1992a). The fused knob of the tubing was fixed to one small stainless screw anchored to the bone, with dental cement (Takemoto 1992a). The outer end of the tubing was occluded with a stainless steel wire stopper. After the intracisternal tubing was implanted, the rats were kept individually in plastic cages (36 × 30.5 × 18 cm) with wood chips on the floor, and the cages were covered with metal wire tops containing pellets and a water bottle. The cage was placed in a room with a 12-h light and 12-h dark cycle. Water and pellets (Standard rat chow, type MF, Oriental Yeast Co., Ltd., Tokyo, Japan) were supplied ad libitum. Seven days after the first operation, the rat was re-anesthetized with sodium pentobarbital (i.p., 50 mg/kg) and a polyethylene catheter (sp 10 of 6 cm, fused to sp 19 of 16 cm) was placed in the terminal aorta to perform arterial pressure measurements, heart rate measurements, and drug administration. The other end of the catheter, which was plugged with a wire stopper was run subcutaneously and exited at the back of the neck. To reduce operation-induced stress, the protocol excluded the venous catheter insertion step used in previous studies (Takemoto 1990, 1995a). The cage was placed in another recovery room with controlled 12-h light and 12-h dark cycles. After each operation, antibiotics (potassium penicillin G 4,000 unit, streptomycin sulfate 20 mg) were subcutaneously injected.

Injections of amino acids

Amino acid solution, D-cysteine hydrochloride monohydrate and L-cysteine (nacalai tesque, Kyoto, Japan) were freshly prepared just before each experiment by dissolving in artificial cerebrospinal fluid (ACSF) (Takemoto 1990). D-Cysteine hydrochloride solution was adjusted to around pH 7.4 with NaOH solution to reach 0.2 M. A sp 19 segment of 10 cm with a wire stopper in one end that contained 10 µl of solution was connected to the intracisternal tubing before the injection. Then, 15 µl of ACSF was injected into the attached tubing to flush amino acid solution into the cisterna magna. This injection system allowed the experimenter to avoid touching the rat when injecting, so that stress could be minimized.

Measurements of blood pressure and heart rate

Experiments with the freely moving rats started 2 days after the final operation. The experiments started between 8:30 a.m. and 10:00 a.m. Each singly housed rat was moved to an experimental room in its home cage. Each rat was given a resting period of at least 60 min while its arterial blood pressure and heart rate were monitored. A polyethylene tubing (sp 19 fused to PE 50 of Intramedic) from a pressure transducer was connected to the arterial catheter via stainless tubing. Pulsatile pressure signal was amplified with an AP-601G (Nihon Kohden, Japan). A polygraph system (RM-6004, Nihon Kohden) was used for continuous recording of mean arterial pressure with an integrator (EI-600G, Nihon Kohden) and heart rate with a cardiotachometer (AT-601G, Nihon Kohden) from the

pulsatile pressure. The pulsatile and mean arterial pressure and heart rate were recorded by a pen-writing oscillograph (RJG-4024, Nihon Kohden) following an injection of either intracisternal L-cysteine (10 µl of 0.2 M) or D-cysteine (10 µl of 0.2 M) for 90 min. Thereafter, an Evans blue dye solution (10 µl, 2 %) was injected to examine the possible distribution of amino acid solution in the brain surface.

Brain slice preparation

Ten minutes after injecting the dye, sodium pentobarbital (50 mg/kg) was slowly infused intra-arterially. The abdominal aorta was blocked with a hemostat secured below the level of the thoracic aorta, and the upper body of the rat was perfused intracardially with isotonic saline containing heparin (10 unit/ml, 20–40 ml) followed by a fixative solution containing freshly prepared 4 % paraformaldehyde buffered with sodium phosphate (200 ml, pH 7).

After observing the dye distribution, each brain block, including the hypothalamus or medulla, was post-fixed for 2 h. The 50 µm coronal slices were made using a vibratome. Alternating sections were used for each immunohistochemical staining series. The sections were kept in order using a 24-hole culture plate customized with a mesh fiber sheet on the bottom.

Immunohistochemistry

The floating sections were pre-treated with 0.3 % H_2O_2 in phosphate buffered saline (PBS) for 30 min, followed by incubation in blocking solution (3 % bovine serum albumin and 0.3 % triton X-100 in PBS pH 7) for 90 min. The sections were incubated overnight with rabbit polyclonal anti-c-Fos antibody (Calbiochem PC38, ex-Oncogene Ab-5) (Godino et al. 2005) diluted in blocking solution containing 0.2 % sodium azide (1:10,000). The sections were then reacted with biotinylated goat anti-rabbit IgG secondary antibody diluted in blocking solution for 2 h, followed by incubation with horseradish peroxidase avidin D reagent diluted with PBS for 1 h. The reacted complex was detected using 3,3′-diaminobenzidine tetrahydrochloride (DAB, Dojindo Laboratories, Japan) with 0.006 % H_2O_2 intensified with 1 % cobalt chloride and 1 % nickel ammonium sulfate (Godino et al. 2005). This treatment allowed a black or darker brown nuclear product to be detected in the cells using a light microscope.

To perform double staining, the sections were further reacted overnight with either a rabbit polyclonal antibody against vasopressin (1:17,000, Chemicon AB 1565, which exhibited less than 1 % cross-reactivity with oxytocin), or a rabbit polyclonal antibody against TH (1:2,500, Protos Biotech Corp, New York, CA-101-bTHrab). A similar reaction was used for c-Fos detection; however, only DAB–H_2O_2 solution was used to produce a detectable brown product in the cytoplasm. Sections were washed thoroughly with PBS between each reaction. Following antibody detection, the sections were mounted on gelatinized glass slides, air-dried overnight, dehydrated, made clear with xylene solutions, and mounted using a coverslip set with Permount SP15 (Fisher Scientific).

Observation and counting of labeled cells

The sections were examined using a light microscope equipped with a mounted digital camera, and images were captured using a DP70 Image Analysis System (Olympus). The cells that positively reacted were manually counted from printed magnified photographs. The corresponding region was kept under the microscope as a reference to adjust the focus on other vertical levels in the section if necessary. The counts were made by a researcher blinded to the treatment.

When counting c-Fos-stained cells in the hypothalamus (Paxinos et al. 1999a), dense areas with DAB and darker brown or black c-Fos nuclei were first encircled in a highly magnified photograph (×100), then counted. The photos of the frontal slices of the medulla were taken in order at low magnification (×20), and then three slices of −14.2 mm to the Bregma (the obex level), −13.6 and −12.6 mm were determined using a brainstem atlas (Paxinos et al. 1999b). After numbering all levels of slices in the photos, TH-positive cells with c-Fos labeling in the medulla were counted at a higher magnification in the corresponding slices.

The results are expressed as a median (upper quartile, lower quartile). The data were analyzed using nonparametric Mann–Whitney's test or Kruskal–Wallis analysis followed by Steel's test. Statistical analysis package, Ekuseru-Tokei 2010 developed by Social Survey Research Information Co., Ltd. (Japan) was used. p values <0.05 were considered statistically significant.

Results

Hemodynamic changes

The changes in mean arterial blood pressure and heart rate of seven L-cysteine-injected rats and five D-cysteine-injected rats are shown in Fig. 1. Intracisternal injection of L-cysteine produced the pressor response between 1 and 20 min and the cardiac response 1 and 5 min. In contrast, intracisternal injection of D-cysteine did not produce significant changes in both variables.

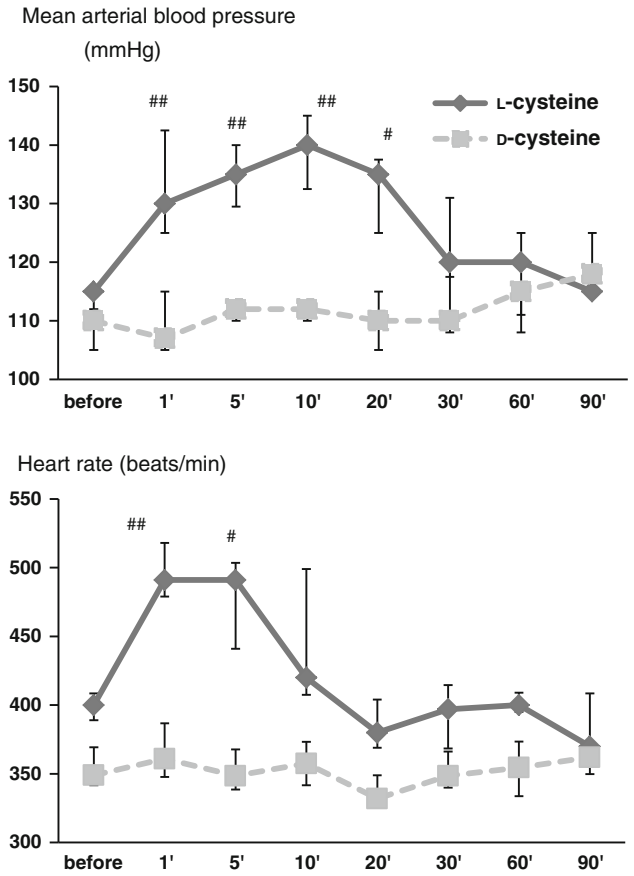

Fig. 1 Changes in mean arterial blood pressure and heart rate following intracisternal injection of L-cysteine or D-cysteine (0.2 M, 10 µl). Results are presented as medians with upper and lower quartiles. $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ by Kruskal–Wallis analysis followed by the Steel's test. The resting values of blood pressure and heart rate between L- and D-cysteine treatments before injection show no significant difference by Mann–Whitney's *U* test

c-Fos synthesis in vasopressinergic neurons of the hypothalamus

Sections showing vasopressinergic neurons in L-cysteine-injected and D-cysteine-injected rats are shown in Fig. 2A. As shown in Fig. 2B, the median number of c-Fos-positive vasopressinergic neurons in both the SON and PVN was higher ($p < 0.01$) in L-cysteine injected rats. Specific significant difference ($p < 0.05$) was obtained in two levels of the most rostral (10 of median, 23 of upper quartile, 7.5 of lower quartile for L-cysteine and 4, 4, 4 for D-cysteine) and caudal (11, 25, 8 for L-cysteine and 2, 8, 1 for D-cysteine) PVN between both treatments.

The numbers of c-Fos-positive cells in the SON per section were 91 (132, 25), 119 (144, 21), 61 (113, 22), and 41 (92, 15) from rostral to caudal levels for L-cysteine treatment and 13 (16, 9), 8 (19, 6), 14 (20, 4), and 9 (23, 9) for D-cysteine treatment. In the PVN, they were 140 (188, 97), 200 (312, 164), 225 (246, 121), and 185 (215, 150) for L-cysteine treatment and 80 (85, 60), 80 (90, 45), 110 (120, 100), and 130 (165, 80) for D-cysteine, respectively. Mann–Whitney's *U* test detected significant difference ($p < 0.05$) between the c-Fos numbers of L-cysteine treatment and D-cysteine treatment in every level of the PVN slices, but not for any pair in the SON.

Double labeling of c-Fos and TH in medullary sections

Figure 3A shows photos of medullary slices from both L-cysteine and D-cysteine-injected rats. The numbers of double-labeled TH neurons in the L-cysteine treatment between −14.4 and −13.4 mm except −13.6 mm were significantly ($p < 0.05$) higher than in the D-cysteine one, as shown in Fig. 3B.

Distribution of Evans blue solution

In all rats, dye spread over the medullary surface and the caudal cerebellar surface facing the cisterna magna, and to the ventral side of the brain or the bottom of the cranial bone.

Discussion

Intracisternal injection of L-cysteine produced increases in arterial blood pressure and heart rate in freely moving rats, as observed in previous studies (Takemoto 1990, 1995a). However, the injection of D-cysteine did not modify these variables in the present examination. The results indicate that the pressor and tachycardiac responses to L-cysteine were stereospecific, not dependent upon the chemical thiol residue alone. This was similar to the results with the pressor response to L-proline but not to D-proline (Takemoto 1995b). The responses with L-proline were completely abolished by the previous intracisternal injection of the ionotropic excitatory amino acid antagonist kynurenate (Takemoto 1999). Although L-cysteine microinjected into the nucleus tractus solitarii (NTS) produced a depressor but not pressor response in anesthetized rats, the response was completely blocked by co-injection of kynurenate (Takemoto and Kan 2000). Excitatory amino acid receptors on the brain cell membrane may also be responsible for the present hemodynamic effects of L-cysteine injected into the cisterna magna.

The current immunohistochemical examination revealed that the L-cysteine-injected rats had significantly higher numbers of c-Fos-positive vasopressinergic neurons both in the SON and PVN than the control D-cysteine-injected rats. The numbers of c-Fos-positive cells both in vasopressin- and TH-positive neurons with the D-cysteine treatment showed no significant difference from those with ACSF

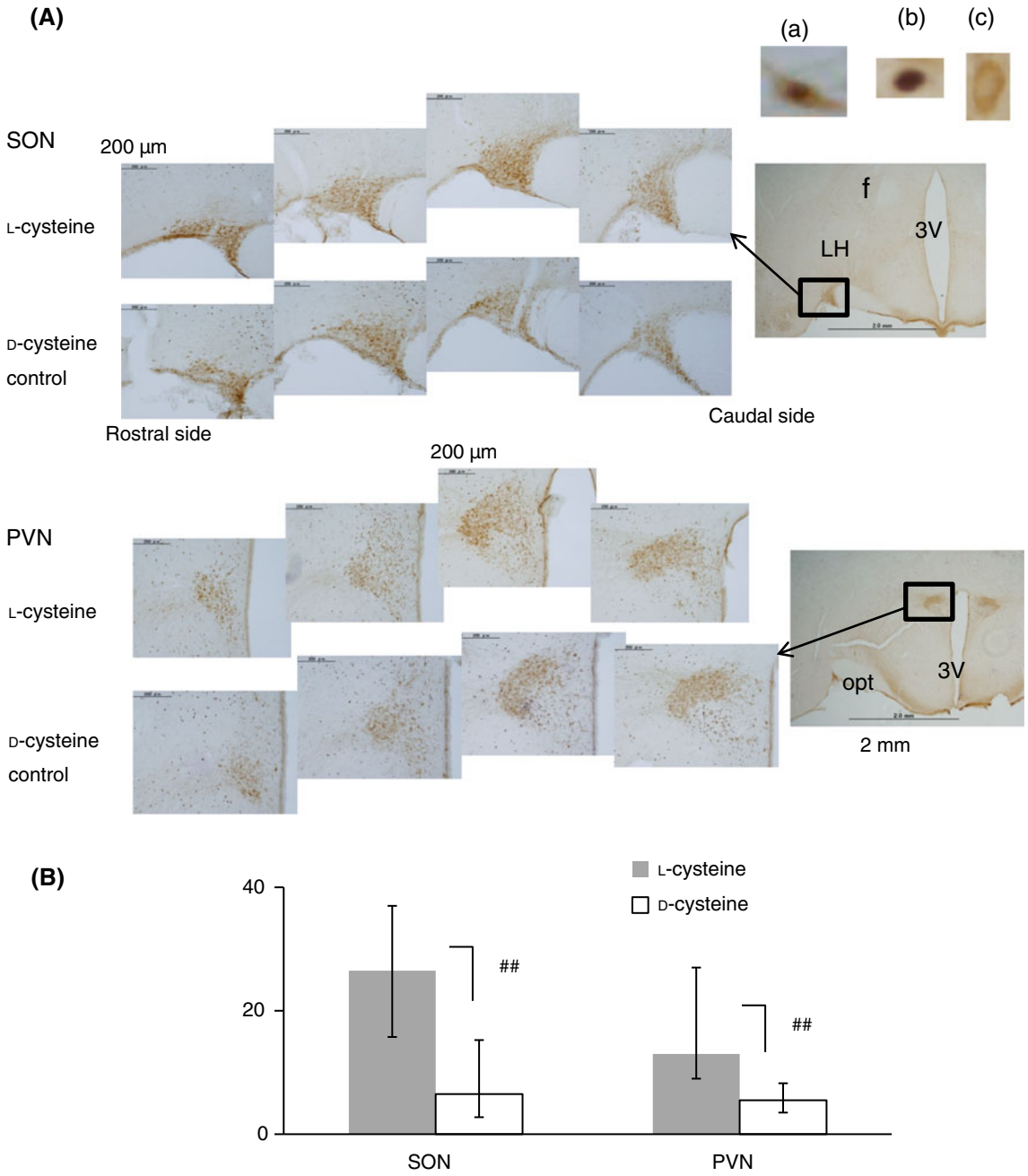

Fig. 2 **A** Frontal sections showing vasopressinergic neurons in the hypothalamic SON and PVN. Representative higher magnification photographs are shown as the double-labeled vasopressinergic neuron (a), as the c-Fos-positive cell (b), and as the vasopressinergic neuron without c-Fos synthesis (c). The *arrows* indicate the location of the magnification in the original photograph. *f* Fornix, *3V* third ventricle, *opt* optic tract, *LH* lateral hypothalamic area. **B** The numbers of c-Fos-positive and vasopressin-positive neurons in 50 μm thickness section of the SON and PVN following L-cysteine or control D-cysteine injection. Data are represented as the medians with upper and lower quartiles. $^{\#\#}p < 0.01$ by Mann–Whitney's *U* test (color figure online)

control in the previous study of the same protocol for the L-proline treatment (Takemoto 2011). The results support the prior pharmacological interpretation that L-cysteine activates vasopressinergic neurons which contribute to the pressor response persisting after ganglionic blockade (Takemoto 1995a).

The current results also showed that TH-positive neurons in the medulla presented significantly greater numbers of c-Fos-labeled cells by L-cysteine than D-cysteine stimulation. According to Buller et al. (1999), the levels between −15.2 mm to the Bregma and −12.6 mm in Fig. 3B include noradrenergic A1 neurons and anterior

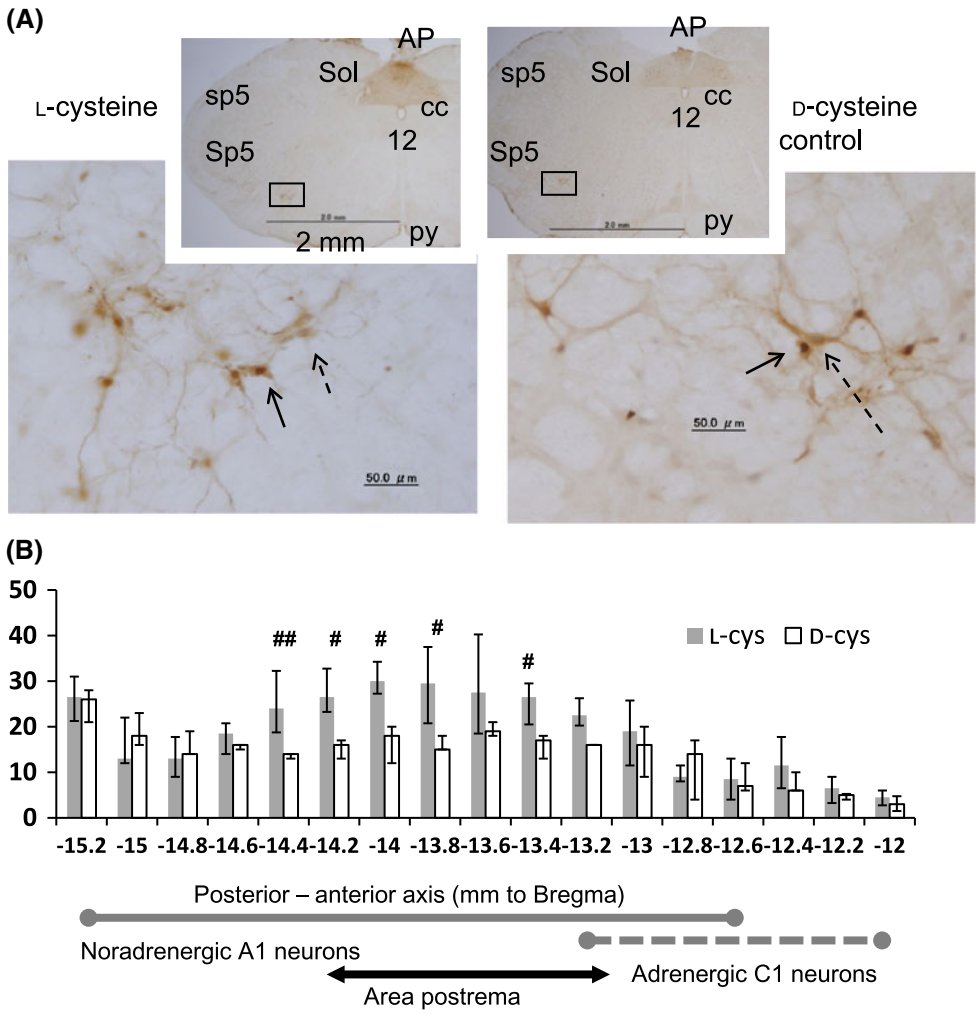

Fig. 3 **A** Double-labeled A1 neurons showing *black* c-Fos nuclei and *brown* TH neurons in medullary sections from the L-cysteine injected rat and the control D-cysteine injected rat. *Arrows with a continuous line* indicate double-stained A1 neurons, and *arrows with a dotted line* indicate A1 neurons without c-Fos synthesis. The *framed regions* in the small photographs were magnified. *AP* Area postrema, *cc* central canal, *py* pyramidal tract, *Sol* nucleus of the solitary tract, *sp5* spinal trigeminal tract, *Sp5* spinal trigeminal nucleus. **B** Numbers of double-labeled TH-containing neurons in the medulla with L- or control D-cysteine. Data are represented as the medians with upper and lower quartiles. $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ by Mann–Whitney's *U* test (color figure online)

levels from −13.2 mm adrenergic C1 neurons. Namely, each slice between −14.4 and −13.4 (except −13.6) that correspond to the level of area postrema and part of A1 neuronal group shows significant difference between treatments of L-cysteine and D-cysteine control. A significantly augmented range of A1 neurons was interestingly restricted at levels of the area postrema within wider distribution of A1-positive neurons in the rostral–caudal axis. A1 neuronal group sends axonal terminals to the hypothalamic vasopressinergic neurons both in the SON and PVN (Armstrong 2004; Cunningham and Sawchenko 1988; Rinaman 2007). In view of this, vasopressinergic nervous activation in both nuclei after intracisternal injection of L-cysteine may be via the A1 neuronal activation in the medulla. However, elucidation of the exact causal relationship from those L-cysteine-activated A1 neurons in the medulla to vasopressinergic neurons in both hypothalamic SON and PVN needs further examination with different approaches.

Distribution of intracisternally injected dye solution after experiments gives clues to possible acting sites of L-cysteine. The dye stained parts of the brain as described previously (Takemoto 1993, 2003, 2011). Namely, it flowed down to the ventral brain surface including the SON, but not the PVN, situated on the bottom of the cranial bone. Therefore, the direct action of L-cysteine on the PVN would be excluded. The SON and PVN receive mainly axons from the A1 neurons in the ventral medulla which connect to the neuronal group in the NTS at the dorsal medulla (Chan et al. 1995; Rinaman 2007). The significant numbers of c-Fos-positive A1 neurons were detected only at the level of the area postrema after L-cysteine stimulation

in the cisterna magna. The dye stained both the A1 and NTS areas. The NTS contains various kinds of visceral afferent terminals from the body (Andresen and Paton 2011). Therefore, there are at least two possibilities for activation of the selected part of A1 neurons: one is direct activation of corresponding A1 neurons and another is through activation of specific neurons in the NTS. Exact acting sites of L-cysteine resulting in vasopressin release remain unknown at present.

L-Cysteine is one amino acid with a pressor response to intracisternal stimulation of conscious rats (Takemoto 1990), but the mode of responses is different from others (Takemoto 1993, 1995a, b, 2011, 2012). First, the pressor response to L-cysteine is equivalent to the typical excitatory amino acid neurotransmitter L-glutamate and stronger than the neurotransmitter candidate L-proline. Second, L-cysteine produces a significant robust tachycardiac response, but L-proline evokes a bradycardiac response and L-arginine various responses. Third, respiratory acceleration is observed together with the pressor response to L-cysteine, which is similar to L-glutamate. Fourth, effects of peripheral pharmacological treatments using vasopressin receptor 1 antagonist alone on the pressor response to L-cysteine were different from those to L-proline and L-glutamate. Ganglionic blockade produced augmented pressor response to all three amino acids but not L-arginine. Then additional vasopressin antagonist completely abolished the augmented responses to three amino acids, suggesting that both autonomic activation and vasopressin release into the blood stream produce the pressor response to three amino acids. However, vasopressin antagonist alone without ganglionic blockade significantly attenuated the pressor response to both L-proline and L-glutamate, but not to L-cysteine. Therefore, vasopressin release into the blood stream could be a major cause of the pressor response to central stimulation of L-proline and L-glutamate, with a minor contribution from autonomic activation. Conversely, autonomic activation could be the major cause to the pressor response to L-cysteine, with minor contribution from vasopressin release. The present study provided the fifth piece of evidence to differentiate the mode of responses to L-cysteine from L-proline on c-Fos synthesis of the vasopressinergic neurons in two nuclei of the hypothalamus. Both the SON and PVN were significantly stimulated by intracisternally injected L-cysteine in the present study, but the SON alone was markedly stimulated by the L-proline (Takemoto 2011), supporting the minor role of vasopressin release on the pressor response to L-cysteine and the major role to L-proline (Takemoto 1995a, b).

The current examination using the c-Fos protein detection indicates that the intracisternal L-cysteine stimulation can activate vasopressinergic neurons in the PVN and SON. Intracisternally applied L-cysteine appears to stimulate some node of neural network for vasopressin release. Vasopressin can be released from the posterior pituitary by various functional factors such as high blood osmolality, reduced arterial pressure, and stress (Cunningham and Sawchenko 1991). In addition, recent progress on vasopressin research in social behaviors reveals vasopressin release not only into the blood but also into the extracellular fluid around both the SON and PVN to various stimulations (Engelmann et al. 2004). Vasopressin release in the PVN secretes corticotropin (ACTH) and might be centrally related to the stress response through activation of the hypothalamic–pituitary–adrenal system (Engelmann et al. 2004). However, it remains unknown at present what physiological functions L-cysteine can modify through vasopressin release and if there is possible vasopressin release into the extracellular space of the SON and PVN after L-cysteine stimulation.

L-Cysteine is one of the basic 20 amino acids for protein synthesis, and is produced from methionine in mammalian cells. Its thiol residue plays an important role in maintaining a stable topographical structure by bonding thiol residues within proteins. Other than these molecular structural roles, L-cysteine has several actions such as neurotoxin, neuroprotection, and neuromodulation (Janáky et al. 2000). The neurotoxic effect of overdosed L-cysteine partly through the NMDA receptors has been well studied (Olney et al. 1990; Janáky et al. 2000). High concentrations of L-cysteine produced brain damage in the neonatal animal several hours after its systemic administration, due to the immature blood–brain barrier (Olney et al. 1990). Adult brains are also sensitive to systemic L-cysteine, but areas of damage are different from the neonatal animals, suggesting effects of cerebral blood flow (Sawamoto et al. 2004). With respect to neuroprotection, L-cysteine makes a contribution through glutathione that is present in millimolar concentrations in mammalian cells (Cooper 1997). L-Cysteine, a rate-limiting precursor for glutathione synthesis, is taken up via a specific transporter into neurons and is synthesized into glutathione together with L-glutamate and glycine (Dringen 2000). In addition to glutathione, L-cysteine is used as a precursor for many other bioactive molecules including taurine, *S*-nitrosocysteine, L-cysteine sulfinic acid, *S*-sulfocysteine, cysteate, and hydrogen sulfide (Griffith 1987; Thompson and Kilpatrick 1996; Janáky et al. 2000; Qu et al. 2008; Zhang and Hogg 2005). However, L-cysteine itself is a potential neuromodulator because it is released from brain slices (Keller et al. 1989; Zängerle et al. 1992), excites neurons (Olney et al. 1990), and is taken up by neurons and glial cells (Sagara et al. 1993). The recruitment of vasopressinergic neurons and A1 neurons may be via such a neuromodulatory action of L-cysteine.

In conclusion, the present study using c-Fos detection suggests that intracisternal injection of L-cysteine but not D-cysteine activates hypothalamic vasopressinergic neurons and A1 neurons in freely moving rats. L-Cysteine may have a functional role as an extracellular signal like neuromodulators in central regulation of blood pressure.

Conflict of interest The author declares that she has no conflict of interest.

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