広島大学学術情報リポジトリ
Hiroshima University Institutional Repository

Title	Muscle vasodilator response via potential adrenaline secretion to L-cysteine microinjected in rostral ventrolateral medulla of rats
Author(s)	Takemoto, Yumi
Citation	Autonomic Neuroscience , 224 : 102644
Issue Date	2020-03
DOI	10.1016/j.autneu.2020.102644
Self DOI	
URL	https://ir.lib.hiroshima-u.ac.jp/00050432
Right	© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/ This is not the published version. Please cite only the published version. この論文は出版社版ではありません。引用の際には出版社版をご確認、ご利用ください。
Relation	

Muscle vasodilator response via potential adrenaline secretion to L-cysteine microinjected in rostral ventrolateral medulla of rats

The corrections made in this section will be reviewed and approved by journal production editor.

Yumi Takemoto yumitake@hiroshima-u.ac.jp

Department of Physiology II, Graduate School of Biomedical and Health Sciences (Medicine), Hiroshima University, Japan

[a]Kasumi 1-2-3, Minami-ku, Hiroshima 734-8551, Japan.

Abstract

We previously found that the thiol amino acid L-cysteine microinjected into rat medullary autonomic areas produces changes in arterial blood pressure (AP) via ionotropic excitatory amino acid receptors (iEAAr), but its effect on vascular beds is still unknown. Rostral ventrolateral medulla (RVLM) pressor area includes adrenal and lumbar presympathetic neurons which activation could cause opposite muscle vascular responses: vasodilation versus vasoconstriction. However, there are few data on the vasodilator response in rats. Ionotropic EAAr activation alone with L-cysteine may be effective to differentiate RVLM sites for those opposite responses. To test it, muscle blood flow responses to L-cysteine were mapped in the ventrolateral medulla (VLM) of rats. In anesthetized rats with a cranial window above the VLM, hindquarter flow (HQF) and AP were monitored, providing hindquarter resistance (HQR) by dividing AP by HQF. L-Cysteine mapping in VLM including caudal depressor vasodilator area defined with L-glutamate showed HQR responses in parallel to AP responses, suggesting the importance of iEAAr in muscle vascular regulation. Microinjections of L-cysteine into RVLM succeeded to detect sites of slower muscle vasodilation and blockade of peripheral β-adrenoceptors abolished this response, indicating potential adrenaline secretion. Although there was no functional topography, the iEAAr activation alone with L-cysteine can differentiate sites of muscle vasodilation from vasoconstriction in rat RVLM. The neuromodulator candidate L-cysteine is a useful tool when chemical stimulation of iEAAr is required.

Keywords: L-Cysteine; ~~i~~Ionotropic excitatory amino acid receptors; RVLM; ~~m~~Muscle vasodilation; ~~a~~Adrenaline secretion; ~~r~~Rats

~~1.~~1 Introduction

The muscle vascular bed shifts the blood flow to the carotid artery when the rat grooms (Mizuma et al., 1987) but it is inversed when the rat spontaneously walks (Takemoto and Iriuchijima, 1989), probably via fast autonomic nervous regulation. The medulla includes a core network of neurons to regulate vascular sympathetic activities (Guyenet, 2006). Hence, we are concerned with the role of the medulla in the regulation of such vascular beds.

The rostral ventrolateral medulla (RVLM) includes one of the most important autonomic areas of vascular presympathetic neurons that regulate the resting arterial blood pressure (AP) and mediate many autonomic reflexes for body homeostasis (Guyenet, 2006; Dampney, 2016). Neuroanatomical evidence indicates that the RVLM of the rat includes both lumbar (Lee et al., 2007) and adrenal (Ross et al., 1984; Strack et al., 1989; Pyner and Coote, 1998) presympathetic motor neurons. It suggests that their excitation with excitatory amino acids (EAA) such as L-glutamate will produce opposite muscle vascular responses, i.e., muscle vasoconstriction caused by lumbar sympathetic nervous activation versus muscle vasodilation caused by adrenaline secretion. In reality, muscle vasoconstriction and adrenaline secretion were observed in response to stimulation with EAA in the cat RVLM (McAllen, 1986). However, there is little evidence of muscle vasodilation contrasting usual vasoconstriction from stimulation with EAA in the rat RVLM (Guyenet, 2006).

Mueller et al. (Mueller et al., 2011) reported one rat RVLM site where L-glutamate stimulation caused activation of the adrenal, but not lumbar or renal, sympathetic neurons with a hypotensive response that could be due to muscle vasodilation caused by adrenaline secretion. This was the site distinct from many microinjected sites that led coactivation of three neurons and an increase in AP, suggesting the existence of distinguishable sites for adrenaline-produced muscle vasodilation in RVLM vasoconstrictor pressor area of rats in response to EAA stimulation.

Thus far, most medullary autonomic reflexes examined are activated mainly through the ionotropic EAA receptors (iEAAr), but not through the metabotropic EAA receptors (mEAAr). Activated responses in many autonomic reflexes and central circuits that include neurons in the RVLM (Koshiya et al., 1993; Kiely and Gordon, 1994; Zanzinger et al., 1994; McCulloch et al., 1999; Zhou et al., 2006; Schreihofer and Sved, 2011; Sabetghadam et al., 2017) and the caudal ventrolateral medulla (CVLM) (Sun and Guyenet, 1985; Miyawaki et al., 1997; Sved et al., 2000; Verberne et al., 1989; Miyawaki et al., 1996; Mandel and Schreihofer, 2009; Verberne and Guyenet, 1992; Peng et al., 2000; Sartor and Verberne, 2003; Mobley and Schreihofer, 2006; Holstein et al., 2014; Owens and Verberne, 2000; Yang and Coote, 1999; Sun and Penneton, 2005; Guyenet, 2011) are inhibited by application of iEAAr blocking agents such as kynurenate. The typical EAA L-glutamate are often used as a chemical stimulant in RVLM and CVLM, but this amino acid activates both of iEAAr and mEAAr. Hence, additional mEAAr activation may interfere or mask the targeted iEAAr responses such as the muscle vasodilation initiating in adrenal presympathetic activation of RVLM.

The previous studies have shown that the thiol amino acid L-cysteine is a functionally active molecule in the brain cardiovascular control regions of rats (Takemoto, 1990; Takemoto, 1995a; Takemoto, 2012; Takemoto, 2013; Takemoto, 2014a; Takemoto, 2014b; Takemoto, 2014c). L-Cysteine microinjections produce a pressor response in the RVLM (Takemoto, 2014a) and depressor responses in the nucleus tractus solitarius (NTS) visceral neuron terminated area in the dorsal medulla (Takemoto, 2014b) and the CVLM autonomic interneuron area (Takemoto, 2014c). These responses to L-cysteine in the medulla were blocked by iEAAr antagonists (Takemoto, 2014a; Takemoto, 2014b; Takemoto, 2014c), indicating that L-cysteine activates iEAAr alone, different from the multiple EAAr activator L-glutamate. It has not yet known about the effect of L-cysteine on muscle vascular bed that may contribute to its AP responses. The iEAAr activator L-cysteine may be useful to find potential differential sites for muscle vasodilation and vasoconstriction in rat RVLM. The current study tested this hypothesis by mapping responses in hindquarter flow resistance (HQR) with L-cysteine in RVLM and CVLM of rats.

Since we identified distinguishable (hormonally-like) slow vasodilator reaction sites in the RVLM pressor area, the potential for adrenaline secretion was pharmacologically assessed by blocking the peripheral β2-adrenoceptors.

The RVLM pressor and CVLM depressor areas were defined from L-glutamate control mapping in the current sino-aortic denervated (SAD) rat and in the previous intact rat that showed only pressor responses in RVLM (Takemoto, 2004). Unexpectedly, quite numbers of sites of RVLM vasodilation were obtained in SAD rats.

~~2.~~2 Materials and ~~M~~methods

All protocols and surgical procedures employed in this study were approved by the president of Hiroshima University and were performed in accordance with the guidelines of the Committee on Animal Experimentation and the Committee on Research Facilities for Laboratory Animal Science in Natural Science Center for Basic Research and Development, Hiroshima University. Following the guidelines, experiments were designed with the lowest possible sample sizes in order to reduce the number of animals killed.

All procedures were performed as previously described (Takemoto, 2014a; Takemoto, 2014b; Takemoto, 2014c; Takemoto, 2004; Takemoto, 1995b; Takemoto, 2016), with slight modifications. Briefly, 20 male Wistar rats (310–360 g) were anesthetized with urethane (1.0~~–~~1.2 g/kg intraperitoneally) and α-chloralose (50 mg/kg intraperitoneally). After a 2-mm-diameter electromagnetic flow probe (Nihon Kohden, Japan) was placed around the aortic terminal by a retroperitoneal approach, the animal was intubated with tubing coated on the outside with atropine sulfate ointment (Santen, Osaka, Japan) to prevent mucosal secretion and placed in the supine position in an electrically isolated stereotaxic frame. A window was then opened above the ventral medulla by scraping the occipital bone using a dental technology engine after retracting the trachea, esophagus, and muscles. Then, the animal was cannulated with polyethylene tubing in the left carotid artery and jugular vein to allow for AP measurement and drug injection, respectively. Sino-aortic denervation (SAD) of both sides was performed in the control experiment of L-glutamate as detailed in ref. (Takemoto, 1995b). The respirator system (model SN-480-7, Shinano, Tokyo, Japan) with Capnocheck Plus (Smiths medical, Minnesota, USA) was connected to the tracheal tubing and controlled the maintenance of normocapnia ($PaCO_2$ 35–45 mmHg), as detailed in the previous study (Takemoto, 2004). Arterial blood pressure (AP) and

hindquarter blood flow (HQF) were amplified with AP-601G and electromagnetic blood flowmeter MFV1100, respectively (Nihon Kohden, Japan). Zero values of HQF were obtained when the rats were killed and blood flow stopped after the completion of the experiments. Recordings of AP and HQF were sampled at 100 Hz with a LabScribe 3 digital system (iWorx, New Hampshire, USA). HQR was calculated as AP/HQF and expressed as percent change. Rectal temperature was maintained between 36.5 °C and 37.5 °C.

Microinjections were performed at a depth of 0.7 mm for the RVLM area and 0.8 mm for the CVLM area using a glass micropipette containing L-cysteine solution (30 mM, 34 nl) or L-glutamate (20 mM, 68 nl) connected to a micromanipulator by polyethylene tubing and a syringe filled with distilled water. Each concentration was decided based on the previous studies (Takemoto, 2014a; Takemoto, 2014c). Care was taken to avoid blood vessels. L-Cysteine was freshly dissolved in commercial artificial cerebrospinal fluid (Artcereb, Otsuka Pharmaceutical Co., Ltd., Japan) that was also used for dissolving L-glutamate and gamma-aminobutyric acid (GABA) (10 mM). The injection interval was longer than 3 ~~minutes~~min to allow the variables to return to their original levels.

Microinjections of L-cysteine were performed initially at 2.5 mm in the anterior–posterior direction and 2.0 mm lateral to the right side of the caudal beginning of the basilar artery to find a site with a typical pressor response and then systematically moved to other regions around this site to find areas responsible for vasodilation in our initial experiments. This was based on the microinjection study by Mueller et al. (Mueller et al., 2011), which found only one potential site for adrenaline secretion within the RVLM, where L-glutamate microinjection produced adrenal, but not lumbar or renal, sympathetic activation as well as a depressor change. Their site was located 200 μm caudal and 200 μm medial to the site responsible for the pressor and three sympathetic activation responses.

A marker (zero) point was set at the caudolateral edge of the beginning of the basilar artery. When the anatomical distance of the basilar artery was apparently altered, the rostral end of the second rootlet of cranial nerve XII was used as the reference point, located 1.11 mm from the basilar artery zero point (Takemoto, 2004).

Following the response-mapping experiment to L-cysteine stimulation, testing of β-adrenoceptor blocking of peripheral adrenaline release was performed in seven rats. L-Cysteine was microinjected into the RVLM vasodilator site, and then the injection was repeated 2 to 7 ~~minutes~~min after intravenous injection of dl-propranolol (2 mg/kg) (nacalai, Kyoto, Japan). To make sure the peripheral β2 adrenoceptor blocking effect of propranolol, adrenaline (0.6 nmol/kg, 0.1 ml) was also injected before and after propranolol treatment in 5 rats. Percent responses in HQR to adrenaline iv injection before and after the treatment were ~~-~~23 ± 7.4 and 47 ± 11 (p = ~~0~~.0003 by paired t-test). The data demonstrated that propranolol blocked β2 adrenergic vasodilation of muscle by adrenaline, converting only to enhanced α1 vasoconstriction.

Supplemental doses of urethane and α-chloralose were administered intravenously as needed. Adequate depth of anesthesia was assessed based on AP stability and/or the absence of a withdrawal response to a firm toe pinch.

At the end of the experiments, Coomassie Brilliant Blue solution was microinjected to mark the sites where the depressor vasodilator responses were obtained. The rats were killed by an overdose of pentobarbital sodium (65 mg/kg intravenously); then a zero value of HQF was obtained, and the upper body was transcardially perfused with isotonic saline containing heparin (20 ml), followed by 8% formaldehyde saline solution (50 ml). The brainstem was blocked out and kept in the same solution at 4 °C. The sliced frontal section (50 μm) was recorded using a digital camera system (DP70, Olympus, Japan).

The values of variables were expressed as means ± SD. The effect of drug treatment was analyzed using a paired *t*-test, and a correlation matrix was applied to evaluate the correlations between the changes in the variables. The statistical analysis package Ekuseru-Tokei 2012, developed by Social Survey Research Information Co., Ltd. (Japan), was utilized, and *p* values <0.05 were considered statistically significant.

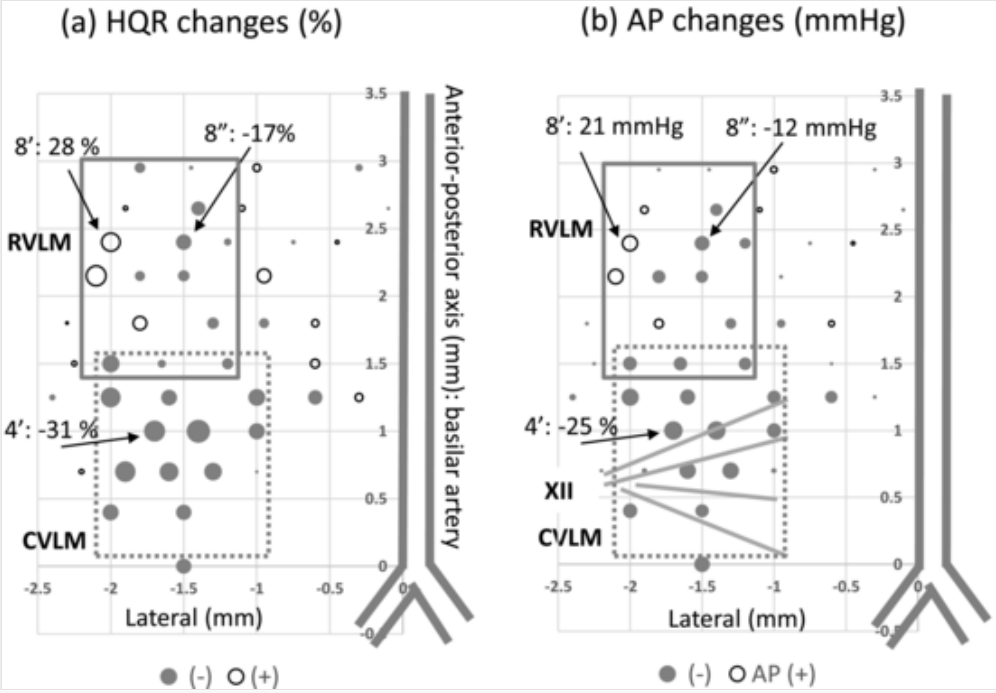
3.3 Results

3.1.3.1 An example of response mapping with L-cysteine

Fig. 1 shows the injection sites at which changes in HQR (a) and AP (b) were produced when L-cysteine (30 mM, 34 nl) was microinjected into the ventral medulla. Microinjections of L-cysteine in 46 sites produced different levels of vasoconstrictor (white circles) or vasodilator (gray circles) responses (a) with corresponding changes in AP (b). The rostral part of an arbitrary area of the RVLM included both the muscle vasodilator and vasoconstrictor sites, but the CVLM area included only the vasodilator sites. In the RVLM area, the distinct sites for vasodilator depressor responses to L-cysteine were obtained. Changes in AP (mmHg) and HQR (%) at 46 sites were highly correlated ($r = 0.9075$, $p < .01$).

alt-text: Fig. 1

Fig. 1.Fig. 1

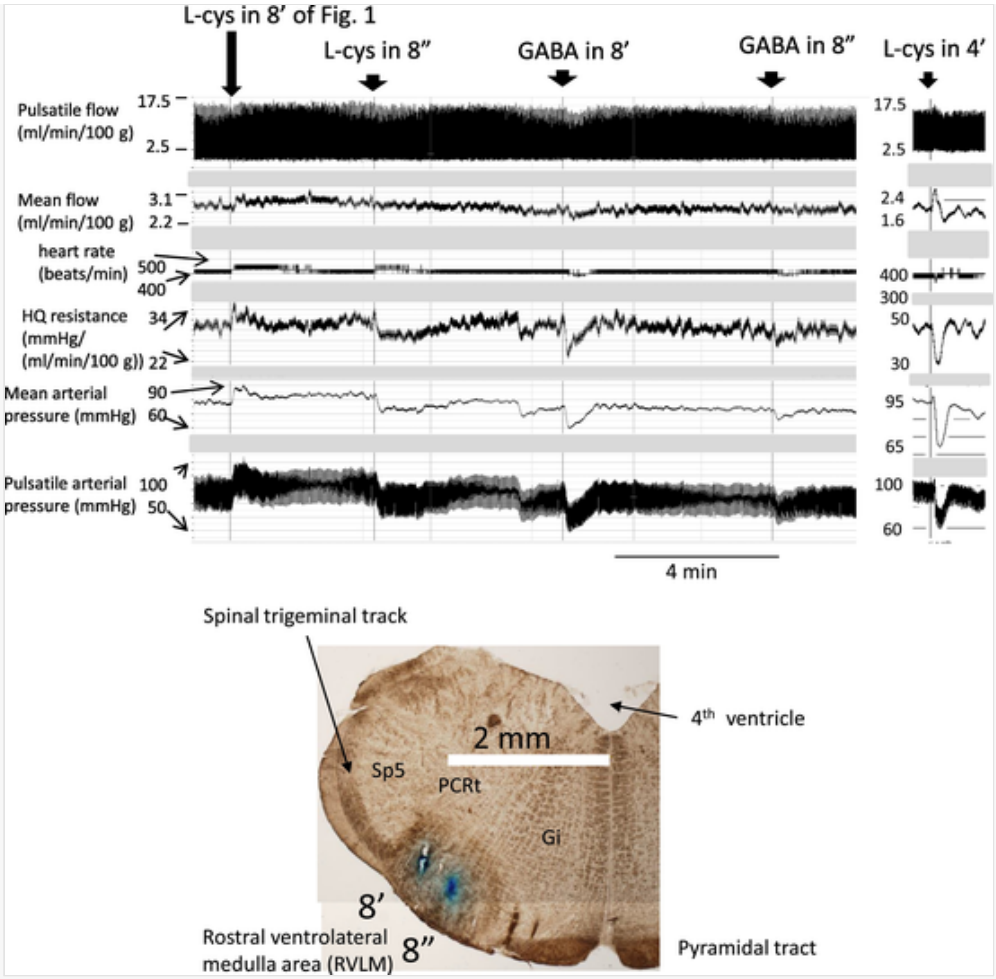


Examples of responses mapping. Changes in hindquarter flow resistance (HQR) (a) and arterial blood pressure (AP) (b) in response to L-cysteine microinjections were mapped over the surface of the ventral medulla of a rat. Circle sizes are equivalent to changes in HQR and AP as shown of values at each two sites. Recording data at sites 8′, 8″ and 4′ were shown in Fig. 2. AP level before microinjections: 87 ± 6.6 mmHg(mean ± SD, n = 46). RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve.

Hemodynamic variables recorded at sites 8′, 8″ and 4′ in Fig. 1 are shown in Fig. 2. At site 8′, L-cysteine microinjection produced increases in AP, HQR, and heart rate. At site 8″, it produced decreases in AP and HQR but an increase in heart rate, suggesting hemodynamic β2-adrenoceptor effects of adrenaline secreted by stimulation at site 8″. Additional microinjections of GABA (10 mM, 34 nl) into sites 8′ and 8″ produced decreases in all three variables, indicating that both sites were included in the RVLM. The last recording shows the typical vasodilator depressor responses to L-cysteine at site 4′ in the CVLM area. Note that site 4′ has a faster vasodilator response than site 8″.

alt-text: Fig. 2

~~Fig. 2.~~Fig. 2



Responses recordings at 8′, 8″ and 4′ in Fig. 1 and a photo of a frontal medulla slice (50 μm) marked at 8′ and 8″ with the dye. Recordings include also responses to GABA at 8′ and 8″. At 8′, arterial pressure (AP), hindquarter resistance (HQR) and heart rate were increased after L-cysteine (L-cys) microinjection, but exactly decreased after GABA microinjection. At 8″, three variables were decreased by GABA microinjections same as at 8′, but L-cysteine microinjection decreased AP and HQR and increased heart rate different from 8′. At 4′, AP and HQR were decreased. Gi: gigantocellular reticular nucleus, PCRt: parvicellular reticular nucleus, Sp5: spinal trigeminal nucleus.

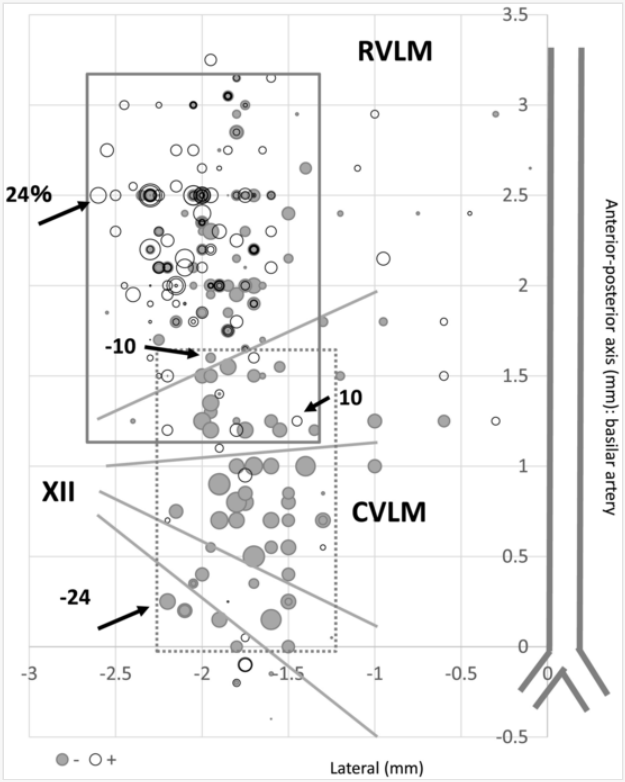
Fig. 2 shows a 50-μm-thick frontal medulla slice that includes sites 8′ and 8″ where Coomassie Brilliant Blue solution was microinjected after the experiment.

~~3.2.~~3.2 Maps summarizing the sites of response of HQR and AP to L-cysteine on the ventral medulla surface

Figs. 3 and 4 illustrate the sites on the ventral surface of the medulla of 11 rats where changes in HQR and AP, respectively, were produced by L-cysteine microinjections (total number, 316 sites). In two rats, there were no sites for depressor vasodilator responses within the RVLM vasoconstrictor area, which may have had sites for depressor vasodilator responses under vessels on the ventral surface of the medulla. The means ± SD of AP and HQR before microinjection were 93 ± 11 mmHg and 33.7 ± 12.8 mmHg/(ml/min/100 g), respectively.

alt-text: Fig. 3

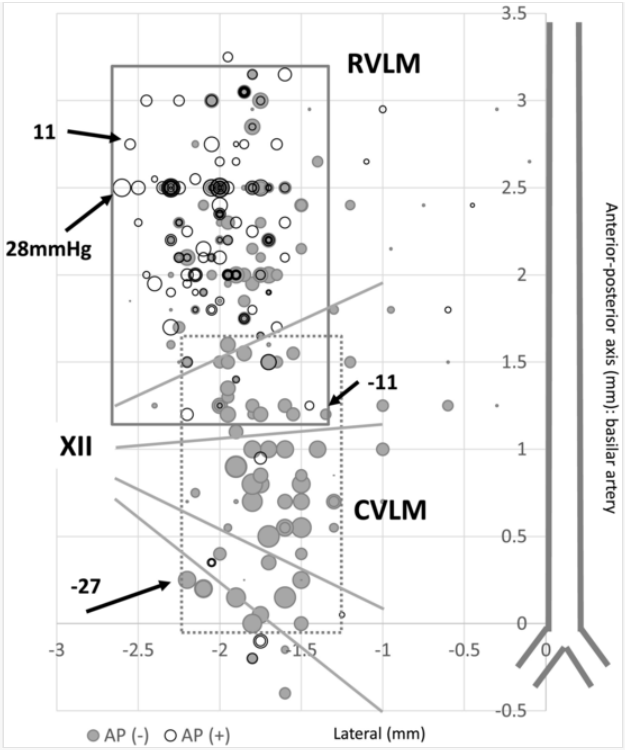
~~Fig. 3.~~Fig. 3



Mapped 316 sites for changes in hindquarters flow resistance (%) with L-cysteine microinjections over the ventral medulla surface of 11 rats. The circle size corresponds to the change in HQR as shown as representative values at the four sites. RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve.

alt-text: Fig. 4

~~Fig. 4.~~Fig. 4



Mapped sites for changes in AP (mmHg) in 11 rats with L-cysteine microinjections over the ventral medulla surface. The circle size corresponds to the change in AP as shown as representative values at the four sites. RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve. Pre-injection level: 93 ± 11 mm Hg (Mean ± SD).

The rectangles of the RVLM and CVLM areas in Figs. 3 and 4 were defined and drawn based on the data in Fig. 7 and ref. (Takemoto, 2004) where pressor and depressor responses to L-glutamate microinjections were obtained.

Within the arbitrary rectangle of the RVLM, there were 39 sites for vasodilation larger than 10% in the anterior area between 1.9 and 3.05 mm rostral to the zero point in Fig. 3 where intermingled sites for vasoconstriction were seen. The posterior RVLM and CVLM depressor areas included mainly sites for vasodilation.

The changes in AP (mmHg) and HQR (%) at 316 sites were significantly and highly correlated (r = 0.8045, p < ~~0~~ .01).

~~3.3.~~3.3 Propranolol test

To explore the possibility that peripheral adrenaline release produces muscle vasodilation via β2-adrenoceptors, L-cysteine microinjection was repeated at the RVLM vasodilator sites after β-adrenoceptor blockade with intravenous injection of dl-propranolol in seven rats after the response-mapping experiment.

Vasodilation from L-cysteine injection was abolished after propranolol treatment. The mean changes in HQR and AP in seven rats were −12% ± 7.9% and −10 ± 7.0 mmHg before propranolol treatment and 5.0% ± 6.5% and 9.8 ± 6.0 mmHg after propanol treatment, respectively. Propranolol treatment significantly abolished vasodilator depressor responses to L-cysteine microinjection into a site in the RVLM area (p < ~~0~~ .01 by paired t-test for both variables). Propranolol also significantly reduced basal preinjection AP from 94 ± 7.0

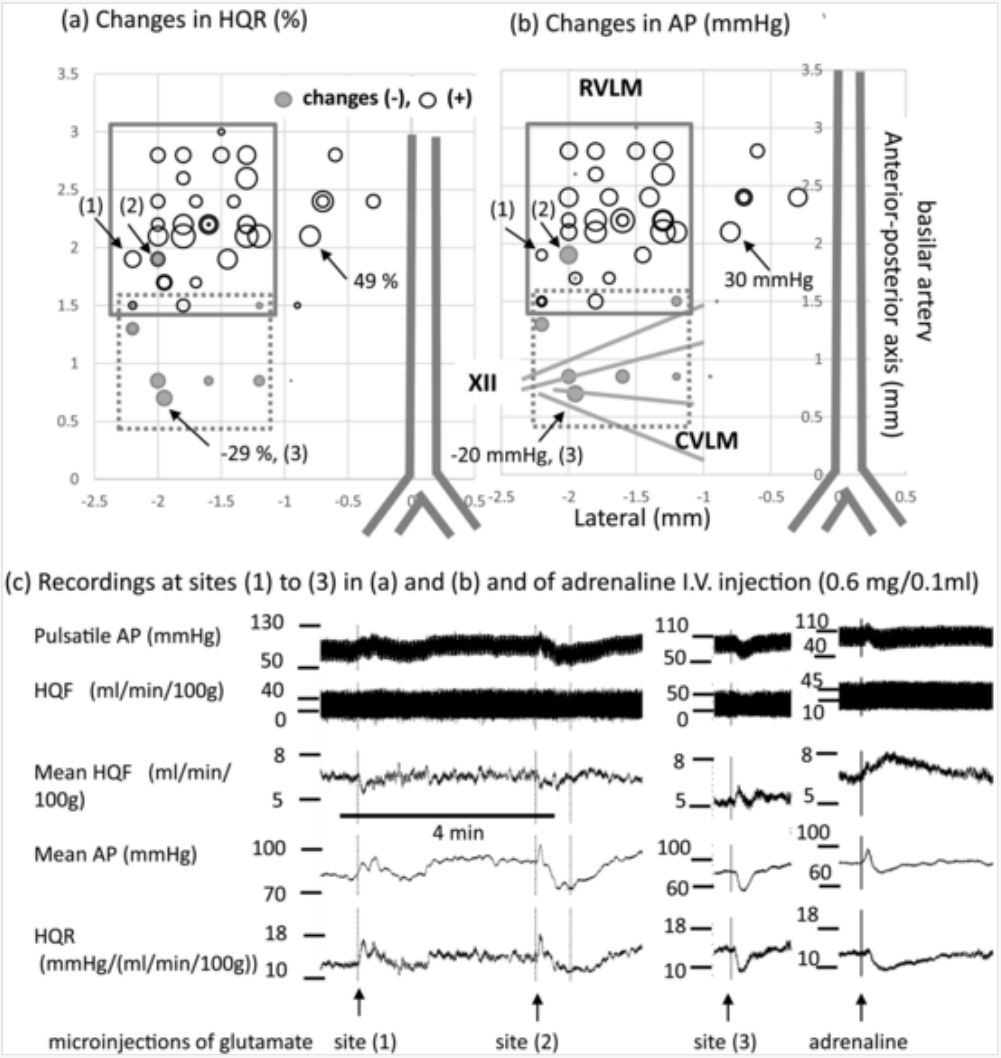
to 84 ± 6.8 mmHg (p <~~0~~.01 by paired *t*-test). The seven vasodilator sites tested were included in the RVLM area from 2.85 to 1.6 mm in the anterior–posterior direction and from −2.1 to −1.7 mm lateral to the right side of the beginning of the basilar artery.

~~3.4.~~3.4 An example of response mapping with L-glutamate in the control SAD rat

Fig. 5 shows the injection sites at which changes in HQR (a) and AP (b) were produced when L-glutamate (20 mM, 68 nl) was microinjected into the ventral medulla. Microinjections of L-glutamate in 47 sites produced different levels of vasoconstrictor or vasodilator responses (a) with corresponding changes in AP (b). The rostral part of the RVLM pressor area included mainly muscle vasoconstrictor sites and one distinct vasodilator site (site 2), and the CVLM depressor area included the vasodilator sites alone. Changes in AP (mmHg) and HQR (%) at 47 sites were highly correlated (r = 0.9043, p <~~0~~.01).

alt-text: Fig. 5

~~Fig. 5.~~Fig. 5



Examples of responses mapping in one SAD rat. Changes in hindquarter flow resistance (HQR) (a) and arterial blood pressure (AP) (b) in response to L-glutamate microinjections were mapped over the surface of the ventral medulla. Circle sizes are

equivalent to changes in HQR and AP as shown of values at each two sites. Recording data were shown in (c). AP level before microinjections: 83 ± 5.9 mm Hg (means ± SD, n = 47). RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve. HQF: hindquarters flow.

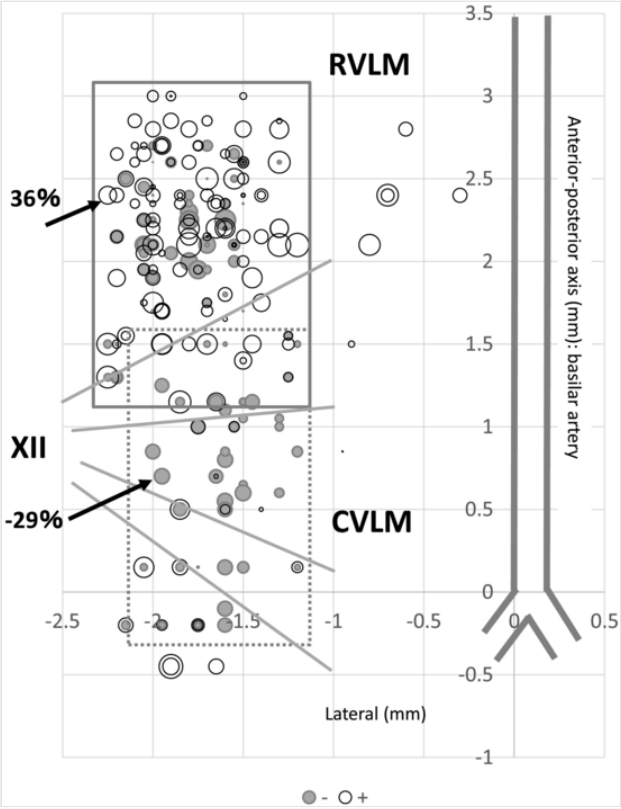
Hemodynamic variables recorded at sites 1 to 3 in Fig. 5 (a) and (b) are shown in (c). At site 1, L-glutamate microinjection produced increases in AP and HQR. At site 2, it produced a gradual decrease following a fast increase in AP and HQR which changes were similar with iv bolus injection of adrenaline (0.6 mg/0.1 ml). At site 3 in the CVLM, AP and HQR were decreased and returned to the original level faster than site 2 in the RVLM. The hemodynamic pattern at site 2 may suggest adrenaline secretion, earlier muscle vasoconstrictor effect through α1 adrenoceptor activation followed by vasodilator effect through β2-adrenoceptor effects.

RVLM pressor and CVLM depressor areas defined with AP responses to L-glutamate in SAD control rats.

Figs. 6 and 7 illustrate the sites on the ventral surface of the medulla of nine SAD rats where changes in HQR and AP, respectively, were produced by L-glutamate microinjections (total number, 246 sites). In one rat, there were no sites for depressor vasodilator responses within the RVLM vasoconstrictor area. The means ± SD of AP and HQR before microinjection were 84 ± 13 mmHg and 20.0 ± 4.0 mmHg/(ml/min/100 g), respectively.

alt-text: Fig. 6

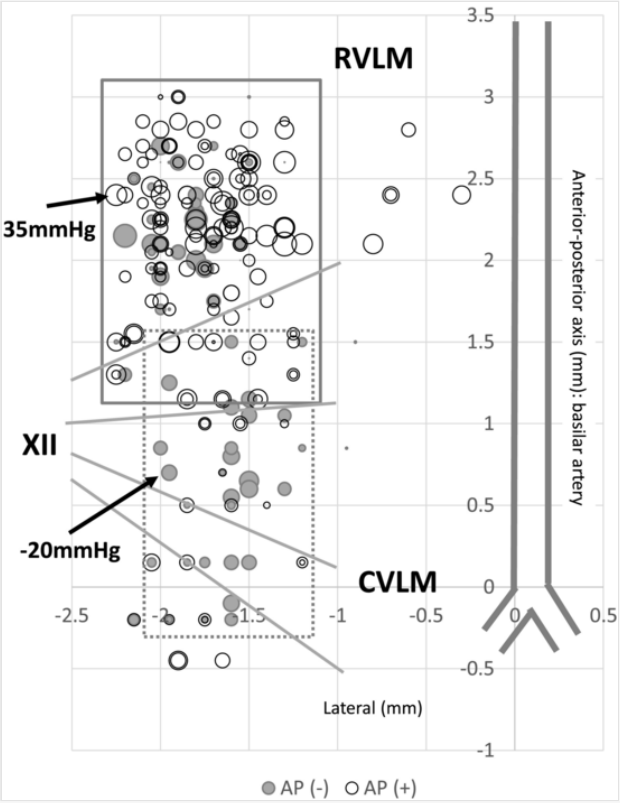
~~Fig. 6.~~Fig. 6



Mapped 246 sites for changes in hindquarters flow resistance (%) with L-glutamate microinjections over the ventral medulla surface of 9 SAD rats. The circle size corresponds to the change in HQR as shown as representative values at the four sites. RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve.

alt-text: Fig. 7

~~Fig. 7.~~Fig. 7



Mapped sites for changes in AP (mm Hg) in 9 SAD rats with L-glutamate microinjections over the ventral medulla surface. The circle size corresponds to the change in AP as shown as representative values at the four sites. RVLM: rostral ventrolateral medulla. CVLM: caudal ventrolateral medulla. XII: the twelfth cranial nerve.

The rectangles of the RVLM and CVLM in Fig. 6 were drawn based on the data where pressor and depressor responses to L-glutamate microinjections were obtained in the current SAD rats (Fig. 7) and in the previous intact rats (Takemoto, 2004).

Within the rectangles of the RVLM, there were 33 sites for vasodilation larger than 10% in the anterior area between 1.9 and 3.05 mm rostral to the zero point in Fig. 6 where intermingled area with vasoconstriction was seen. The posterior RVLM or intermediate area included also vasodilator and vasoconstrictor sites, different from the result of only vasodilation in L-cysteine (Fig. 3). The CVLM depressor areas included mainly sites for vasodilation as in L-cysteine.

~~4.~~4 Discussion

The present study originally aimed to know if there are localized sites regarding muscle vascular responses to the iEAAr activator L-cysteine stimulation within the VLM autonomic areas, since we are interested in the central nervous system mechanism of redistribution of muscle blood flow during behaviors of the rat such as

grooming and spontaneous walking (Mizuma et al., 1987; Takemoto and Iriuchijima, 1989). The RVLM adrenal (Ross et al., 1984; Strack et al., 1989; Pyner and Coote, 1998) and lumbar (Lee et al., 2007) presympathetic excitation with EAA was predicted to cause opposite effects of muscle vasodilation through β2 effect of secreted adrenaline and of vasoconstriction via lumbar sympathetic activation. However, despite the long history of adrenaline research (Verberne, 2016), there was little evidence on muscle vasodilation or adrenaline secretion with EAAr stimulation of RVLM presympathetic area in the case of rats, except one potential report using multiple EAAr activator L-glutamate (Mueller et al., 2011). Hence, we hypothesized that the iEAAr stimulation alone with L-cysteine could detect that muscle vasodilator site distinct from muscle vasoconstrictor site in the RVLM pressor area.

The results demonstrate that the iEAAr activator L-cysteine microinjection into the RVLM succeeded to detect the sites of muscle vasodilation distinct from vasoconstriction, supporting this hypothesis.

With respect to a possible role of iEAAr in RVLM adrenal presympathetic neurons or adrenaline secretion of rats, there are some indirect data by experiments monitoring HQ flow (Cravo et al., 2003) and adrenal sympathetic nerve activity (Sabetghadam et al., 2017). Namely, electrical stimulation of perifornical hypothalamus produces HQ vasodilation probably via adrenaline secretion like a defense response, but that vasodilation was completely abolished by the iEAAr blocking agent kynurenate injection into the bilateral RVLM area (Cravo et al., 2003). The same strategy using kynurenate showed the similar result in the rat stimulated with hypoglycemia or lowered plasma glucose, in which adrenal medulla is expected to secrete adrenaline to help normal glucose level restore after sensing shortage of glucose in the hypothalamus (Verberne, 2016). Hypoglycemic stimulation with a drug (2-deoxy-D-glucose) increased adrenal sympathetic nerve activity possibly followed by adrenaline secretion, but kynurenate microinjection into the bilateral RVLM abolished this evoked adrenal sympathetic nerve activity (Sabetghadam et al., 2017). Both functional experiments indirectly demonstrate that potential adrenaline secretion activated by the central neuronal circuit would be dependent on iEAAr in RVLM adrenal presympathetic neurons. The RVLM vasodilator sites in the current study might be responsible for those hypothalamus- or hypoglycemia- evoked adrenaline secretion for vascular control and glucose restoration.

When compared the vasodilator recordings in RVLM and in CVLM after L-cysteine microinjection (Fig. 2), the vasodilator change in RVLM is slower than in CVLM, suggesting a hormonal slow reaction via adrenaline secretion. Namely, the vasodilation from the iEAAr activator L-cysteine in RVLM may be ascribable to adrenal presympathetic activation. This vasodilation was completely abolished with β-adrenoceptor blocking agent dl-propranolol that effectively blocked the muscle vasodilation from iv adrenaline injection, supporting the adrenal secretion by L-cysteine. However, the non-specific β-adrenoceptor blocker of propranolol may have a central inhibitory effect on adrenaline secretion via inhibition of β1 adrenoceptors in RVLM neurons (Oshima et al., 2014). To draw conclusions, further testing may be needed using specific β2 antagonists that do not affect the central nervous system.

The arbitrary frame in the CVLM depressor area mainly includes vasodilator sites that respond to L-cysteine, but the frame in the RVLM pressor area includes many depressor vasodilator gray sites in addition to pressor vasoconstrictor sites by L-cysteine stimulation, especially in the most posterior part (Figs. 3 and 4). The

frames were drawn according to our data on L-glutamate control mapping in SAD-rats (Fig. 7) and intact rats (Takemoto, 2004). In intact rats (Takemoto, 2004) with L-glutamate which could activate both of mEAAr and iEAAr, the frames mainly included pressor responses in the RVLM and depressor responses in the CVLM. Therefore, the most posterior vasodilator part of the RVLM in Fig. 3 with L-cysteine may result from iEAAr adrenal presympathetic activation alone. In reality, one site for propranolol test in L-cysteine experiment was included in this posterior RVLM area. However, considering the variation in the anatomy of small and even large vessels in the ventral medulla of individual rats, neuroanatomical arrangements may vary in similar ways among rats. Further examination is required focusing this intermediate area of RVLM and CVLM defined with L-glutamate.

Changes in HQR induced by stimulation of L-cysteine in all the VLM autonomic areas were highly correlated with the changes in AP. This means that the hindquarter or muscle vascular bed is among the important vascular beds that directly influence AP levels with L-cysteine. Unlike the original expectation, there was no localized distribution of sites just for the muscle vascular bed in the rat RVLM presympathetic area and CVLM interneuron areas, which differed from the AP-sensitive area reflecting changes in multiple vascular beds. Rather, it suggests that iEAAr activated by L-cysteine may play an important role in the VLM muscle vascular regulation, keeping proper levels of AP.

In this study, responses mapping over the VLM using L-cysteine was performed for the first time. Thus, as mentioned above, RVLM and CVLM areas were based on pressor and depressor responses to L-glutamate, according to key references (Guyenet, 2006; Ross et al., 1984; Mueller et al., 2011; Schreihofer and Sved, 2011; Willette et al., 1983).

Unexpectedly we detected depressor and muscle vasodilator RVLM sites with L-glutamate using the SAD or no baroreflex rat in the control mapping (Figs. 5-7). Mueller et al. (Mueller et al., 2011) reported only one depressor site with activation of only adrenal sympathetic neurons, and many pressor sites with co-activation of adrenal, renal and lumbar sympathetic neurons in a narrow RVLM area located within 0.5 mm of the facial nucleus. In addition, our previous AP mapping data with L-glutamate demonstrated no RVLM depressor sites in intact rats (Takemoto, 2004). Surprisingly, the quite number of vasodilator sites was obtained with L-glutamate in SAD rats in the present control experiment. The slow depressor vasodilator responses to L-glutamate in RVLM were close to those with iv injected adrenaline, different from those in CVLM (Fig. 5), suggesting potential involvement of adrenaline secretion. Baroreflex might have been another cause of producing no or few depressor sites in the RVLM of SAD-intact rats.

Although there were distinct RVLM sites of muscle vasodilation and vasoconstriction with L-cysteine in individual rats, it showed no clear topography between both functional sites in the RVLM of all rats (Fig. 3). Rather it showed the intermingle existence of vasodilator and vasoconstrictor sites or maybe of adrenal and lumbar presympathetic neurons, in agreement with the functional evidence in cats (McAllen, 1986) and in rats (Verberne, 2016).

With regard to differential functional localization of RVLM presympathetic neurons, microinjections of EAA in cats have shown that topography such as for muscle, renal, and cardiac vascular beds (Dampney and McAllen, 1988; McAllen and Dampney, 1990; McAllen and May, 1994). However, in rats, there was no

topography between renal and splenic presympathetic neurons when activated with dl-homocysteic acid (Belluli and Weaver, 1991). Then, differential and concurrent activation of renal, lumbar and adrenal sympathetic nerves of rats were reported after RVLM stimulation with L-glutamate (Mueller et al., 2011), indicating no topography. Rather, in addition to densely mixed but separate distribution of presympathetic neurons to T2 and T8, the minor intermingled existence of the 'generalist' presympathetic neurons to coactivate multiple functional presympathetic neurons were recently identified (Farmer et al., 2019), supporting anatomical evidence of the central command neurons in rat RVLM (Jansen et al., 1995). In view of the information presented so far, the rat RVLM appears to be packed with mixed populations of multiple functional presympathetic neurons. As we observed robust blood flow shift in rat behaviors of grooming and spontaneously walking (Mizuma et al., 1987; Takemoto and Iriuchijima, 1989), fast recruitments of distinct autonomic neurons are quite important in animal daily life, keeping body homeostasis. To understand the mechanisms of control of such differential vascular beds, it may be necessary to clarify the relationship between the basic arrangements of individual vascular beds and higher centers. The present study demonstrated a possible segregation of RVLM sites of muscle vasodilation and vasoconstriction using the iEAAr activator L-cysteine. It may suggest the important role of chemicals such as receptors and neurotransmitters rather than the anatomical localization of RVLM neurons in case of rats.

With respect to the possible central role of L-cysteine, we first found its strong pressor, tachycardiac, and visceral vasoconstrictor actions when it was injected into the cisterna magna of the freely moving rat (Takemoto, 1990; Takemoto, 1995a; Takemoto, 2012). Further studies demonstrated that intracisternal L-cysteine, but not D-cysteine, also activates hypothalamic neurons releasing vasopressin into the blood (Takemoto, 2013). However, it is not clear if L-cysteine directly or indirectly produce the functional effects. A neurotransmitter candidate, L-cysteine sulfinic acid (CSA), is known to be synthesized from L-cysteine, suggesting a possible precursor role of L-cysteine. However, CSA showed a mechanism resisting the iEAAr blocker kynurenate from its depressor response in the NTS, the mechanism of which is different from that in L-cysteine (Takemoto, 2014b). Moreover, L-AP4 receptors, which are among the metabotropic EAAr, seemed to have a possible binding site for L-cysteine in the brain, according to a biochemical binding study (Pullan et al., 1987); hence, the effect was expected through L-AP4-sensitive receptors. However, their mechanisms in the CVLM were different, indicating no involvement of L-AP4-sensitive receptors in response to L-cysteine stimulation (Takemoto, 2014c). Furthermore, L-homocysteine, one of the L-cysteine homologue molecules, has hemodynamic effects similar to those of L-cysteine when microinjected into the VLM autonomic areas, but is acting through an NMDA receptor-mediated mechanism (Takemoto, 2016), different from that in L-cysteine. These pieces of evidence suggest a strict requirement at the molecular level for functional actions of L-cysteine in the central nervous system. What the present study added is a functional role of L-cysteine to regulate the muscle vascular bed in RVLM and CVLM of rats leading changes in AP. The general multiple functions of L-cysteine as precursors for glutathione, hydrogen sulfide, proteins, reductants, and others in body homeostasis and as a neurotoxin (Janáky et al., 2000) are quite similar to the wide range of functions of the authentic neurotransmitter L-glutamate in the body, suggesting a potential endogenous neuromodulator or neurotransmitter role of L-cysteine (Janáky et al., 2000) especially in iEAAr (Takemoto, 2014a; Takemoto, 2014b; Takemoto, 2014c).

The iEAAr in the RVLM and CVLM autonomic neurons are known to play a key role in responses to recruitments of autonomic reflexes and/or autonomic central circuits (Koshiya et al., 1993; Kiely and Gordon, 1994; Zanzinger et al., 1994; McCulloch et al., 1999; Zhou et al., 2006; Schreihofer and Sved, 2011; Sabetghadam et al., 2017; Sun and Guyenet, 1985; Miyawaki et al., 1997; Sved et al., 2000; Verberne et al., 1989; Miyawaki et al., 1996; Mandel and Schreihofer, 2009; Verberne and Guyenet, 1992; Peng et al., 2000; Sartor and Verberne, 2003; Mobley and Schreihofer, 2006; Holstein et al., 2014; Owens and Verberne, 2000; Yang and Coote, 1999; Sun and Penneton, 2005; Guyenet, 2011). In detail, iEAAr are divided into two types receptors: NMDA receptors, and non-NMDA receptors that include AMPA receptors and kainate receptors. Interestingly, a lot of such cardiovascular or sympathetic responses are eliminated by iEAAr antagonism. Microinjection of antagonists such as the broad type kynurenate or NMDA/ non-NMDA types into the RVLM abolished chemoreceptor pressor reflex (Koshiya et al., 1993), the somatic pressor reflex (Kiely and Gordon, 1994), the somato-sympathetic reflex (Zanzinger et al., 1994), the nasopharyngeal reflex (McCulloch et al., 1999), and the gall bladder-stimulated pressor reflex (Zhou et al., 2006). The same strategy in the CVLM abolished the baroreflex (Sun and Guyenet, 1985; Miyawaki et al., 1997; Sved et al., 2000), a vagal cardiopulmonary reflex (Verberne et al., 1989), cardiorespiratory coupling (Miyawaki et al., 1996), hypoxia (Mandel and Schreihofer, 2009) or Bezold-Jarisch reflex (Verberne and Guyenet, 1992), depressor responses to the splanchnic nerve afferent and CCK stimulations (Peng et al., 2000; Sartor and Verberne, 2003; Mobley and Schreihofer, 2006), and vestibulo-sympathetic reflex (Holstein et al., 2014). The medial prefrontal area (Owens and Verberne, 2000), the paraventricular nucleus (Yang and Coote, 1999), the caudal pressor area (Sun and Penneton, 2005) and the respiratory pattern generator (Guyenet, 2011) also send information to the CVLM neurons but iEAAr antagonism here abolished their responses to the corresponding activation. A body of evidence indicates a critical role of iEAAr in regulating autonomic homeostasis at the brain level.

The typical EAA neurotransmitter L-glutamate has been often used as the chemical stimulant, stimulating both of mEAAr and iEAAr. However, it may be unsuitable for an iEAAr stimulant in above mentioned studies, considering additional stimulation of mEAAr which may produce unpredictable responses. In reality, microinjection of L-AP4, one of synthetic mEAA, into rat CVLM (Takemoto, 2014c) and RVLM (Tsuchihashi et al., 2000), produced changes in AP the same as L-glutamate, meaning responses to L-glutamate stimulation cannot be ascribable to iEAAr activation alone. In that regard, interpretation would become simple and helpful if the natural iEAAr activators such as L-cysteine were used as a chemical tool, as shown in the current study.

In conclusion, using iEAAr activator L-cysteine, muscle vasodilator responding sites possibly from adrenaline secretion were obtained in rat RVLM pressor area distinct from vasoconstrictor sites, although those vasodilator sites have been long missed. The muscle vascular response to iEAAr activated by L-cysteine in rat VLM parallels the AP response, suggesting a role of iEAAr in the VLM in muscle vascular regulation. It also shows that the potential neuromodulator L-cysteine could be a useful chemical tool as a stimulant for functional studies which need stimulation of iEAAr alone.

Acknowledgments

This work was supported by JSPS Kakenhi grant number JP18K06854.

~~Conflict~~ Declaration of competing interest

The author declares that there are no conflicts of interest.

References

The corrections made in this section will be reviewed and approved by journal production editor.

Belluli, D.J., Weaver, L.C., 1991. Differential control of renal and splenic nerves without medullary topography. ~~Am J Physiol~~Am. J. Phys. 260, H1072–H1079.

Cravo, S.L., Possas, O.S., Ferreira-Neto, M.L., 2003. Rostral ventrolateral medulla: an integrative site for muscle vasodilation during defense-alerting reactions. ~~Cell Mol Neurobiol~~Cell. Mol. Neurobiol. 23, 579–595.

Dampney, R.A.L., 2016. Central neural control of the cardiovascular system: current perspectives. ~~Adv Physiol Educ~~Adv. Physiol. Educ. 40, 283–296.

Dampney, R.A., McAllen, R.M., 1988. Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. ~~J Physiol~~J. Physiol. 395, 41–56.

Farmer, D.G.S., Pracejus, N., Dempsey, B., Turner, A., Bokiniec, P., Paton, J.F.R., Pickering, A.E., Burguet, J., Andrey, P., Goodchild, A.K., McAllen, R.M., McMullan, S., 2019. ~~J Physiol~~J. Physiol. 597, 3407–3423.

Guyenet, P., 2006. The sympathetic control of blood pressure. ~~Nat Rev Neurosci~~Nat. Rev. Neurosci. 7, 335–346.

Guyenet, P.G., 2011. Cardiorespiratory integration. In: Llewellyn-Smith, I.J., Verberne, A.J.M. (Eds.), Central Regulation of Autonomic Functions, 2nd ed. Oxford University Press, New York, pp. 180–201.

Holstein, G.R., Friedrich, V.L., ~~Jr.~~Jr., Martinelli, G.P., 2014. Projection neurons of the vestibulo-sympathetic reflex pathway. ~~J Comp Neurol~~J. Comp. Neurol. 15, 2053–2074.

Janáky, R., Varga, V., Hermann, A., Saransaari, P., Oja, S.S., 2000. Mechanisms of L-cysteine neurotoxicity. ~~Neurochem Res~~Neurochem. Res. 25, 1397–1405.

Jansen, A.S.P., Van Nguyen, X., Karpitskiy, V., Mettenleiter, T.C., Loewy, A.D., 1995. ~~Central command neurons of the sympathetic nervous system: Basis of the fight-or-flight response~~Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 270, 253–260.

Kiely, J.M., Gordon, F.J., 1994. Role of rostral ventrolateral medulla in centrally mediated pressor responses. ~~Am J Physiol~~Am. J. Phys. 267, H1549–H1556.

Koshiya, N., Huangfu, D., Guyenet, P.G., 1993. Ventrolateral medulla and sympathetic chemoreflex in the rat. ~~Neurosci Lett~~Neurosci. Lett. 232, 103–106.

Lee, T.-K., Lois, J.H., Troupe, J.H., Wilson, T.D., Yates, B.J., 2007. Transneuronal tracing of neural pathways that regulate hindlimb muscle blood flow. ~~Am J Physiol~~Am. J. Phys. 292, R1532~~–1541~~R1541.

Mandel, D.A., Schreihofer, A.M., 2009. Modulation of the sympathetic response to acute hypoxia by the caudal ventrolateral medulla in rats. ~~J Physiol~~J. Physiol. 587, 461–475.

McAllen, R.M., 1986. Action and specificity of ventral medullary vasopressor neurones in the cat. Neurosci 18, 51–59.

McAllen, R.M., Dampney, R.A., 1990. Vasomotor neurons in the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed bot not body region. ~~Neurosci Lett~~Neurosci. Lett. 10, 91–96.

McAllen, R.M., May, C.N., 1994. Differential drives from rostral ventrolateral medullary neurons to three identified sympathetic outflows. ~~Am J Physiol~~Am. J. Phys. 267, R935~~–944~~R944.

McCulloch, P.F., Panneton, W.N., Guyenet, P.G., 1999. The rostral ventrolateral medulla mediates the sympathoactivation produced by chemical stimulation of the rat nasal mucosa. ~~J Physiol (Lond)~~J. Physiol. Lond. 516 (Pt 2), 471–483.

Miyawaki, T., Minson, J., Arnolda, L., Chalmers, J., Llewellyn-Smith, I., Pilowsky, P., 1996. Role of excitatory amino acid receptors in cardiorespiratory coupling in ventrolateral medulla. ~~Am J Physiol~~Am. J. Phys. 271, R1221–R1230.

Miyawaki, T., Suzuki, S., Minson, J., Arnolda, L., Chalmers, J., Llewellyn-Smith, I., Pilowsky, P., 1997. Role of AMPA/Kainate receptors in transmission of the sympathetic baroreflex in rat CVVLM. ~~Am J Physiol~~Am. J. Phys. 272, R800–R812.

Mizuma, E., Takemoto, Y., Iriuchijima, J., 1987. Redistribution of cardiac output during grooming of the rat. Jpn J Physiol 37, 49–57.

Mobley, S.C., Schreihofer, A.M., 2006. Systemic cholecystokinin differentially affects baro-activated GABAergic neurons in rat caudal ventrolateral medulla. ~~J Neurophysiol~~J. Neurophysiol. 96, 2760–2768.

Mueller, P.J., Mischel, N.A., Scislo, T.J., 2011. Differential activation of adrenal, renal, and lumbar sympathetic nerves following stimulation of the rostral ventrolateral medulla of the rat. ~~Am J Physiol~~Am. J. Phys. 300, R1230~~–1240~~R1240.

Oshima, N., Onimaru, H., Yamamaoto, K., Takechi, H., Nishida, Y., Oda, T., Kumagai, H., 2014. Expression and functions of β1- and β2-adrenergic receptors on the bulbospinal neurons in the rostral ventrolateral medulla. ~~Hypertens Res~~Hypertens. Res. 37, 976–983. doi:10.1038/hr.2014.112.

Owens, N.C., Verberne, A.J.M., 2000. Medial prefrontal depressor response: involvement of the rostral and caudal ventrolateral medulla in the rat. J Auton Nerv SystJ. Auton. Nerv. Syst. 78, 86–93.

Peng, Y.J., Wang, N., Gong, Q.L., Li, P., 2000. Caudal ventrolateral medulla mediates the depressor response elicited by the greater splanchnic nerve afferent stimulation in rats. Neurosci LettNeurosci. Lett. 325, 134–138.

Pullan, L.M., Olney, J.W., Price, M.T., Compton, R.P., Hood, W.F., Michel, J., Monahan, J.B., 1987. Excitatory amino acid receptor potency and subclass specificity of sulfur-containing amino acids. J NeurochemJ. Neurochem. 49, 1301–1307.

Pyner, S., Coote, J.H., 1998. Rostroventrolateral medulla neurons preferentially project of target-specified sympathetic preganglionic neurons. Neurosci 83, 617–632.

Ross, C.A., Ruggiero, D.A., Joh, T.H., Park, D.H., Reis, D.J., 1984. Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. J Comp NeurolJ. Comp. Neurol. 228, 168–185.

Sabetghadam, A., Korim, W.S., Verberne, A.J.M., 2017. Perifornical hypothalamic pathway to the adrenal gland: role for glutamatergic transmission in the glucose counter-regulatory response. Auton NeurosciAuton. Neurosci. 203, 67–73.

Sartor, D.M., Verberne, A.J., 2003. Phenotypic identification of rat rostroventrolateral medullary presympathetic vasomotor neurons inhibited by exogenous cholecystokinin. J Comp NeurolJ. Comp. Neurol. 465, 467–479.

Schreihofer, A.M., Sved, A.F., 2011. The ventrolateral medulla and sympathetic regulation of arterial pressure. In: Llewellyn-Smith, I.J., Verberne, A.J.M. (Eds.), Central Regulation of Autonomic Functions, 2nd ed. Oxford University Press, New York, pp. 78–97.

Strack, A.M., Sawyer, W.B., Hughes, J.H., Platt, K.B., Loewy, A.D., 1989. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral injections. Brain ResBrain Res. 491, 156–162.

Sun, M.-K., Guyenet, P.G., 1985. GABA-mediated baroreceptor inhibition of reticulospinal neurons. Am J PhysiolAm. J. Phys. 249, R672–R680.

Sun, W., Penneton, W.M., 2005. Defining projections from the caudal pressor area of the caudal ventrolateral medulla. J Comp NeurolJ. Comp. Neurol. 482, 273–293.

Sved, A.F., Ito, S., Madden, C.J., 2000. Baroreflex dependent and independent roles of the caudal ventrolateral medulla in cardiovascular regulation. Brain Res BullBrain Res. Bull. 51, 129–133.

Takemoto, Y., 1990. Amino acids with central pressor effect in conscious rats. Jpn J Physiol 40, 561–565.

Takemoto, Y., 1995. The central effect of L-cysteine on cardiovascular system of the conscious rat. Jpn J Physiol 45, 771–783.

Takemoto, Y., 1995. Regional hemodynamic changes and vasopressin release induced by central injection of L-proline in the conscious rat. Jpn J Physiol 45, 743–758.

Takemoto, Y., 2004. L-Proline microinjected into the rat ventrolateral medulla induces a depressor response distinct from L-glutamate. Jpn J Physiol 54, 339–345.

Takemoto, Y., 2012. Amino acids that centrally influence blood pressure and regional blood flow in conscious rats. ~~J Amino Acids~~J. Amino Acids 2012 (831759), 1–14. (doi: 10.1155-2012/831759).

Takemoto, Y., 2013. Pressor response to L-cysteine injected into the cisterna magna of conscious rats involves recruitment of hypothalamic vasopressinergic neurons. Amino Acids 44, 1053–1060.

Takemoto, Y., 2014. Functional cardiovascular action of L-cysteine microinjected into pressor sites of the rostral ventrolateral medulla of the rat. Amino Acids 46, 863–872. doi:10.1007/s00726-013-1651-5.

Takemoto, Y., 2014. Cardiovascular actions of L-cysteine and L-cysteine sulfinic acid in the nucleus tractus solitarius of the rat. Amino Acids 46, 1707–1713. doi:10.1007/s00726-014-1733-z.

Takemoto, Y., 2014. ~~L-Cysteine and L-AP4 microinjections in the rat caudal ventrolateral medulla decrease arterial blood pressure~~L-cysteine and L-AP4 microinjections in the rat caudal ventrolateral medulla decrease arterial blood pressure. Auton. Neurosci. 186, 45–53. doi:10.1016/j.autneu.2014.09.018.

Takemoto, Y., 2016. Central cardiovascular actions of L-homocysteine microinjected into ventrolateral medullary autonomic areas of the rat. Amino Acids 48, 2215–2225.

Takemoto, Y., Iriuchijima, J., 1989. Blood flow redistribution during spontaneous wheel walk of the rat. Jpn J Physiol 39, 847–855.

Tsuchihashi, T., Liu, Y., Kagiyama, S., Matsumura, K., Abe, I., Fujishima, M., 2000. Metabotropic glutamate receptor subtypes involved in cardiovascular regulation in the rostral ventrolateral medulla of rats. ~~Brain Res Bull~~Brain Res. Bull. 52, 279–283.

Verberne, A.J.M., 2016. Adrenaline: insights into its metabolic roles in hypoglycaemia and diabetes. Brit J Pharmacol 173, 1425–1437.

Verberne, A.J.M., Guyenet, P.G., 1992. Medullary pathway of the Bezold-Jarisch reflex in the rat. ~~Am J Physiol~~Am. J. Phys. 263, R1195–R1202.

Verberne, A.L.M., Beart, P.M., Louis, W.J., 1989. Excitatory amino acid receptors in the caudal ventrolateral medulla mediate a vagal cardiopulmonary reflex in the rat. ~~Exp Brain Res~~Exp. Brain Res. 78, 185–192.

Willette, R.N., Barcas, P.P., Krieger, A.J.K., Sapru, H.N., 1983. Vasopressor and depressor areas in the rat medulla. Neuropharmacol 22, 1071–1079.

Yang, Z., Coote, J.H., 1999. The influence of the paraventricular nucleus on baroreceptor dependent caudal ventrolateral medullary neurons of the rat. Pfluegers Arch 438, 47–52.

Zanzinger, J., Czachurski, J., Offner, B., Seller, H., 1994. Somato-sympathetic reflex transmission in the ventrolateral medulla oblongata: special organization an receptor types. ~~Brain Res~~Brain Res. 656, 353–358.

Zhou, W., Fu, L.W., Tjen-A-Looi, S.C., Guo, Z.L., Longhurst, J.C., 2006. Role of glutamate in a visceral sympathoexcitatory reflex in rostral ventrolateral medulla of cats. ~~Am J Physiol~~Am. J. Phys. 291, H1309–H1318.

Highlights

- L-Cysteine in rat RVLM and CVLM changed muscle blood flow
- RVLM produced both muscle vasoconstriction and vasodilation
- CVLM produced muscle vasodilation alone
- Muscle vasodilation in RVLM was blocked by intravenous propranolol
- L-Cysteine in RVLM may activate muscle/adrenaline presympathetic neurons

Queries and Answers

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact n.bhaskaran@elsevier.com immediately prior to returning your corrections.

Answer: Yes

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order, and please carefully verify the spelling of all authors' names.

Answer: Yes

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

Answer: Yes

Query: Have we correctly interpreted the following funding source(s) and country names you cited in your article: "JSPS".

Answer: Japan Society for the Promotion of Science (JSPS), Grant-in-Aid for Scientific Research (Kakenhi), grant number JP18K06854