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Spike Responses of Sympathetic Vertebral Nerve Activities during Stimulation of the Pressor Dorsomedial and Rostral Ventrolateral Medulla Differ in Cats

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Abstract

In this study, we developed a differential integrator unit containing a window discriminator (WPI-121) and three integrators (sample/hold, Gould) to characterize the difference in spikes involved in sympathetic vertebral nerve activity (VNA) and integrated VNA (Int.VNA) during stimulation of the dorsomedial (DM) and rostral ventrolateral (RVLM) medulla. The upper level (UL) in the window discriminator was set at a value, 80% of the VNA amplitude above the zero. The low level (LL) was set at a value 10% above the zero. The number of VNA spikes above the UL in the window discriminator was defined as F*a*, while the number of VNA spikes within the UL and LL as F*w*. In 26 cats anesthetized with urethane and α-chloralose, results showed that electrical stimulation and microinjections of glutamate (Glu, 0.25 M, 50 nl) or glycine (Gly, 1.0 M, 50 nl) at the same point in DM or RVLM increased both systemic arterial pressure (SAP) and Int.VNA. However, the increase of Int.VNA on DM stimulation was contributed by increases of both F*a* and F*w*, while on RVLM stimulation the increase of Int.VNA was mainly contributed by the increase of F*a*, against a decrease of F*w*. As compared to Glu, stimulation of DM by Gly produced more increase in F*w*, while stimulation of RVLM by Gly produced more decrease in F*w*. During RVLM stimulation the VNA was usually transferred to a huge synchronized oscillation. These findings suggest that although stimulations of the sympathetic-pressor neurons in DM and RVLM produce similar increases in SAP and VNA, the nature of these neurons is different.

Key Words : glutamate, sympathetic vertebral nerve activity, rostral ventrolateral medulla, dorsomedial medulla, pressor area

Introduction

It has been well established that the rostral ventrolateral medulla (RVLM) plays a very important role in the maintenance of sympathetic tone and systemic arterial pressure (SAP) (15, 31). However, in recent years there has been increasing evidence showing that sympathetic neurons in the pressor area of dorsomedial medulla (DM) share the basal functions of cardiovascular integration (7, 27). Evidence includes: (i) Microinjection in the DM with various chemicals, i.e., glutamate (Glu), angiotensin II (10) and glycine (Gly) (35) in cats (7, 27, 8), and Glu in rats (37) and rabbits (19, 21), increases the systemic arterial pressure (SAP) similarly to that of microinjection in RVLM. (ii) Lesioning sympathetic preganglionic neurons in DM also produces a fall in the resting SAP although the extent of reduction is less than that of lesioning the RVLM (26). (iii) Neurons in DM have direct projection to the thoracolumbar intermediolateral cell column in the spinal cord (32, 22). (iv) In the pressor areas of DM and RVLM, there also co-exist neural mechanisms which integrate and/or modulate non-cardiovascular functions, i.e., visceral urinary-bladder motility (11), somatic spinal reflex (33) and visceral and somatic respiratory responses (28).

Besides monitoring the SAP change, measuring the integrated sympathetic vertebral nerve activity (Int.VNA) is also an essential procedure to study

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functions of the pressor DM and RVLM. The value of Int.VNA is based on the firing rate and the amplitude of the nerve spike. In a previous study, we have shown that in most of the cases, microinjections of Glu and Gly into the same pressor area of DM and RVLM produced similar increases in the amplitude of SAP and Int.VNA. The onset latency in Gly, however, was about 3 sec longer than that of Glu (35). In a recent study, we used Glu and Gly antagonists to study the mechanism of these agents. We found that Gly increased SAP and VNA through inhibition of the strychnine-sensitive inhibitory neurons. Gly might also act through the strychnine-insensitive site of the N-methyl-D-aspartate (NMDA) to potentiate the NMDA-induced SAP and VNA increases (36). However, in these investigations we simply used an integrator to study the Int.VNA. We have not found any difference in the VNA between DM and RVLM following Glu and Gly activation.

The window discriminator is designed and becomes a useful tool to discriminate nerve activities (spikes) from simultaneously recorded smaller number of fibers or neurons, or to eliminate the stimulus artifact through its preset upper level (UL) and low level (LL). In this study, we developed a differential integrator unit that contained a window discriminator (WPI-121) and three integrators (Gould) on-line real time to quantify, classify and integrate the sympathetic VNA during electrical stimulation or chemical injection to the pressor areas of DM and RVLM. In this study, we defined F*a* as the number of spikes of VNA per second recorded above the UL, and F*w* as the number of spikes of VNA per second recorded within the UL and LL. With such a design, we counted the number of spikes above the UL and within the LL and UL individually.

Results show that the VNA responses following electrical stimulation or chemical injection on DM and RVLM are significantly different in F*a* and F*w*. This may suggest that the nature of sympathoexcitatory neurons in DM and RVLM is different.

Material and Methods

General Procedure

Experiments were performed on twenty-six cats of either sex, weighing 2.5 ~ 4.0 kg. Each animal was anesthetized intraperitoneally with α-chloralose (40 mg/kg) and urethane (400 mg/kg) and paralyzed with gallamine triethiodide (2 mg/kg/30 min). The general procedures of experimentation have been described previously (35). These include monitoring of the SAP, mean SAP (MSAP), heart rate (HR), cannulation of the right femoral vein for infusion, intubation of the trachea for artificial ventilation with the end-expiratory CO_2 maintained at 4%, and the rectal temperature held at 37.5 °C. All recordings were made on a recorder (Gould ES-1000).

Neural Recordings

The left sympathetic vertebral nerve was exposed along its origin from the stellate ganglion as previously described (35). The nerve, cut at its distal end and desheathed, was hooked by a bipolar platinum electrode that was connected to a preamplifier (WPI DAM 60, bandpass frequency 3 Hz ~ 3 kHz) to amplify the whole-bundle nerve activities. The amplified signals of VNA were sent to a window discriminator (WPI-121). The latter has three output terminals; above, within and multiplex. In this study, we used two output terminals; above and within. The upper level (UL) of the window discriminator was set at a value 80% of the VNA amplitude, while the low level (LL) was set at 10% VNA amplitude above the baseline in resting condition (Fig. 1). Signals smaller than LL were considered to be background noise and were omitted. Spikes larger than UL, or within LL and UL were individually converted to a transistor-transistor logical (TTL) pulse (5 volt, 1 ms) by the window discriminator, and then integrated by an integrator (sample/hold, Gould) with a reset time of 1 s. The number of spikes above UL was defined as F*a* (spike/s), while the number of VNA spikes located within LL and UL was defined as F*w* (spike/s). The absolute values in F*w* and F*a* were calibrated by a series of pulses (5 volt, 1 ms) generated from a function generator (TEK., FG 507). The VNA was rectified and integrated by an integrator (sample/hold, Gould) with a reset time of 1 s and defined as Int.VNA. All data were stored on a tape recorder

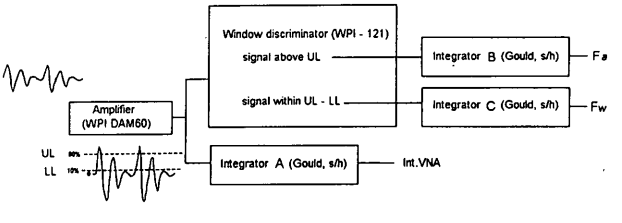

Fig. 1. Schematic diagram using a differential integrator unit to analyze the sympathetic vertebral nerve activity (VNA).

VNA signals (left plane) were passed through an amplifier (DAM60, WPI) and a window discriminator (WPI-121). Part of the signals coming from the amplifier was passed through the integrator A (s/h, Gould) and was rectified and integrated to become the Int.VNA. Part of the signals coming from the amplifier was passed through a window discriminator (WPI-121), in which the UL and LL were preset based on the nature of VNA within the window discriminator. Signals above the preset UL were channeled to an integrator B (s/h, Gould) to become F*a*. Signals within the range of LL and UL were channeled to integrator C (s/h, Gould) to become F*w*.

system (Neuro Data DR-890, Sony slv-400) for later analysis.

Brain Stimulation

The brain was explored and stereotaxically stimulated by a David - Kopf stereotaxic apparatus as described previously (35). Two areas in the medulla were stimulated, namely; DM and RVLM. The stereotaxic coordinates of these structures were: DM, 3.0 to 4.0 mm rostral to the obex, 1.5 to 2.5 mm lateral to the midline and 1.5 to 2.5 mm ventral to the dorsal surface of the medulla; RVLM, 4.0 to 5.0 mm rostral to the obex, 3.7 to 4.5 mm lateral to the midline and 3.7 to 4.5 mm ventral to the dorsal surface of the medulla.

Stimulations were delivered through a set of three-barrel glass micropipetts (outside tip diameter, 40 - 50 μm), mounted on a carrier of the stereotaxic apparatus (David Kopf) (34). The micropipette for brain stimulation was set at an angle of 34 degrees. Among the three barrels, one containing 3.0 M saline was connected to an isolation unit of a rectangular pulse generator (Grass S-88) through a piece of silver wire for electrical stimulation. The others, containing sodium glutamate (Glu, 0.25 M) and glycine (Gly, 1.0 M) in artificial CSF (aCSF) (12) with 1% pontamine sky blue, at pH 7.4, were connected separately each to a pneumatic pressure system (miniframe model PPS-2, PPM-2) through a piece of PE 50 plastic tubing for microinjection. Control injection of vehicle (aCSF) did not produce any discernible effect. Correct localization of cardiovascular site for activation was identified first by electrical stimulation (100 μA, 1.0 ms, 80 Hz and 15 s) and then confirmed by microinjections of Glu and Gly at the same point. Injection (50 nl) was accomplished in 10 to 15 s under visual guide through a microscope (Wild M650) with a reticule. Such injection procedures produced a stain of 100 - 300 μm in diameter as verified histologically.

Histology

At the end of each experiment, the animal was sacrificed with an overdose of pentobarbital. The brain was removed and immersed in 10% formalin-saline for 8 hrs. The chemical injection sites were verified using two consecutive frozen sections at 50 μm thickness, one stained with cresyl violet for identification of nuclei, and the other unstained for determination of the stimulating site and dye diffusion.

Data Analysis

Percentage changes of MSAP, F*w*, F*a* and Int.VNA in response to electrical, Glu and Gly stimulations were calculated with the following formula: (response value - control value) ÷ (control value) × 100%. Data are presented as means±SEM. Significant changes were determined by the Student's *t*-test and one-way ANOVA. Changes were considered significant when the *P* value was less than 0.05.

Results

General Description

Activities of vertebral nerve were analyzed and measured by a differential integrator unit designed as shown in Fig. 1. The changes of SAP, Int.VNA and the F*a* and F*w* of VNA following electrical, Glu and Gly stimulations on DM and RVLM are compared and summarized in Table 1, and illustrated in Figs. 2-3. Twenty-four points in DM and 22 points in RVLM responded to electrical stimulation and microinjections of Glu and Gly by increases of SAP and Int.VNA. At these points, electrical stimulation often produced a greater SAP rise in DM than in RVLM. In contrast, Glu usually produced a greater rise in SAP in RVLM than in DM. The present study counted only those results with changes in SAP or VNA larger than 5% of their controls. Under resting conditions, the averaged values of F*a* and F*w* were 12 and 73, respectively.

Changes of Fw and Fa of the VNA during DM and RVLM stimulations

A. DM

Electrical stimulation in 24 DM points produced increases in F*w* (95.8%) and F*a* (294.2%) as listed in Table 1. In 19 of the 24 DM points, microinjection of Glu increased both F*w* (74.5%) and F*a* (280.7%). Five of the 24 points produced decrease in Int.VNA associated with an increase in F*w* (22.4%) and a decrease in F*a* (27.1%). In 18 of the 24 DM points, microinjection of Gly increased both F*w* (128.8%) and F*a* (112.3%). Six of the 24 points produced decrease in Int.VNA associated with an increase in F*w* (21.3%) and a decrease in F*a* (29.2%). The points inducing a decrease of Int.VNA were found close to the region near the nucleus of tractus solitarius and dorsal motor nucleus. Figure 2 shows an example of changes in SAP and Int.VNA under electrical stimulation and microinjections of Glu and Gly on DM. In this cat, the values of F*w* and F*a* under resting conditions were 55 and 12 spikes/s, respectively. The sum of F*w* and F*a* was 67 spikes/s. During electrical stimulation SAP increased 36.4% and Int.VNA increased 355.7% (Fig. 2A). The F*w* was increased from 55 to 110 spikes/s, or increased 100%, while the F*a* change was from 12 to 54 spikes/s, or increased 350%. The sum of F*w* and F*a* increases was 164

Table 1. Effects of Electrical (E) and Chemical (Glu and Gly) Stimulations on the Pressor Areas of DM and RVLM.

Brain Stimulation		MSAP increase (%) (n)	Int.VNA (%) (n)	Fw (%)	Fa (%)
DM	E	47.2±13.4 (24)	273.5±56.7 (24)	95.8±33.8	294.2±64.2
	Glu	41.3±10.4 (24)	266.5±78.5 (19)	74.5±26.7	280.7±46.3
			-29.8±8.1 (5)	22.4±20.4	-27.1±4.5
	Gly	32.6±11.2 (24)	223.1±68.6 (18)	128.8±33.8[b]	112.3±24.5[a,b]
			-37.5±20.7 (6)	21.3±11.8	-29.2±7.2
RVLM	E	42.3±12.5 (22)	295.3±74.3 (22)	-67.5±22.7*	344.2±73.3
	Glu	55.4±23.7 (22)	275.4±84.7 (18)	-45.8±18.2*	315.7±65.4
			-23.1±4.1 (4)	-10.8±8.5*	-18.5±7.1*
	Gly	45.7±25.8 (22)	285.5±48.2 (17)	-62.5±26.7*	28.2±82.3*
			-22.5±3.5 (5)	-5.1±2.5*	-12.2±9.1*

Data showing changes of SAP, Int.VNA, and Fw and Fa in percentage (%) against their own control, during electrical stimulation or after microinjection of Glu or Gly in the pressor areas of DM and RVLM.

Abbreviations: DM: dorsomedial medulla; RVLM: rostral ventrolateral medulla; MSAP: mean systemic artierial pressure (mmHg); E: electrical stimulation; Glu: glutamate; VNA: vertebral nerve activity; Int.VNA: integrated vertebral nerve activity; Fw: the number of spikes of the VNA within LL and UL; Fa: the number of spikes of the VNA above the UL. - indicates decrease. All values are mean±S.E.M. (n)=number of points in the brain receiving electrical current or Glu or Gly stimulation. *indicates the values between DM and RVLM activation under electrical stimulation or microinjection of Glu or Gly, in the same direction (+ or -) of Int.VNA are statistically significant with $p<0.05$ by one-way ANOVA. [a]Values of the responese stimulated by electrical stimulation and Gly in DM or RVLM are significant different with $p<0.05$ by paired Student's t-test. [b]Values of the responses stimulated by Glu and Gly in DM or RVLM are significant different with $p<0.05$ by paired Student's t-test.

spikes/s, or increased 145% (from 67 to 164 spikes/s). Microinjection of Glu at the same point also produced an elevation of SAP (36.4%) and a prolonged (5 min) increase of the Int.VNA (340%) (Fig. 2B). The Fw rosed from 55 to 116 spikes/s, or increased 111%, and Fa rose was from 12 to 36 spike/s, or increased 200%. The sum of Fw and Fa elevations was 152 spikes/s, or an increase of 127% (from 67 to 152 spikes/s). Microinjection of Gly at the same point also produced marked elevation of SAP (45.8%) and prolonged (10 min) increases of the Int.VNA (210%) (Fig. 2C). The Fw rose from 55 to 165 spike/s, or increased 200%, and Fa rose from 12 to 25 spikes/s, or increased 108%. The sum of Fw and Fa elevations was 190 spikes/s, or increased 183% (from 67 to 190 spikes/s). This suggests that the increase of Int.VNA resultion from DM stimulation was contributed by the increases of both Fw and Fa.

B. RVLM

Electrical stimulation on 22 points in RVLM produced increases of SAP (42.3%) and Int.VNA (295.3%) associated with an increase in Fa (344.2%) and a decrease in Fw (67.5%) (Table 1). In 18 of the 22 points, Glu stimulation increased the Int.VNA (275.4%) and Fa (315.7%) and with a reduction of Fw (45.8%). However, in 4 of the 22 points, Glu produced a decrease in Int.VNA 23.1% associated with decreases in both Fw (10.8%) and Fa (18.5%). In 17 of the 22 points, microinjection of Gly produced increases of SAP (45.7%) and Int.VNA (285.5%) associated with a Fa increase (328.2%) and Fw decrease (62.5%). However, in 5 of the 22 points, Gly produced a decrease in Int.VNA 22.5% associated with decreases

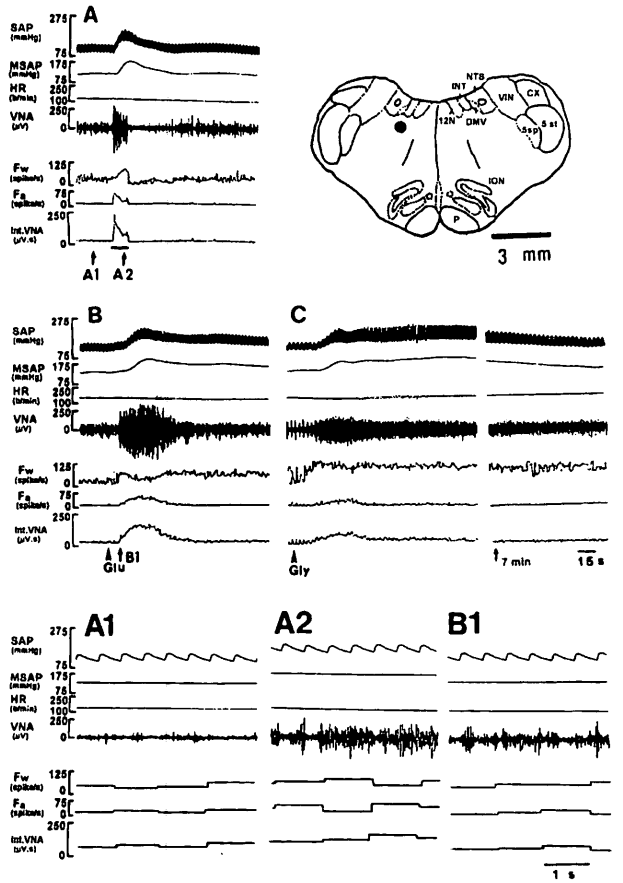

Fig. 2. Electrical stimulation and Glu and Gly microinjections on the same point of DM produced marked increases of SAP and Int.VNA associated with increases of F*w* and F*a*.

In this and following figures, tracings from top to bottom are: SAP, systemic arterial blood pressure; MSAP, mean SAP; HR, heart rate. Bar (–, 15 sec) and arrow (↑) under the tracings indicate the time of delivering electrical stimulation, microinjection of Glu or Gly, respectively. Dot (•) in the brain drawing shows the site of stimulation.

In the lower panel of this figure; A1, A2 and B1 indicate VNA in fast run taken at the time marked by arrow: A1, before electrical stimulation, A2, and B1 during electrical stimulation and Glu microinjection, respectively. Note that electrical stimulation immediately increased SAP and VNA but the duration of actions was short. The electrical stimulation and Glu microinjection produced discrete oscillation in VNA. Gly stimulation took a longer latency of about 7 sec but the increases in SAP and VNA were prolonged (7 min). Data show that during electrical, Glu and Gly stimulations, both F*w* and F*a* were increased.

Abbreviations: CX: external cuneate nucleus; DMV: Dorsal motor nucleus of vagus; INT: nucleus intercalatus; ION: Inferior olivary nucleus; NTS: Nucleus tractus solitarius; P: Pyramidal tract; PH: nucleus praepositus hypoglossi; VIN: Inferior vestibular nucleus; VMN: Medial vestibular nucleus; 5sp: Spinal trigeminal nucleus; 5st: Spinal trigeminal tract; 12 N: Hypoglossal nucleus.

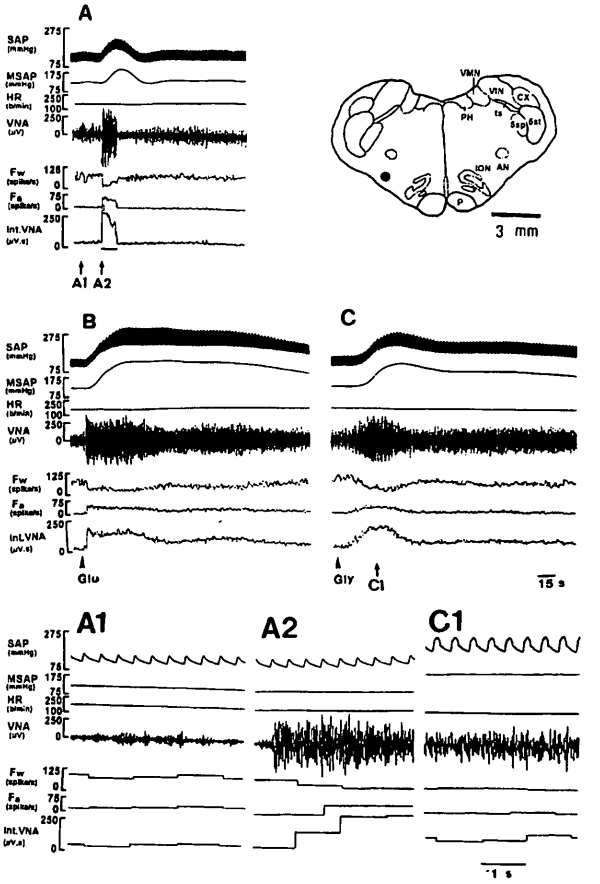

Fig. 3. Electrical and chemical (Glu and Gly) stimulations on RVLM produced marked increases of SAP and Int.VNA associated with a decrease in F*w* and an increase in F*a*.

In the lower panel of this figure; A1, A2 and C1 indicate VNA in fast run taken at the time marked by the arrow: A1, before electrical stimulation, A2 and C1 during electrical stimulation and Gly microinjection, respectively. Note that electrical stimulation and Gly microinjection produced continuous oscillation in VNA. Electrical, Glu and Gly stimulations produced F*a* increase, while F*w* decreased during the actions.

of both F*w* (5.1%) and F*a* (12.2%). These points are located near the nucleus ambiguus. Figure 3 shows an example of RVLM stimulation. In this cat, the averaged resting values of F*w* and F*a* were 85 and 10 spikes/s, respectively. The sum of F*w* and F*a* was 95 spikes/s. Electrical stimulation produced rises of SAP (55%) and Int.VNA (450%) (Fig. 3A) associated with a large oscillation of VNA (Fig. 3 A2). The F*w* decreased 65% (from 85 to 30 spikes/s), while the F*a* increased 400% (from 10 to 50 spikes/s). The sum of F*w* and F*a* was 80 spikes/s, or a decrease of 15.8% (from 95 to 80 spikes/s). Microinjection of Glu at the same point produced a marked elevation of SAP (110%) and Int.VNA (350%). The F*w* was decreased 58% (from 85 to 35 spikes/s), while F*a* was increased 350% (from 10 to 45 spikes/s). The sum of F*w* and F*a* was 80 spikes/s, or a decrease of 15.8% (from 95 to 80 spikes/s). Microinjection of Gly at the same point also produced increases of SAP (95.8%) and Int.VNA (380%) (Fig. 3C). During the period of increase in Int.VNA the F*w* was decreased 70% (from 85 to 25 spikes/s) and F*a* was increased 320% (from 10 to 42 spikes/s). The sum of F*w* and F*a* was 67 spikes/s or

a decrease 29.5% (from 95 to 67 spikes/s). This suggests that during stimulation of the RVLM the increase of Int.VNA was mainly contributed by the increase of F*a* and decrease of F*w*.

Discussion

The conventional ways to quantify or analyze the changes in the peripheral nerve activity are as follows: (i) The discharge of the multifibers is rectified and integrated. (ii) The number of discharges above a preset level or within two preset levels is counted. (iii) The discharge is analyzed using a software of power spectrum.

The off-line analysis of power spectrum is a convenient method for analyzing nerve discharge. Barman et al. suggested that two main frequencies ranges of 2 - 6 Hz and 10 Hz exist in the brain stem (2, 38). However, the signals are often attenuated by artifacts of low frequency arising from the artificial ventilator, muscle movement, pulsation of the vessel, or heart beat near the recorded sympathetic vertebral nerve. In particular, the heart rate, whose frequency is close to 3 Hz, may disturb the analysis by power spectrum.

In this study, we combined the former two conventional methods in analyzing nerve activities and developed an on-line analysis of differential integrator unit to quantity, classify and integrate the VNA. We set the bandwidth of preamplifier at 3 to 3 kHz to minimize the possible artifacts mentioned above. The frequency resolution of the window discriminator was 1000 Hz because the standard TTL output of window discriminator was 5 volts, 1.0 ms, which was four times the maximum summation of F*w* and F*a* of VNA that was simultaneously less than 250 spikes/s. In the present system of analysis, the value of LL was set at a level 10% of the VNA amplitude above zero to prevent the background noise. The value of UL was set at 80% of the VNA amplitude to provide a proper arbitrary for analysis of the nerve activity. The setting of optimal UL depends entirely upon the need of the experiment. If the UL is set at a higher level, the F*a* value will be lower. On the contrary, when the UL is set at a lower level, the F*a* value will be higher. In this study we found that our setting in UL (80% of the VNA) and the LL (10% of the VNA) provided optimal values for F*a* and F*w*. However, this approach has its drawback. The recorded signal of VNA was a summated signal derived from the bundle of multifibers that displaced simultaneously at the recording electrode. Therefore, the discharge of an individual fiber could not be demonstrated. This problem exists in most electrophysiological studies, especially in peripheral nerve recording.

By using the concurrent method for VNA, we observed some interesting features of VNA in terms of changes in F*a* and F*w* during activation of the DM and RVLM either by electrical stimulation or by microinjections of Glu and Gly: (i) Electrical stimulation and microinjections of Glu and Gly into the pressor areas of DM and RVLM produced increases of SAP and Int.VNA. (ii) The increase of Int.VNA on DM activation was contributed by the increases of F*w* and F*a*. The sum of F*w* and F*a* during DM stimulation was 145 % higher than that of the resting state. (iii) In RVLM, the increase of Int.VNA was principally contributed by the increase of F*a* and a decrease of Fw. The sum of F*a* and F*w* during RVLM stimulation was 15.8 % less than that of the resting state. It is very important that although the amplitudes of Int.VNA increase during DM and RVLM stimulations were similar but the F*w* and F*a* have an inverse effect. (iv) Gly stimulation on RVLM produced more increase in F*a* and more decrease in F*w* than that of Glu did. (v) Electrical and chemical stimulations usually produced huge synchronized oscillation in VNA on RVLM more than on DM. Therefore, the nature of the VNA between RVLM and DM differs.

Characteristics of DM

The latency onsets of responses in SAP and VNA subsequent to electrical current were shorter than that by Glu and Gly. Electrical stimulation immediately increased SAP and VNA, and the responses subsided rapidly when the stimulation was terminated. On the other hand, Glu produced prolonged responses in SAP and VNA (7 min, Fig. 2). Furthermore, the threshold current-intensity for VNA (15 μA) activation was smaller than that for SAP (30 μA) activation. This may suggest that neurons in the sympathetic pressor area are more susceptible to changes of VNA than of SAP. Furthermore, the changes of SAP and VNA induced by different current-intensity differs between DM and RVLM. In DM, electrical stimulation often produced a greater increase in SAP or VNA than Glu, while in RVLM Glu often induced greater responses than electrical current. Since electrical current activates not only cell bodies and dendrites but also passing fibers, while Glu activates only the former two excluding passing fibers (18), this suggests that DM contains more passing fibers than that of RVLM. Indeed, anatomical studies have shown that DM contains sympathetic neurons whose axons project to RVLM, locus coeruleus of the pons and paraventricular nucleus of the hypothalamus (16). In addition, the area of DM receives various ascending inputs including many peripheral, visceral, nociceptive and chemoreceptive sources (16). As a result, stimulation on DM increases both F*w* and F*a*.

The higher content of passing fibers in DM is also reflected by the fact that lesioning the DM with direct current (DC) produces more marked decrease of the resting SAP than lesioning this region by kainic acid (26, 6). Unlike the latter which destroys the perikarya only, electrical lesioning destroys not only perikarya but also projecting fibers passing from everywhere, including those from DM to RVLM (7, 13). Administration of kainic acid in DM also decreases resting SAP, and the SAP increase induced by Glu or other chemicals. This supports the contention that neurons in DM also play a role in integrating SAP (9).

Glu activation in DM also increases F*w* and F*a*. Since Glu activates cell bodies and dendrites, the increases are not related to activation of passing fibers around the area receiving stimulation. One possibility may be that there exists a great number of non-cardiovascular neurons in the DM innervating other structures through the sympathetic vertebral nerve. When these neurons are activated by Glu, the VNA increase despite an absence of SAP increase.

Characteristics of RVLM

It has been well established that RVLM plays an essential role in maintaining basal vasomotor tone (15) and phasic regulation of blood pressure (13, 23). RVLM mediates and/or generates tonic sympathetic activity and integrates circulatory reflexes. Thus, it is considered to be the main supraspinal sympathoexcitatory common pathway. Neurons in the RVLM may have pacemaker properties suggesting of sympathetic excitation (24, 3, 4). RVLM contains bulbospinal neurons that project to the preganglionic sympathetic neurons of the spinal cord (14, 25). This has been shown in anatomical (1) and electrophysiological studies (3, 4, 5).

Two possibilities have been considered regarding the high tonic activity in vasomotor neurons of RVLM. First, the blood flow and capillary density within the RVLM are significantly higher (17), and the sympathetic neurons are very sensitive to oxygen (30), pH, pO_2, and pCO_2 (13). Secondly, RVLM contains pacemaker cells (29) or non-pacemaker sympathoexcitatory cells that are highly correlated with spontaneous sympathetic vasomotor activity (20).

The present study observed a marked oscillation of VNA following electrical or Glu and Gly stimulations on RVLM. This oscillation may be derived from a group of neurons that discharge in a synchronous rate. Under resting conditions, a large number of these neurons remain silent. Thus, the change of neuron activity in RVLM falls in the range of high F*w* (averaged 72 Hz), and low F*a* (averaged 12 Hz). During activation, either electrical or chemical stimulations, many silent neurons are activated and fired actively with their signals activity exceeding the upper level of the window discriminator. As a result, a great portion of signals originally presented in F*w* are converted to F*a* and, thus, create a much lower level in F*w* than the resting state (from 72 to 35 Hz). This may also suggest that there exist more neurons in RVLM that discharge synchronously with the VNA.

Conclusion

The present study illustrates an on-line analysis of differential integrator unit beyond the original design of window discriminator to distinguish different characteristics of the spike response of VNA consequent to electrical current or Glu and Gly stimulations on the pressor areas of DM and RVLM. With this method, some differences in the nature of sympathoexcitatory neurons between DM and RVLM can be discriminated.

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References

1. Amendt, K., J. Czachueski, K. Dembowsky, and H. Seller. Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *J. Auton. Nerv. Syst.* 1: 103-117, 1979.
2. Barman, S.M., and G.L. Gebber. Rostral ventrolateral medullary and caudal medullary raphe neurons with activity correlated to the 10-Hz rhythm in sympathetic nerve discharge. *J. Neurophysiol.* 68: 1535-1547, 1992.
3. Barman, S.M., and G.L. Gebber. Axonal projection patterns of ventrolateral medullospinal sympathoexcitatory neurons. *J. Neurophysiol.* 53: 1551-1566, 1985.
4. Brown, D.L., and P.G. Guyenet. Cardiovascular neurons of brain stem with projections to spinal cord. *Am. J. Physiol.* 247: R1009-1016, 1984.
5. Brown, D.L., and P.G. Guyenet. Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circ. Res.* 56: 359-369, 1985.
6. Chai, C.Y. The role of different portions of lower brain stem of the cat in central cardiovascular control mechanism. *Chin. J. Physiol.* 19: 55-64, 1963.
7. Chai, C.Y., R.H. Lin, A.M.Y. Lin, C.M. Pan, E.H.Y. Lee, and J.S.

Kuo. Pressor responses from electrical and glutamate microinjection of the dorsal or ventrolateral medulla. *Am. J. Physiol.* 255, R709-717, 1988.

8. Chai, C.Y., S.Y. Chen, J.C. Liu, S.D. Wang, C.J. Tseng, R.H. Lin, S.P. Mao, H.T. Horng, J.C. Liu, and J.S. Kuo. Precollicullar decerebration reduces the pressor responses evoked by stimulation of rostral pons but not medulla in cats. *J. Auton. Nerv. Syst.* 46, 147-159, 1993.
9. Chai, C.Y., C. Yuan, H.T. Horng, S.D. Wang, C.J. Tseng, and J.S. Kuo. Neurons in the pressor sites of dorsal and ventrolateral medulla mediate pressor actions of the periaqueductal grey of midbrain. *Chin. J. Physiol.* 36: 37-45, 1993.
10. Chai, C.Y., S.Y. Chen, A.M.Y. Lin, and C.J. Tseng. Angiotensin II activates pressor and depressor sites of the pontomedulla that react to glutamate. *Clin. Exp. Pharmacol. Physiol.* 23: 415-423, 1996.
11. Chen, S.Y., S.D. Wang, C.L. Cheng, J.S. Kuo, W.C. de Groat, and C.Y. Chai. Glutamate activation of neurons in CV- reactive areas of cat brain stem affects urinary bladder motility. *Am. J. Physiol.* 265: F520-529, 1993.
12. Chiang, C.H., P. Pappagianopoulos, B. Hoop, and H. Kazemi. Central cardiorespiratory effects of glutamate in dog. *J. Appl. Physiol.* 60: 2056-2062, 1986.
13. Dampney, R.A.L., and E.A. Moon. Role of the ventrolateral medulla in vasomotor response to cerebral ischemia. *Am. J. Physiol.* 239: H349-358, 1980.
14. Dampney, R.A.L., J. Czachurski, K. Dembowsky, A.K. Goodchild, and H. Seller. Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. *J. Auton. Nerv. Syst.* 20: 73-86, 1987.
15. Dampney, R.A.L. Functional organization of central pathways regulating the cardiovascular system. *Physiol. Rev.* 74: 323-364, 1994.
16. Felder, R.B. Excitatory and inhibitory interactions among renal and cardiovascular afferent nerves in dorsomedial medulla. *Am. J. Physiol.* 250: R580-588, 1986.
17. Gobel, U., H. Schrock, H. Seller, and W. Kuschinsky. Glucose utilization, blood flow and capillary density in the ventrolateral medulla of the rat. *Pflü gers Arch.* 416: 477- 480, 1990.
18. Goodchild, A.K., R.A.L. Dampney, and R. Bandler. A method of evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. *J. Neurosci. Methods* 6: 351-363, 1982.
19. Goodchild, A.K., and R.A.L. Dampney. A vasopressor cell group in the rostral dorsomedial medulla of the rabbit. *Brain Res.* 360: 24-32, 1985.
20. Haselton, J.R., and P.G. Guyenet. Electrophysiological characterization of putative C1 adrenergic neurons in the rat. *Neuroscience* 30: 199-214, 1989.
21. Hirooka, Y., J.W. Polosa, and R.A.L. Dampney. Pressor response from rostral dorsomedial medulla is mediated by excitatory amino acid receptors in rostral VLM. *Am. J. Physiol.* 267: R309-315, 1994.
22. Lan, C.T., W.C. Wu, E.A. Ling, and C.Y. Chai. Evidence of a direct projection pathways from the cardiovascular-reactive dorsal medulla to the intermediolateral cell column of the spinal cord in cats as revealed by light and electron microscopy. *Neuroscience* 77: 521-533, 1997.
23. McAllen, R.M. Action and specificity of ventral medullary vasopressor neurons in the cat. *Neuroscience* 18: 51-59, 1986.
24. Morrison, S.F., T.A. Milner, B.M. Pickel, and D.J. Reis. Reticulospinal vasomotor neurons of the rat rostral ventrolateral medulla (RVL): Relationship to sympathetic nerve activity and the C1 adrenergic group. *J. Neurosci.* 8: 1286-1301, 1988.
25. Ross, C.A., D.A. Ruggiero, T.H. Joh, D.H. Park, and D.J. Reis. Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *J. Comp. Neurol.* 228: 168-184, 1984.
26. Su, C.K., A.M.Y. Lin, R.H. Lin, J.S. Kuo, and C.Y. Chai. Contribution between dorsal and ventrolateral regions of medulla oblongata in vasomotor function of cat. *Brain Res. Bull.* 23: 447-456, 1989.
27. Su, C.K., C.T. Yen, J.C. Hwang, J.S. Kuo, and C.Y. Chai. Differential effects on sympathetic nerve activities elicited by activation of neurons in the pressor areas of dorsal and rostral ventrolateral medulla in cats. *J. Auton. Nerv. Syst.* 40: 141-154, 1992.
28. Su, C.K., S.Y. Chen, J. M. Yang, R.H. Lin, H.T. Horng, J.C. Hwang, and C.Y. Chai. Correlation of cardiovascular and respiratory responses to glutamate excitation of pressor areas of the medulla in cats. *Chin. J. Physiol.* 35: 279-289, 1992.
29. Sun, M.K., B.S. Young, J.T. Hackett, and P.G. Guyenet. Reticulospinal pacemaker neurons of the rat rostral ventrolateral medulla with putative sympathoexcitatory function: an intracellular study in vitro. *Brain Res.* 422: 229-239, 1988.
30. Sun, M.K., I.T. Jeske, and D.J. Reis. Cyanide excites medullary sympathoexcitatory neurons in rats. *Am. J. Physiol.* 262: R182-189, 1992.
31. Sun, M.K. Central neural organization and control of sympathetic nervous system in mammals. *Neurobiology* 47: 157-233, 1995.
32. Wang, S.D., K.D. Yen, C.M. Pan, J.C. Liu, J.S. Kuo, and C.Y. Chai. Descending pressor pathways from the dorsomedial and ventrolateral medulla of cats. *Chin. J. Physiol.* 39: 17-28, 1996.
33. Wu, W.C., S.D. Wang, J.C. Liu, H.T. Horng, M.J. Wayner, J.C. Ma, and C.Y. Chai. Activation of neurons in cardiovascular areas of brain stem affects spinal reflexes. *Brain Res. Bull.* 33: 393-402, 1994.
34. Wu, W.C., M.L. Tsai, C.P. Liu, and C.T. Chai. A device supplementing Narishige PE-2M glass microelectrode puller for making better multibarrel micropipettes. *Biomed. Engng - Applic. Basis, Commun.* 7, 519-523, 1995.
35. Wu, W.C., S.Y. Chen, J.S. Kuo, and C.Y. Chai. Glycine produced pressor responses when microinjected in the pressor areas of pons and medulla in cats. *J. Auton. Nerv. Syst.* 59: 1-11, 1996.
36. Wu, W.C., J.S. Kuo, Y. Wang, and C.Y. Chai. Glycine inhibits the strychnine-sensitive pressor inhibitory mechanism to produce hypertension and augments the NMDA-pressor actions in medulla. *J. Auton. Nerv. Syst.* (in press), 1998.
37. Yardley, C.P., R.D. Stein, and L.C. Weaver. Tonic influences from the rostral medulla affect sympathetic nerves differentially. *Am. J. Physiol.* 256: R323-31, 1989.
38. Zhong, S., S.M. Barman, and G.L. Gebber. Effects of brain stem lesions on 10 Hz and 2-6 Hz rhythms in sympathetic nerve discharge. *Am. J. Physiol.* 262: R1015-1024, 1992.