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Cardiovascular Neurons Mediating Somatosympathetic Reflex in Rostral Ventrolateral Medulla

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= ABSTRACT=

The rostral ventrolateral medulla (RVLM) includes vasopressor neurons, which transmit activation signals to the intermediolateral nucleus (IML) of the spinal cord, where the preganglionic sympathetic nucleus is located, to raise arterial blood pressure (BP). However, controversy exists as to the possible depressor area in the RVLM and the pathway involved. The present study persued evidence for the location of depressor neurons and the pathway by simultaneously observing changes in BP and the firing rate (FR) of cardiovascular neurons (CVNs) in the RVLM during the somatosympathetic reflex (SSR) elicited by peripheral nerve stimulation, since CVNs are known to contribute to the generation of the sympathetic nerve discharge. In 42 cats, anaesthetized with α-chloralose, single unit recording was performed, using carbon filament electrodes inserted into the RVLM, enabling estimation of the post R wave unit histogram (PR-UNIT) and the spike triggered average of sympathetic nerve discharge (STA-SND), allowing identification of CVNs. Antidromic stimulation of spinal T_2 segment was followed to determine whether the identified CVN projects axonal endings to the spinal cord (reticulospinal neuron). The sciatic nerve was electrically stimulated at Aδ-intensity (1 mA, 0.1 ms), 1 Hz and C-intensity (10 mA, 0.5 ms), 20 Hz to elicit the depressor, and pressor responses of the SSR, respectively. Simultaneous measurement of CVN firing rate was made. Experimental results are summarized as follows.

1) 20 out of 98 CVNs had axonal projections to the spinal cord and 17 out of 98 CVNs showed FR changes during SSR.

2) Response patterns of FR and BP during SSR were classified into 8 types.

3) These 8 different response patterns could be further classified into those from pressor and depressor neurons.

These results demonstrate that some CVNs were identifiable as reticulospinal neurons responding to antidromic stimulation and that CVNs operating as depressor neurons as well as pressor neurons exist in the RVLM, both of which are involved with SSR mediation. Therefore, evidence was found that an independent depressor pathway might be involved in the mediation of SSR.

Key Words: Rostral ventrolateral medulla (RVLM), Cardiovascular neuron (CVN), Somatosympathetic reflex (SSR), Pressor neuron, and Depressor neuron.

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INTRODUCTION

The center for blood pressure regulation is located in the medulla oblongata (Dittmar, 1873). Several lines of evidence support the contention that the neurons of the rostral ventrolateral medulla (RVLM) have a key role in blood pressure regulation. First, electrical or L-glutamate induced stimulation of this area increases blood pressure while its destruction decreases blood pressure (Guertzenstein, 1973; Guertzenstein & Silver, 1974; Feldberg & Guertzenstein; 1976), Secondly, a pathway from the RVLM to the intermediolateral nucleus (IML) of the thoracolumbar spinal cord has been demonstrated with anterograde and retrograde transport techniques (Dahlstrom & Fuxe, 1964; Amendt et al, 1979; Ross et al, 1984; Dampney et al, 1987). These suggest that the pressor response is mediated by RVLM neurons, supplying a tonic excitation to the sympathetic preganglionic neurons in the IML of the spinal cord.

Electrical excitation of A1 noradrenergic neurons or microinjection of L-glutamate in the caudal ventrolateral medulla (CVLM) lowers blood pressure, whereas the impairment of neuronal activity by electrolytic lesions or kainic acid produces hypertension (Blessing & Reis, 1982). In addition, the neurons of the CVLM tonically inhibit those in the RVLM (Granata et al, 1986). Therefore, the neurons in the RVLM tonically inhibited by the CVLM may excite the sympathetic nerve less. The depressor response may, therefore, occur due to a decreased sympathetic nerve activity following the tonic inhibition. On the other hand, the raphe neurons exert a sympatho-inhibitory function by directly projecting axons to the IML of the spinal cord (Morrison & Gebber, 1984). Areas can be identified in the RVLM that exhibit either the pressor or depressor response when electrically or chemically (glutamate injection) stimulated (Park et al, 1990). Furthermore, these areas have been demonstrated to participate in the somatosympathetic pressor and depressor responses (Park et al, 1990). Therefore, it may be possible for a group of neurons in the RVLM to independently mediate the depressor response and form a depressor pathway without tonic inhibition from the CVLM.

RVLM neurons participating in blood pressure regulation named the cardiovascular neurons (CVN) show spontaneous activity synchronous to the cardiac rhythm (Barman & Gebber, 1981). These have been demonstrated to mediate the somatosympathetic reflex (Morrison & Reis, 1989; Kim et al, 1992). If the pressor and depressor neurons operate independently in the RVLM, this should result in different response patterns in the firing rate and blood pressure change elicited by the somatosympathetic reflex (SSR). Nonetheless, the firing rate of both groups of neurons would be synchronized to the cardiac cycle. To test these hypotheses, the present study identified CVNs in the RVLM and measured changes in firing rate of these neurons and blood pressure during SSR elicited by peripheral nerve stimulation.

METHODS

Animal preparation

Forty two adult cats of either sex (2~3 kg, body weight) were used. After pre-anaesthetic treatment with atropine (0.1 mg/kg, i.m.), to minimize secretion and sedation with a single dose of ketamine (ketalar, 20 mg/kg, i.m.), the animal was anaesthetized with α-chloralose (60 mg/kg, i.v.). The trachea, the femoral artery, and vein were cannulated for artificial ventilation, blood pressure monitoring and intravenous injection of drugs, respectively. The animal was paralyzed by intravenous administration of pancuronium bromide (Mioblock, Organon, initial dose: 0.4 mg, maintenance dose: 0.4 mg/hour). End-expiratory CO_2 concentration was maintained at 3~4% and rectal temperature was kept at 37±1℃ with a ther-

mostat controlled blanket. Hartmann solution was infused continuously throughout the experiment (10~15 ml/hour).

The electrocardiogram (ECG) was recorded with subcutaneous pin electrodes. A Schmitt-trigger circuit was employed to provide pulses triggered with each R wave on ECG. The upper thoracic spinal cord was exposed by a laminectomy on the T_2 vertebra. The stellate ganglion and the inferior cardiac nerve were isolated to record the sympathetic nerve activity, then an occipital craniectomy was performed. To expose the floor of the fourth ventricle, the cerebellum was removed by pneumatic suction. The sciatic nerve was isolated and exposed for electrical stimulation in the left hindlimb. At the completion of these operative procedures, the animal was mounted on a stereotaxic apparatus and mineral oil pools were made with incised skin flaps over the exposed area. A water circulating heating coil in the thoracic pool prevented heat loss out to the exposed area. A thoracotomy reduced the movements, related to the respiratory pump, of the tissue near the recording electrode. A schematic diagram of the experimental set-up is shown in Fig. 1.

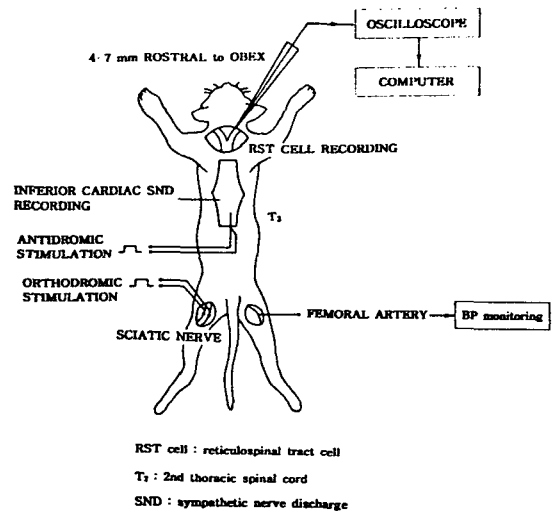

Fig. 1. Experimental set-up.

Neural recordings

RVLM neuronal activity: The facial nucleus was located by recording, with a teflon coated silver electrode, the antidromic field potential elicited in the facial motor neuron by the stimulation (1 mA, 0.1 ms, 1 Hz) of the mandibular branch of the facial nerve. The RVLM was assumed be located from 200 μm caudal to the site where the facial field potential was no longer detectable, since it is known to lie directly caudal to the facial nucleus (Ruggiero et al, 1989).

Single cell activity in the RVLM was recorded with a carbon filament electrode (tip resistance: 2~3 MΩ). The obex was used as a surface landmark to place the electrode. It was positioned on the dorsal surface of the medulla, 4~7 mm rostral to the obex and 3~4.5 mm lateral from the midline. The electrode was lowered down with desired steps by a hydraulic microdrive (PC-5N, Narishige) until any single cell activities were differentiable. Usually, the single cell activities were picked up at depths of 3~7 mm from the dorsal surface. The electrical activities were amplified (band pass 0.3~10 KHz; gain 10,000, DAM-80, WPI) and displayed and stored by a digital oscilloscope (4094C, Nicolet). The single cell activities were extracted using a window discriminator (Frederick Haer & CO), sampled at a rate of 67 kHz (1401, CED), and stored on an IBM-PC/AT computer for further analysis.

Sympathetic nerve discharge (SND): Recordings were made from the central endings of the sectioned left inferior cardiac postganglionic sympathetic nerve. The inferior cardiac nerve was isolated retropleurally at its exit from the stellate ganglion after removing the head of the first rib. Electric potentials were recorded with a bipolar platinum hook electrode. Filtered (bandpass 1~1000 Hz) and amplifed (× 10,000, DAM-80, WPI) potentials enabled identification of the synchronized discharges of sympathetic nerve fibers in the form of a slow wave.

Electrical stimulation

Spinal cord: A micromanipulator (MO-81, Narishige) lowered a concentric biplor tungsten electrode (diameter: 500 μm, tip diameter: 100 μm) to a usual depth of 1.5 mm where the IML was assumed to exist. RVLM neurons that project to the spinal cord (reticulospinal neurons) were antidromically identified by delivering 0.1~0.5 ms electric pulses to the IML in the T_2 segment through the electrode using a stimulator (Pulsemaster A300, WPI) coupled with an isolator (A360, WPI). The reticulospinal neurons were identified based on three basic criteria: 1) constant onset latency, 2) high frequency following, and 3) collision with spontaneous activity (Morrison & Gebber, 1984).

Peripheral nerve: The sciatic nerve was dissected and hooked with a tripolar platinum electrode for electrical stimulation to elicit the somatosympathetic reflex (SSR).

Data reduction

Data were analyzed with an IBM-PC/AT computer and hardcopied to a color plotter (Colorpro, Hewlett Packard). Analysis techniques employed are described below.

1) Spike triggered average of sympathetic nerve discharge (STA-SND): The inferior cardiac SND following spontaneous spike of a single RVLM neuron was averaged for 500~1,000 sweeps with each sweep of 500 ms duration before and after the spontaneous spike (Barman & Gebber, 1981).

2) Post R wave interval histogram of unit discharges (PR-UNIT): The time intervals between the trigger pulses coincident with each ECG R-wave and the following RVLM unit discharges were collected for 500 sweeps with each sweep lasting for 1,000 msec. These data were counted and normalized to construct averages of the interval histograms of the RVLM unit discharges (Barman & Gebber, 1981).

3) Post stimulus time histogram of unit discharge (PSTH): The time intervals between the sciatic nerve stimulation (Aδ-intensity or C-intensity) and the following RVLM discharges were collected for 3 seconds to construct the time interval histogram (Morrison & Reis, 1989).

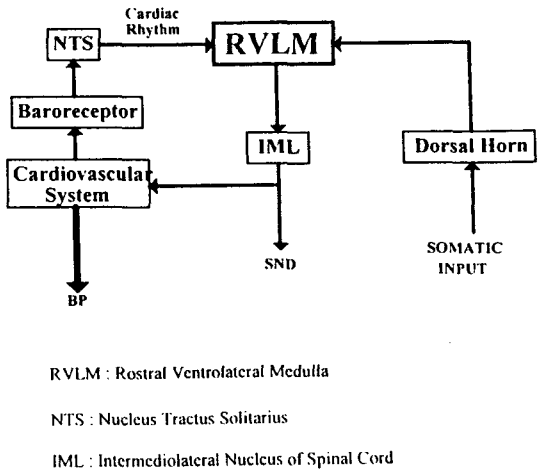

Fig. 2. Functional block diagram of the somatosympathetic reflex (SSR) related to blood pressure (BP) regulation.

Identification of cardiovascular neuron (CVN)

CVNs were identified as those neurons in the RVLM that showed a meaningful waveform in STA-SND with an adequate deflection, and also, whose neuronal activities varied in a close temporal association with the cardiac rhythm as observed on PR-UNIT. These two criteria have been successfully applied previously (Barman & Gebber, 1983; Kim et al, 1992), and the background reasoning is illustrated in Fig. 2. The afferent input concerning the cardiac rhythm is delivered from the cardiovascular system to the RVLM via the baroreceptors and NTS. Since RVLM neurons have axonal projections to the IML of the spinal cord, where the nucleus of the sympathetic preganglionic neuron is located, the temporal relationship is established between the discharges of RVLM neurons and the afferent cardiac rhythm information or SND. This can be observed in the

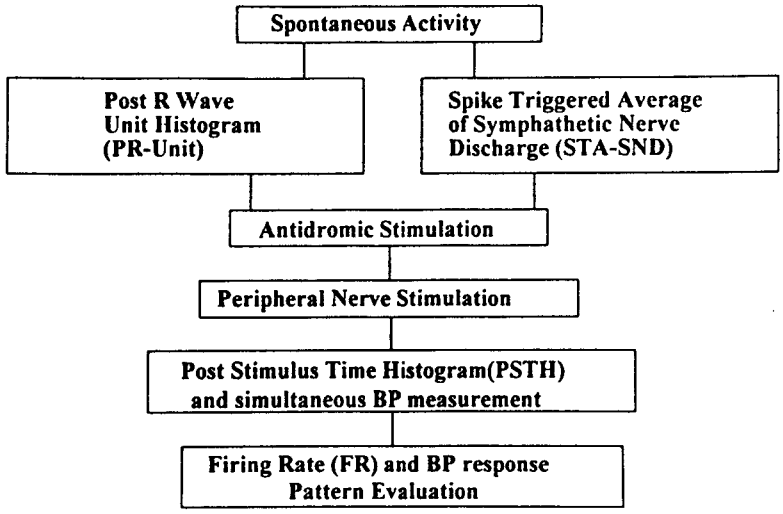

Fig. 3. Flow chart of the present experimental protocol.

PR-UNIT or the STA-SND, respectively.

Evoking somatosympathetic reflex (SSR)

The pressor and depressor responses of SSR were elicited by stimulating the sciatic nerve at C-intensity (10 mA, 0.5 ms) at a high frequency (20 Hz), and at Aδ-intensity (1 mA, 0.1 ms) at a low frequency (1 Hz) respectively. The blood pressure (BP) responses (pressor or depressor responses) were determined by comparing the mean BP before and following the stimulation. Changes in firing rate (△ FR) of RVLM neurons were also determined by taking the differences of the mean FR's on the PSTH before and following the stimulation.

Experimental protocol

Action potentials of RVLM neurons were displayed on an oscilloscope screen. When a neuron showing spontaneous activity was encountered, STA-SND and PR-UNIT were taken. If these met with the previously described criteria, that particular neuron was identified as a CVN. To examine whether this CVN projected its axonal endings to the spinal cord, antidromic stimulation was performed in the spinal cord. The sciatic nerve was stimulated with either Aδ-1 Hz or C-20 Hz (SSR evokation), during which the changes in BP and FR of the identified CVN were simultaneously monitored on PSTH. These response patterns were classified and analyzed. This experimental protocol is summarized in Fig. 3.

Histology

At the end of each experiment, electrolytic lesions were made to mark the recording site in the medulla by passing a DC current of 100 μA through the recording electrode for 20 seconds. The medulla was removed and fixed in 10% formalin solution for at least a week. Frontal sections of 100 μm thickness each were obtained using a vibratome and stained with cresyl violet. The recording sites in RVLM were reconstructed by referring to the electrolytic lesions. After microscopic investigation, photomicrographs were taken (×16, Diaplan, Leitz).

RESULTS

Cardiovascular neuron (CVN)

As previously described, two criteria were applied to identify CVN: 1) synchronous firing

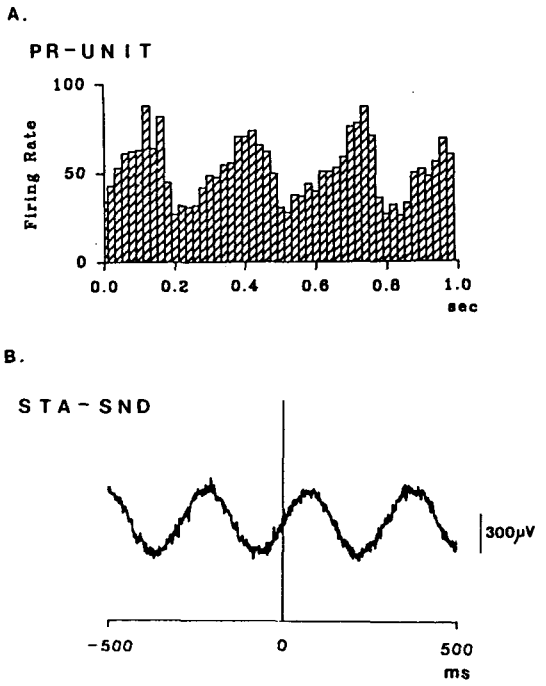

Fig. 4. Post R wave unit histogram (PR-UNIT) and spike triggered average of sympathetic nerve discharge (STA-SND) of a typical cardiovascular neuron (CVN). Note that both traces show temporal synchronization.

A. PR-UNIT. Number of trials was 500 and bin width, 200 ms.

B. STA-SND. Unit spikes at zero lag. Number of trials was 250.

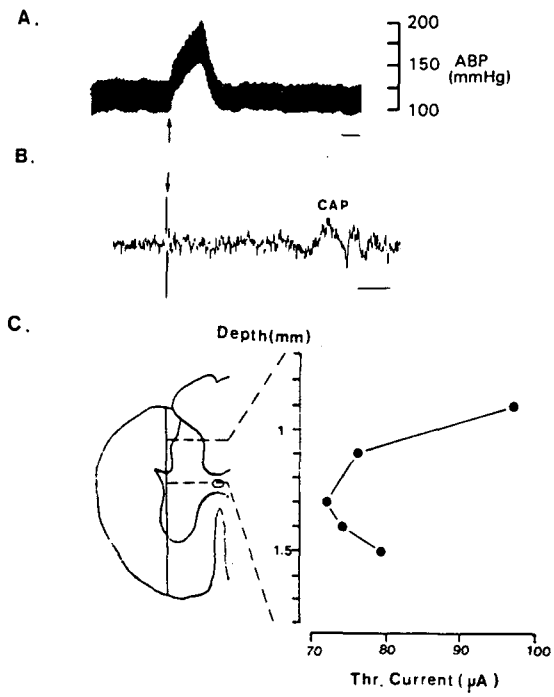

Fig. 5. Confirmation of the electrode location in the intermediolateral nucleus (IML) during antidromic stimulation (arrow).

A. BP increase in response to an electrical stimulus (1 mA, 0.1 ms, 20 Hz) for 10 sec to the ipsilateral T_2 IML. Horizontal calibration is 10 sec.

B. Corresponding compound action potential (CAP) evoked in the inferior cardiac nerve SND. Latency of the CAP was 76 ms. The horizontal calibration is 10 ms.

C. Depth-threshold (Thr.) current curve. The ordinate represents the distance (depth) from the surface of spinal cord, while the abscissa is the current evoking CAP of the inferior cardiac nerve (refer to B).

pattern to the cardiac cycle as determined on PR-UNIT and 2) temporal association of spontaneous spikes with SND observed as a meaningful waveform on STA-SND having an adequate deflection. Both criteria were met in the first 57 neurons which were identified as CVNs. Typical waveforms of PR-UNIT and STA-SND of these neurons are presented in Fig. 4. In the course of this procedure, no neuron was found to satisfy either criterion, leading to a decision that STA-SND was not necessary to identify CVNs (discussed later). From the 58th neuron and thereafter, the PR-UNIT criterion above was applied and an additional 41 neurons were found to satisfy this criterion. Therfore, all 98 neurons were considered CVNs.

Reticulospinal neuron

The spinal cord was antidromically stimulated to examine if CVNs had axonal projections to the IML of the spinal cord. An accurate location of the stimulating electrode was confirmed

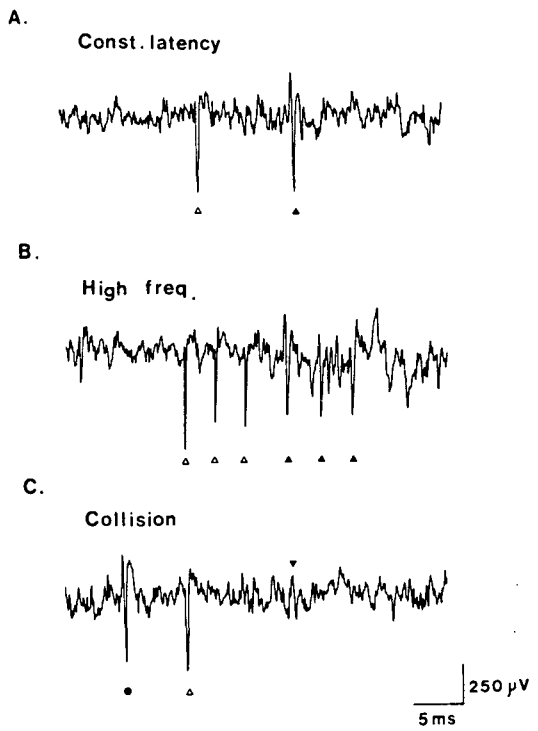

Fig. 6. Cardiovascular neuron (CVN) activity in response to antidromic stimulation of the T_2 segment. Open and closed triangles represent spinal stimulus and induced action potential, respectively.

A. An action potential showing a constant latency of 10 ms. Stimulus intensity, 1 mA with the threshold observed at 600 μA.

B. Each successive stimulus (333 Hz) resulting in the corresponding action potential at the intensity of 1 mA.

C. Collision of spontaneous activity (closed circle) with stimulus (open triangle) induced action potential. Note that no antidromically induced action potential is observed where it should have been (closed triangle).

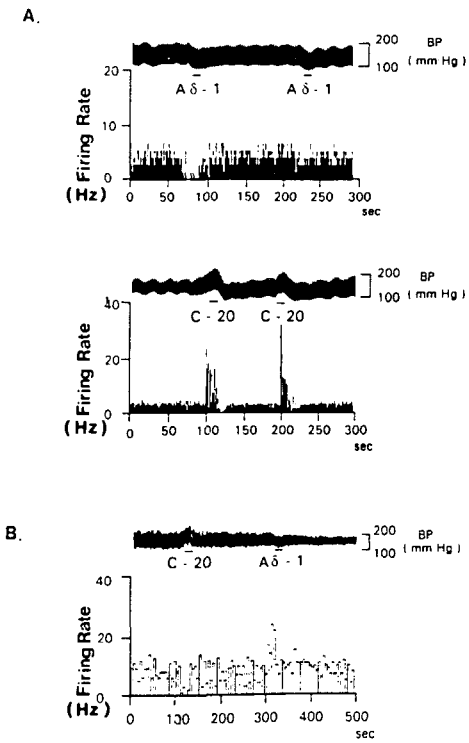

Fig. 7. Examples of cardiovascular neuron (CVN) firing rate (FR) response during somatosympathetic reflex (SSR).

A. CVN showing prominent and moderate decreases in both FR and BP due to successive Aδ-1 Hz stimuli and increase in both FR and BP due to C-20 Hz stimulus.

B. CVN showing an increase in FR and a decrease in BP in response to Aδ-1 Hz stimulus and a decrease in FR and an increase in BP in response to a C-20 Hz stimulus.

by the largest pressor response and the lowest threshold current evoking the compound action potential (CAP) of the inferior cardiac nerve (Fig. 5). Based on the three previously mentioned criteria, i.e., 1) constant onset latency, 2) high frequency following, and 3) collision with spontaneous activity (Fig. 6), 20 CVNs were identified as reticulospinal neurons.

Somatosympathetic reflex (SSR)

We tried to elicit SSR by stimulating the sciatic nerve at Aδ-intensity (1 mA, 0.1 ms) at a low frequency (1 Hz) or C-intensity (10 mA, 0.5 ms) at a high frequency (20 Hz) after identifying a CVN. 17 trials were successful with no technical difficulties. In these 17 neurons, the firing rate (FR) and blood pressure (BP) were

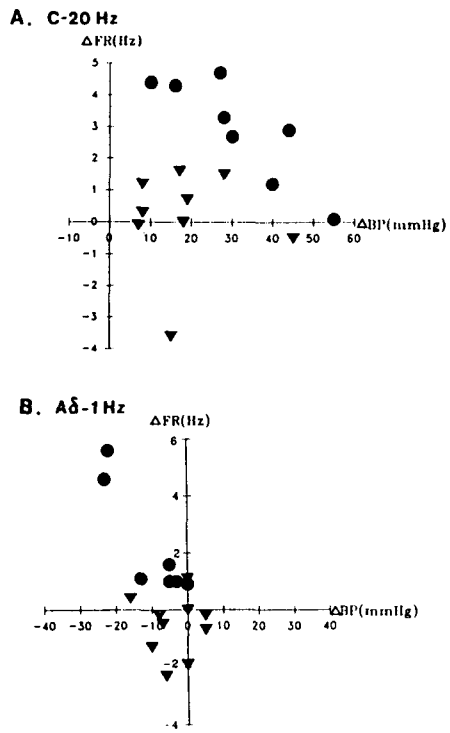

Fig. 8. Relationship between firing rate (△ FR) and blood pressure (△ BP) changes of identified cardiovascular neurons (CVNs). Closed circles represent CVNs which showed increases in FR (△ FR>0) during both pressor and depressor responses, while closed triangles represent those showing differing response patterns.
A. C-20 Hz
B. Aδ-1 Hz

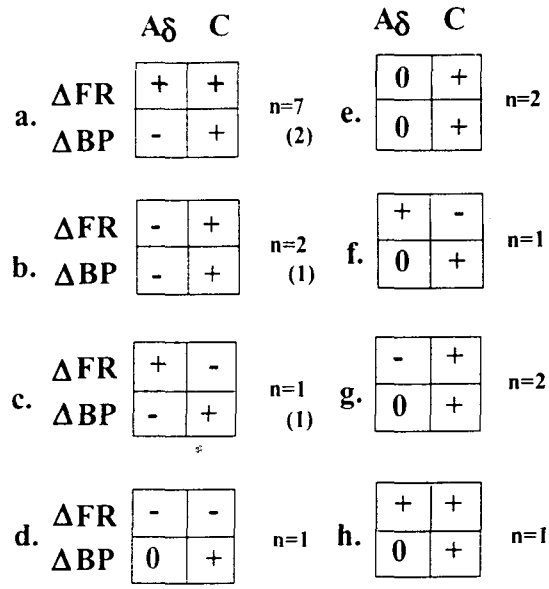

Fig. 9. Pattern characterization of cardiovascular neuron (CVN) firing response and blood pressure change during the somatosympathetic reflex (SSR) elicited by Aδ-1 Hz and C-20 Hz stimuli. CVNs were characterized by one of 8 (a through h) patterns. n represents the number of neurons fitting each pattern and the number in parenthesis represents those that responded to antidromic stimulation.

simultaneously monitored through changes in PSTH. The changes, in both variables (△ FR and △ BP) due to stimulation were measured by averaging for 100 ms before and during the stimulation and taking the differences between these averaged values. An example is shown, in Fig. 7 (A), of a CVN which showed a decreased FR (△ FR<0) with a concomitant decrease in BP (△ BP<0) in response to an Aδ-1 Hz stimulus and increases in both variables (△ FR>0 and △ BP>0) in response to a C-20 Hz stimulus. Another example is shown in Fig. 7 (B). In this CVN, FR increased (△ FR>0) and BP decreased (△ BP<0) in response to an Aδ-1 Hz stimulus, and FR decreased (△ FR<0) and BP increased (△ BP>0) in response to a C-20 Hz stimulus. BP always significantly increased (△ BP>0, $P<0.005$, using paired student's t-test), when stimulated C-20 Hz whilst FR increased (△ FR>0, $P<0.005$) and decreased (△ FR<0, $P<0.05$) in 14, and, 3 neurons respectively. Although stimulation at Aδ-1 Hz did not always decrease BP, an increased FR (△ FR>0, $P<0.01$) was observed in 10 neurons. In response to the same stimulus, 5 neurons decreased their FR (△ FR<0, $P<0.05$) and the remaining 2 neurons showed no measurable changes in either FR or BP as shown in Fig. 8. For a futher study, these response patterns of FR and BP were classified, and we presented in Fig. 9. Out of possible 81 response patterns

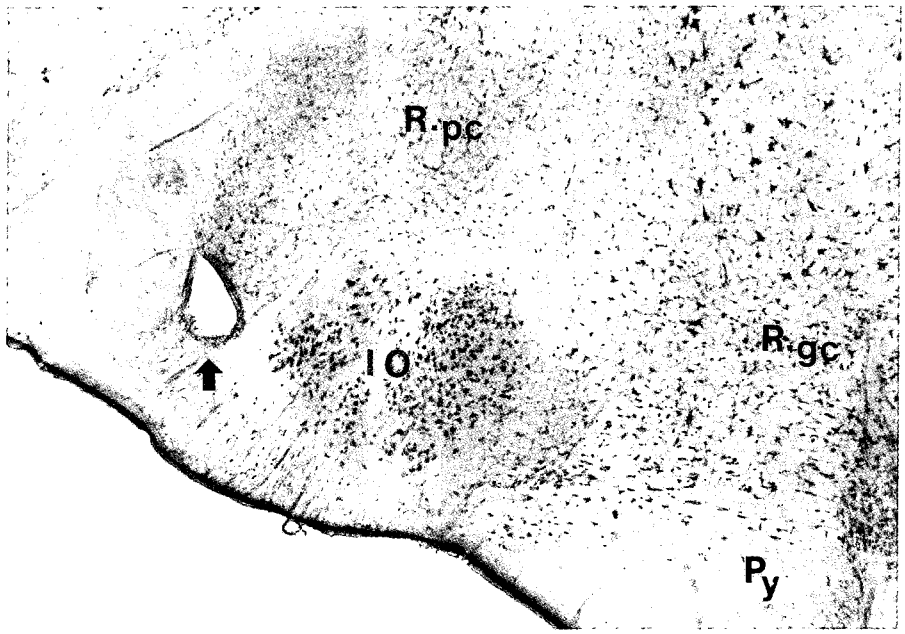

Fig. 10. Photomicrograph taken from a 100 μm frontal section of rostral ventrolateral medulla (RVLM) stained with cresyl violet: 10 mm posterior to the interaural line. Electrical lesion is seen as a white hole at the arrow; IO, inferior olive; Py, pyramid; R. gc, nucleus reticularis gigantocellularis; R. pc, nucleus reticularis parvocellularis.

$[(3^2)^2=81$ for two variables taking three (increase, decrease, and no change) possible values in response to two different stimuli], all 17 CVNs belonged to one of the 8 types of response pattern shown in Fig. 9.

Location of CVN's in RVLM

When reconstructed and reviewed based on the aforementioned electrolytic lesions, the recording sites in the RVLM were all located within the area dorsolateral to the inferior olivary complex (IO), lateral to the nucleus reticularis gigantocellularis (R. gc), and medial to the ventral extension of the nucleus reticularis parvocellularis (R. pc). Fig. 10 shows an example of the medullary section photomicrograph electrically lesioned at a site 5 mm rostral to the obex, 3.75 mm lateral from the midline, and approximately 4.5 mm deep from the dorsal surface. Since the area including the recording sites in theis experiment is well consistent with the so-called anatomic RVLM area (Barman & Gebber, 1985; Ruggiero et al, 1989), all our CVNs were RVLM neurons.

DISCUSSION

While the mechanism of the somatosympathetic pressor response is well understood, how the cardiovascular neurons (CVNs) are involved with the mediation of the somatosympathetic depressor response is still in question. In the present study, we elicited the somatosympathetic reflex (SSR) by stimulating a peripheral nerve and attempted to determine the role of CVNs in the mediation of SSR. The presence of the postulated depressor neuron is demonstrated in the subsequent discussion of the present experimental results.

Cardiovascular neuron (CVN)

CVNs were identified as those neurons in RVLM that showed a meaningful waveform in STA-SND with an adequate deflection, and also, whose neuronal activities varied in a close temporal association with the cardiac rhythm as observed on PR-UNIT (Fig. 4). These are based on the concept that a neuron involved with blood pressure regulation would contribute, if such a neuron (CVN) exists in the RVLM, to the generation of SND, and the same neuron should receive an afferent feedback from the cardiovascular system, since SND deeply affects the blood pressure. As the SND would be an integrated output of neurons having this property, which are not likely to fire all at the same time, the unit discharges of a CVN do not correlate with the SND fluctuation on a one-to-one basis, but the component of a single CVN in the SND emerges only when the SND signals following each unit spike of the CVN are averaged to amplify that particular CVN's SND component (Barman & Gebber, 1981). This is further supported by the fact that a meaningless low deflection waveform (dummy average) was obtained when averaging SND triggered by a random pulse series having approximately the same mean frequency as the unit discharges of CVN (Barman & Gebber, 1981). In the present study, it was not necessary to confirm our STA-SND's with this dummy average procedure, since they all showed a great similarity in both the wave shape and size to the representative STA-SND measured in the inferior cardiac nerve by Barman & Gebber (1985). Since BP is determined by and should vary in the same way as SND in time, the STA-SND of a CVN should keep a similar periodic fluctuation with the cardiac cycle locked to BP variation. The cardiac rhythm, or the periodic cardiovascular variation in general, should be fed in turn to the RVLM for balanced regulation. The input of the cardiac rhythm to the RVLM would lead CVNs to have a firing pattern synchronous to the cardiac cycle, which can be observed on a PR-UNIT. Therefore, the PR-UNIT of a CVN has to be temporally related to the corresponding STA-SND. In our first 57 CVNs, both the STA-SND and the PR-UNIT were in good correlation with the cardiac rhythm without exception. Thus, the next 41 CVNs were identified only on the basis of a PR-UNIT synchronous to the cardiac cycle. This lessened the technical difficulties to a great degree. Therefore, all our 98 neurons were considered CVNs.

Antidromic stimulation

Anatomic projection of CVN to the spinal cord can be examined by antidromic stimulation. When 51 RVLM-spinal neurons were antidromically stimulated by Barman & Gebber (1985), 34 neurons were activated by the stimulation of the T_2 level and of more caudal thoracic spinal segments (T_{11} and/or T_6), while the other 17 were activated only by stimulating in the T_2 level. Since all the RVLM-spinal neurons, some of which exerted widespread excitatory influence on the sympathetic outflow, were activated at the T_2 level, it may not be necessary to stimulate the lower thoracic segments. Furthermore, only one out of 51 neurons terminated its projection at the contralateral rather than the ipsilateral IML. When we delivered the antidromic stimulation through the IML of the ipsilateral T_2 segment, 20 CVN's were activated and identified as reticulospinal neurons. Their mean axonal conduction velocity was 4.3 ± 0.5 m/s (mean $\pm$ SE, $n=20$), quite similar in value to 3.5 ± 0.3 m/s of Barman & Gebber (1985).

Somatosympathetic reflex (SSR)

SSR is the reflex response of the sympathetic nervous system to the somatic or visceral afferent stimulation. Physiological responses following SSR can be observed in the cardiovascular system and other internal organs as well. SSR has been traditionally evaluated by measuring BP, which is one of the most important variables reflecting the integrated cardiovascular responses to SSR. Stimulating the peripheral

nerve at C-intensity at a high frequency (>20 Hz) and at Aδ-intensity at a low frequency (< 20 Hz) increased (pressor response), and decreased (depressor response) BP in vagotomized cats respectively (Johansson, 1962; Chung & Wurster, 1976). Therefore, we intended to induce the pressor and depressor responses of SSR by stimulating the sciatic nerve at C-20 Hz and Aδ-1 Hz, respectively. While BP always substantially increased with C-20 Hz stimulation, the decrease in BP due to Aδ-1 Hz stimulation was much smaller (20~30% of C-20 Hz stimulus) and sometimes no measurable change was observed in our cats with the vagi intact.

Since BP is not only determined by SND through peripheral resistance variation, but also affected by the heart via the cardiac output, intact vagi should have lessened the BP change compared to the vagotomized animals due to the feedback control of BP by the heart. Furthermore, the contribution of C-fibers to SSR is much larger than that of Aδ-fibers in cats (Kim et al, 1992), and an additional temporal facilitation occurs when C-fibers are stimulated at a high frequency such as the C-20 Hz stimulus in our experiment. Therefore, a much weaker depressor response compared to the pressor response is to be expected in the present experimental design. To test this hypothesis, a bilateral cervical vagotomy was performed in four animals, the BP of which did not decrease in response to Aδ-1 Hz stimulus with the intact vagi. As a result of vagotomy, all four animals revealed substantially larger (>6 times compared to the intact vagi) depressor responses.

However, the intact vagi are a preliminary preparation necessary for identification of CVN, without which the PR-UNIT loses the temporal association with the cardiac rhythm due to the lack of an afferent transmission pathway to the RVLM. We think that the preparation and experimental protocol presently employed are still valid as far as the purpose of our study is concerned, since BP actually decreased enough in most animals, to allow differentiation of the depressor neurons, as discussed in the following.

Role of CVN in SSR

The Aδ-1 Hz and C-20 Hz stimuli, presumably eliciting the depressor and pressor responses of the SSR, respectively, produced changes in blood pressure (△ BP) and CVN firing rate (△ FR), which were evaluated simultaneously. If different groups of CVNs mediate SSR in different ways, CVNs showing different response patterns in response to each stimulus are expected. Response patterns of both variables fell into 8 categories as presented in Fig. 9. A primary guideline, in an attempt to differentiate CVNs mediating the pressor response of SSR, may be an increase in FR (△ FR>0) with a concomitant increase in BP (△ BP>0) induced by C-20 Hz stimulus. On the contrary, the CVN mediating the depressor response would decrease its FR (△ FR <0) in response to the same stimulus. This criterion classified the CVNs showing the response patterns, a, b, e, g, and h, of Fig. 9, as pressor neurons and c, d and f as depressor neurons. We tried to further support this differentiation by reviewing the response patterns to Aδ-1 Hz stimulation, presumed to cause opposite changes. Unfortunately, however, in a few cases the response patterns were not consistent with the C-20 Hz stimulation results. It is expected that CVNs with the response pattern, a, classified as pressor neurons based on the observation of △ FR>0 to C-20 Hz stimulus, would decrease FR (△ FR< 0) in response to Aδ-1 Hz stimulus, but in fact, an increased FR was observed (△ FR>0). Similar inconsistency is found in the response pattern, d, where FR decreased in response to both C-20 Hz and Aδ-1 Hz stimuli. Sometimes, e.g., in the response pattern, h, there was no measurable change in BP during the stimulation with Aδ-1 Hz, making it difficult to determine whether the depressor response has really occurred. This variability, we think, is to be expected to some degree in view of the fact that the depressor response was much weaker and sometimes inconsistent compared to the pres-

sor response.

Nevertheless, it is necessary to take a special look at the response pattern, c, which showed a clear depressor neuron characteristic in response to both stimuli. Neurons with the response pattern, c, decreased and increased FR during C-20 Hz and Aδ-1 Hz stimuli, respectively, with corresponding increase and decrease in BP. Furthermore, one of these neurons also had an axonal projection to the IML of the spinal cord, as verified by antidromic stimulation. This strongly suggests the possibility of independent depressor neurons associated with the depressor pathway being present. Therefore, a group of CVNs operating as depressor neurons could be involved with an independent depressor pathway without tonic inhibition from the CVLM in the mediation of the SSR.

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