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Discharge Patterns and Peripheral Nerve Inputs to Cardiovascular Neurons in the Medulla of Cats: Comparison between the lateral and medial medulla

Sang Jeong Kim, Wonil Lim, Myoung Kyu Park, Jin Lee and Jun Kim

Department of Physiology & Biophysics, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-799, Korea

=ABSTRACT=

The discharge patterns and peripheral nerve inputs to cardiovascular neurons were investigated in rostral ventrolateral medulla (RVLM) and raphe nucleus of cats. The data from the two were compared to determine their roles in cardiovascular regulation and the endogenous analgesic system. Animals were anesthetized with α-chloralose and single cell activities were recorded by carbon-filament microelectrode and their relationships with cardiovascular activity were analyzed. In RVLM area, a total of thirty-three cells were identified as cardiovascular neurons. During one cardiac cycle, the mean discharge rate of the neurons was 1.96±0.29 and the peak activity was observed 45 ms after the systolic peak of arterial blood pressure. Thirteen cells could be activated antidromically by stimulation of the the T_2 intermediolateral nucleus. Forty-three raphe neurons were identified as cardiovascular neurons whose mean discharge rate during one cardiac cycle was 1.02±0.12. None of these cells could be activated antidromically. Study of the interval time histogram of RVLM neurons revealed that the time to the first peak was 128±20.0 ms, being shorter than the period of a cardiac cycle. The same parameter found from the raphe neurons was 481±67.2 ms, which was much longer than the cardiac cycle length. Of seventeen RVLM neurons examined ten received only the peripheral Aδ-afferent inputs, whereas six RVLM neurons received both Aδ-and C-inputs; the remaining one cell received an inhibitory peripheral C-input. In contrast, nine of eleven raphe neurons were found to receive Aδ-inputs only. We conclude that the main output of cardiovascular regulatory influences are mediated through the RVLM neurons. The cardiovascular neurons in the raphe nucleus appear to serve as interneurons transferring cardiovascular afferent information to the raphespinal neurons mediating the endogenous analgesic mechanisms.

Key Words: RVLM, Raphe neuron, Cardiovascular neurons, Rhythmic activity, Peripheral afferent inputs

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INTRODUCTION

The rostral ventrolateral medulla (RVLM) has been known to play a key role in maintaining the arterial blood pressure. The RVLM neurons involved in blood pressure regulation show spontaneous rhythmic discharge pattern synchronous to the sympathetic activity and cardiac rhythm (Barman & Gebber, 1981). Many of them send their terminals to the intermediolateral nucleus (IML) of the thoracic spinal cord. Most of them are vasopressor neurons and an increase in their activity elevates the arterial blood pressure and an decrease lowers it. The RVLM neurons are involved in the somatosympathetic reflex (Morrison & Reis, 1989; Stornetta et al, 1989; Sun & Spyer, 1991; Kim et al, 1992). These findings suggest that the medullospinal cells in RVLM act as a pre-autonomic neurons to the sympathetic preganglionic neurons in the IML.

In contrast to the vasopressor pathways mediated by the RVLM, several areas in the ventral medulla seem to be involved in vasodepressor pathways. For example, there are neurons in the caudal ventrolateral medulla (CVLM) which are involved in the baroreflex pathway (Blessing & Reis, 1982; Willette et al, 1984; Granata et al, 1986; Gordon, 1987; Agarwal et al, 1990; Cravo et al, 1991) and inhibit the IML sympathetic activity indirectly through inhibition of the RVLM neuronal activity. Raphespinal neurons are well known for their involvement in the endogenous analgesic system. In view of a close interaction between pain and sympathetic activity (Randich & Maixner, 1984), it is possible that raphe neurons participate in that interaction. Experimental evidence in support of such conjecture is that the raphe neurons exert a sympathoinhibitory effect by directly projecting to the IML (Morrison & Gebber, 1982; 1984; 1985).

In the present study we compared the electrophysiological characteristics of cardiovascular neurons by measuring the spontaneous firing pattern and responses to peripheral nerve stimulation. A specific purpose of our study was to determine the possible role of these neurons in cardiovascular regulation.

MATERIAL AND METHODS

Animal preparation

Twenty-five adult cats of either sex (2-3 kg) were used. The animals were pretreated with atropine (0.1 mg/kg, i.m.), sedated with ketamine (Ketalar, 20 mg/kg, i.m.) and anesthetized with α-chloralose (60 mg/kg, i.v.). The trachea, femoral artery and vein were cannulated for artificial ventilation, blood pressure monitoring and drug injection, respectively. The animals were paralyzed by intravenous administration of pancuronium bromide (Mioblock, Organon, initial dose 0.4 mg, maintenance dose 0.4 mg/hour). End-tidal PCO_2 was monitored (Normocap Co_2 & O_2 monitor, Datex) and maintained within 3.5~4.5 % by adjusting the respirator. Rectal temperature was monitored and maintained within 37~38 °C by an electric blanket (Homeothermic Blanket Control Unit, Harvard). The electrocardiogram was recorded using subcutaneous pin electrodes.

The upper thoracic spinal cord was exposed by a laminectomy on the T_1-T_3 vertebrae. An occipital craniectomy was performed, cerebellum was removed to expose the floor of the IVth ventricle. The left sciatic nerve was isolated for electrical stimulation.

The animal was mounted on a stereotaxic frame and mineral oil pools were made with incised skin flaps over the exposed areas. A water circulating heating coil was used in the thoracic pool to compensate for heat loss. Bilateral thoracotomies were done to reduce the movement related to the respiration.

Electrical stimulation

A tripolar platinum electrode was hooked around the sciatic nerve for electrical stimulation. For the activation of Aδ-fiber, stimuli of 1 mA, 0.1 ms square pulses were generated (Pulsemaster A 300, WPI) and delivered to the electrode and for C-fiber activation, stimuli of 10 mA, 0.5 msec were applied. For antidromic stimulation of medullospinal tract cell, a concentric bipolar electrode was positioned at the dorsolateral sulcus of T_2 spinal cord and lowered ventrally by 1.5 to 2 mm, while delivering 2 Hz stimuli of 500 μA and 0.1 ms. The final position was determined by the maximum pressure change elicited. The conventional criteria of antidromic activation (Fuller & Schlag, 1976) was used for identification of the medullospinal cells.

Neural recordings

Single cell activity in the RVLM was recorded with a carbon-filament electrode (tip resistance 2-3 M Ω at 1 kHz). The obex was used as a surface landmark and the electrode was placed on the dorsal surface of the medulla, 2-6 mm rostral to the obex and 0-1 mm from the midline for raphe neurons and 3-4 mm for RVLM neurons. The electrode was lowered down with a pulse motor microdrive (PC-5N, Narishige). Usually the single cell activities related to the cardiovascular activity were picked up at depth of 3-7 mm from the dorsal surface. The electrical signals were amplified (band pass 0.3-10 kHz, gain 10,000, DAM-80, WPI) and displayed on oscilloscopes and stored on an IBM-PC/AT computer through a window discriminator (Frederick Haer & Co) and a laboratory interface (CED 1401). For identification of cardiovascular neurons, post R-wave histogram (500 sweeps, bin width 10 ms, bin number 100) and interval time histogram (1000 sweeps, bin width 10 ms, bin number 100) were compiled. The peripheral nerve inputs were studied by compiling post stimulus time histogram (bin width 2 ms, bin number 1500, 20-40 sweeps) and single pass time histograms (bin width 1 s, bin number 500).

Histology and statistics

At the end of each experiment, electrolytic lesions were made to mark the location of the electrode tip by passing a DC current of 100 μA for 20s. Then the animal was sacrificed with sufficient dose of anesthetics and the medulla was removed and fixed in 4% formalin solution for at least a week. Frontal sections, of 50 μM thickness , were obtained using a vibratome and stained with cresyl violet. The recorded sites were reconstructed by referring to the electrolytic lesions. The results are expressed as means$\pm$SE. Significant difference between groups were determined with Student's *t*-test.

RESULTS

Cardiovascular neurons in RVLM

Figure 1 shows an example of a typical cardiovascular neuron recorded in the RVLM area. The location of the cell was: 7.0 mm rostral to the obex, 3.75 mm from the midline and 6.929 mm depth (we would like to indicate the coordinate such as 7.0-3.75-6.929, hereafter). It could be activated antidromically with a latency of 15.6 ms (conduction velocity was 7.8 m/s). A post R-wave histogram shows cardiac-related rhythmic discharge. The first peak of arterial blood pressure was at 70 ms and that of neuronal activity was at 120 ms after R-wave. A total of thirty-three cells was identified as cardiac-related neurons. Of these thirteen cells were activated antidromically from the T_2 spinal cord with a mean conduction velocity of 7.95$\pm$1.55 m/sec. The first peak of arterial pressure and neuronal activity were 66$\pm$3.1 and 111$\pm$10.4 ms, respectively.

A representative cardiac-related neuronal

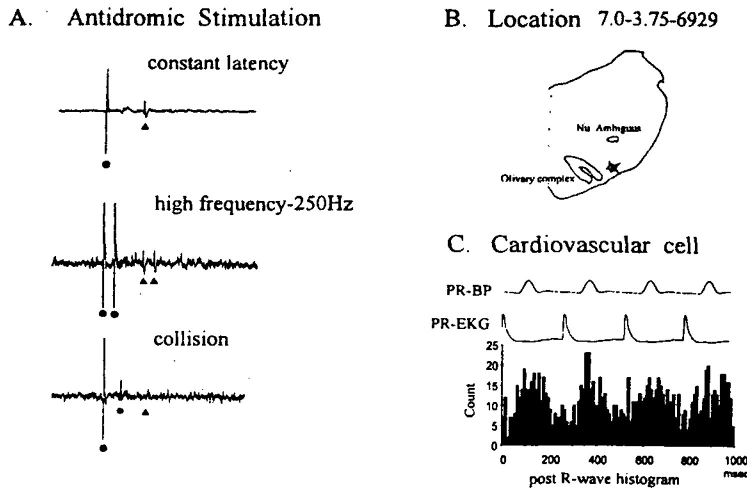

Fig. 1. An example of a pressor cell recorded from rostral ventrolateral medulla. A. Evidences for antidromic excitation from T_2 spinal cord. (●) represents stimulation artefact. (▲) represents cell activity evoked by antidromic stimulation. (*) represents spontaneous cell activity. B. Location of recorded site: 7.0 mm rostral to the obex, 3.75 mm lateral from the midline and 6930 μm in depth from dorsal surface of the medulla (7.0-3.75-6930). C. Post R-wave histogram identifies the unit as a cardiovascular neuron (500 sweeps, 100 bin, 10 msec). PR-BP: post R-wave blood pressure, PR-EKG: post R-wave EKG.

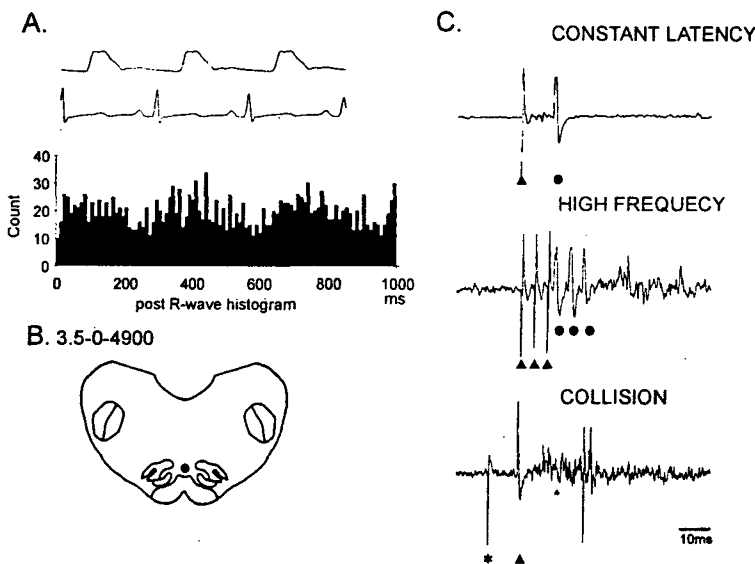

Fig. 2. An example of a cardiovascular neuron recorded from the raphe nucleus. A. Post R-wave histogram of the neuronal activity, blood pressure and electro-cardiogram justify the cardiovascular neuron in the raphe nucleus. B. Location of the recorded site (3.5-0-4900). C. Antidromic stimulation test of nearby raphe neuron that lacks cardiovascular activity. (▲) represents stimulation artefact. (●) represents cell activity evoked by antidromic stimulation. (*) represents spontaneous cell activity.

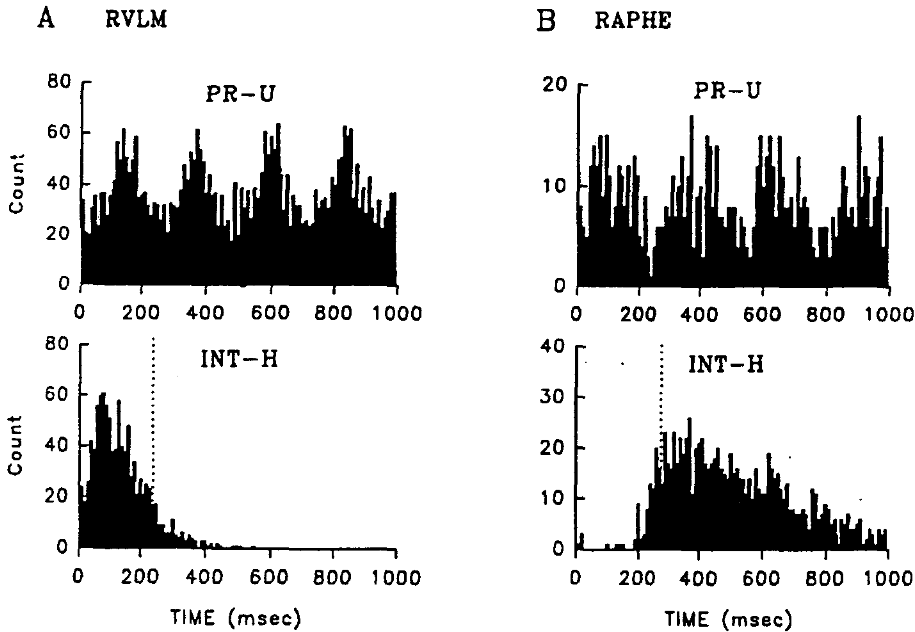

Fig. 3. Comparison of firing pattern between RVLM and raphe cardiovascular neurons. A. post R-wave unit histogram (PR-U; 500 sweeps, 100 bin, 10 msec bin width) and interval time histogram (INT-H; 1000 intervals) of a typical RVLM neuron. The time to peak in INT-H that means the most frequent firing interval is 80 msec. The peak interval is shorter than the cardiac cycle length that is shown as dotted line in INT-H. B. PR-U and INT-H of a typical raphe neuron. The time to peak in INT-H (360 msec) is longer than the cardiac cycle length.

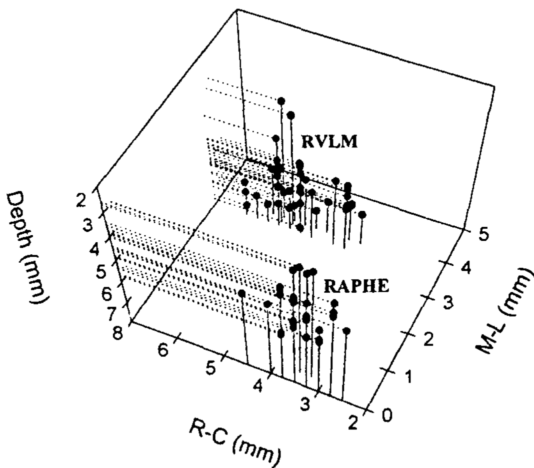

Fig. 4. Location of RVLM and raphe cardiovascular neurons plotted using the three dimensional stereotaxic coordinates. The median location of the RVLM and raphe cells are 5.0-3.6-6.000 and 3.4-0-4.200, respectively. M-L: mediolateral coordinate, R-C: rostrocaudal coordinate, Depth: depth from the dorsal surface.

Table 1. Difference in firing pattern between lateral and medial medullary cardiovascular neurons

	Lat. medulla	
Time to 1st peak of interval time histogram(ms)	128±20.0	481±67.2
No. of discharge during one cycle	1.8±0.46	1.1±0.12

activity recorded in the raphe area is illustrated in Figure 2. Its stereotaxic coordinate was 3.5-0-4.900. The first peaks of arterial pressure and cell activity were at 90 and 133 ms after R-wave, respectively. In forty-three such cells the difference between the peaks of arterial pressure and cellular activities was the same as those of RVLM cardiovascular neurons. This cell could

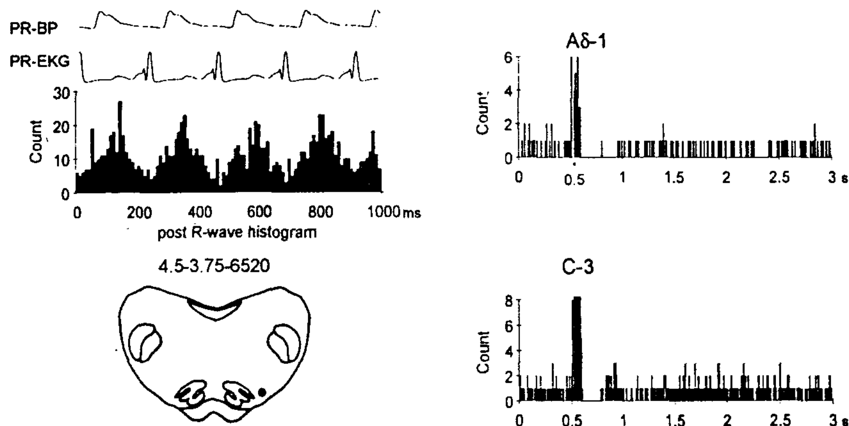

Fig. 5. A representative cardiovascular neurons recorded from the RVLM area (4.5-3.75-6520). The rhythmic pattern in post R-wave histogram is well correlated with cardiac cycle that is shown in PR-BP and PR-EKG. Note that this cell received only peripheral Aδ-afferent inputs. Further increases in stimulation intensity to C-fiber activation range did not increase the firing any more.

not be activated antidromically by T_2 IML stimulation. As indicated on Figure 2C, however, we could activate antidromically some nearby cells, which were not spontaneously active and hence did not show any cardiac related activities.

Interspike interval histograms were constructed to compare spontaneous firing patterns of the raphe and RVLM neurons. As shown in Figure 3, the time to peak in RVLM neuronal group (80 ms in A) was much shorter than that in raphe neuronal group (360 ms in B). That of RVLM cell was shorter than the cardiac cycle length while that of raphe neuron was longer than the cardiac cycle length. The data obtained from all of the recorded cells were summarized in Table 1. Times to peak were 128 ± 20.0 ms (n=19) and 481 ± 67.2 ms (n=22) in RVLM cell and raphe neuron, respectively, and this difference was statistically significant ($p<0.05$). The mean firing frequencies during one cycle length were 1.96 ± 0.29 (n=43) in RVLM cell while that in raphe cells was 1.02 ± 0.12 (n=28). These values differed significantly ($p<0.05$). Some cells exhibited several peaks in interval time histogram (six cells in raphe and three cells in RVLM). In Figure 4, a map of the recorded cells was reconstructed using the three dimensional stereotaxic coordinates. The representative location of the RVLM and raphe cells were 5.0-3.6-6.000 and 3.4-0-4.200, respectively.

Finally we observed responses of cardiovascular neurons to the peripheral nerve stimulation. Shown in Figure 5 is an example of RVLM neuronal responses. The cell was located at 4.5-3.75-6.520. It responded to an excitatory Aδ-afferent inputs but increasing the stimulation intensity to C-fiber activation range failed to produce an aditional increase in the firing rate. Of seventeen RVLM cells tested this type of response was observed in ten cells. On the other hand, six cells received both excitatory Aδ- and C-afferent inputs and remaining one cell received excitatory Aδ- and inhibitory C-input. Among eleven raphe neurons, nine received only Aδ-inputs and the remaining two cells received both Aδ- and C-inputs. On the interval time histograms these two cells showed periodicity.

DISCUSSION

During the last two decades RVLM has been studied extensively as a key locus for the maintenance and regulation of the arterial blood pressure. Following contentions are the generally accepted: 1) various inputs from many sources such as baroreceptor, peripheral nociceptors, hypothalamus etc., are known to converge on the RVLM, 2) the medullospinal cells in RVLM are the main sources of sympathetic outflow from the medulla to the intermediolateral nucleus of spinal cord, and 3) the increase in their discharge rate elevates the arterial blood pressure and the decrease results in blood pressure drop.

The raphe nucleus also contains cardiac related cells and their activation is known to inhibit the sympathetic activity (Morrison & Gebber, 1982; 1984). In our lab we observed that lesions of the RVLM area abolished the vasopressor response to the peripheral nerve stimulation, while vasodepressor response remained (Jun et al, 1991) and that the firing rate of some medullospinal cells in RVLM increased during vasodepressor response and decreased during the vasopressor responses to the peripheral nerve stimulation (Kim et al, 1993). One may suggest several possibilities on the role of cardiac related cells in raphe area. Raphe has been well known for activating the endogenous analgesic system (Basbaum & Fields, 1978; 1984) and there exists a close interaction between pain and the sympathetic system (Randich & Maixner, 1984). Thus it seems to be reasonable to postulate that cardiac related cells in raphe are involved in the interaction of the pain-sympathetic system. Another possibility is that they are involved in vasodepressor pathways which are independent from RVLM vasopressor pathway. If it is true, RVLM may not be the premotor nucleus for the spinal sympathetic outflow. In any case a cardiac related neurons in raphe should also have the same properties as those of RVLM vasopressor cells: 1) they should have spontaneous activities, 2) receive baroreceptor inputs and 3) show rhythmic discharge coincident with cardiac rhythm.

In the present study, RVLM and raphe neurons exhibited similar time to the first peaks after R-wave triggering. This suggests that afferent inputs from baroreceptors are linked to the RVLM and raphe not in series but in parallel pathways. In the cat the distance between the solitary nucleus, which receives baroreceptor inputs, and the RVLM or raphe is approximately 5 mm (estimated using stereotaxic atlas) and the conduction velocity seems not faster than 1 m/s.

The mean number of action potentials in the RVLM neurons during a cardiac cycle was 1.8 that is similar to the 2-6 Hz sympathetic activity (Gebber & Barman, 1980). On the contrary, that of the raphe neurons was 1.1. This suggests that the rhythmic activities of raphe neurons do not determine the sympathetic activity. This idea is further supported by the finding that the time to peak of interval time histogram of RVLM neurons was 128 ms which is much shorter than the cardiac cycle length, while that of raphe neuron was 481 ms which is much longer than cardiac cycle length. This observation also suggests that the baroreceptor inputs to the raphe nucleus are not directly involved in the cardiovascular regulation process, although we could not rule out the possibility that the oscillatory neuronal network (Selverston & Moulins, 1985) in the raphe area is involved in generation of the basic rhythm of sympathetic system. In the present study we could not activate the raphe cardiac related neurons antidromically from the T_2 spinal level, suggesting that no direct connection exists between the raphe neurons and the sympathetic preganglionic neurons. The result is not consistent with those of Morrison & Gebber (1982; 1984). They described two types of cardiac related raphe neurons (type I and II)

and a quarter of type I cell could be activated antidromically. Although we could not activate cardiac related cells we could activate many raphespinal neurons just nearby the recorded cardiac-related cells. Antidromically activated cells, however, were not spontaneously active.

It is well known that noxious peripheral afferent inputs can evoke changes in arterial blood ressure (Sato & Schmidt, 1973). In α-chloralose anesthetized cats, low frequency stimulation of peripheral nerves at the Aδ-intensity, elicits depressor response, while high frequency stimulation at the C-intensity, elicits pressor response (Chung & Wurster, 1976; Chung et al, 1979). The noxious inputs should be transmitted to vasomotor area to elicit changes in arterial pressure. Earlier studies have provided evidence that the terminals of spinoreticular tract cells are distributed in the medial reticular formation within 2.5 mm of the midline and most of these neurons receive only Aδ-inputs (Casey, 1969; Goldman et al, 1972; Fields et al, 1975). In the present study most of the raphe cardiac-related cells received only Aδ-input. Whereas, a third of the RVLM cardiac related cells received both Aδ-and C-inputs which confirms Ammons' (1988) recent study. These results, combined with demonstration that a lesion of the RVLM area abolishes pressor response elicited by peripheral C-fiber stimulation (Jun et al, 1991) and the activity of RVLM neurons increases during C-pressor response (Kim et al, 1992), support the idea that the RVLM neurons receive both Aδ- and C-inputs while raphe neurons receive only Aδ-inputs. The RVLM, not raphe, neurons seem to be involved in mediation of somatosympathetic pressor response, while their mediation of somatosympathetic depressor response remains to be elucidated.

In conclusion, the medullary mechanism of arterial blood pressure regulation is mediated mainly through RVLM cardiac related neurons, while the raphe cardiac related neurons may be involved in the feeding of baroreceptor inputs to the endogenous analgesic system.

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