Coexistence of Neurons Integrating Urinary Bladder Activity and Pelvic Nerve Activity in the Same Cardiovascular Areas of the Pontomedulla in Cats

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Abstract

The present study examines the coexistence of neurons in the same cardiovascular point of the pontomedulla that integrates urinary bladder (UB) motility, and pelvic nerve activity (PNA). Microinjection of monosodium L-glutamate (Glu) into the locus coeruleus (LC), the gigantocellular tegmental field (FTG), the rostral ventrolateral medulla (RVLM), and the dorsomedial medulla (DM) produced pressor responses, whereas injection into the lateral tegmental field (FTL), the nucleus of tractus solitarii (NTS), and the caudal ventrolateral medulla (CVLM) produced depressor responses. However, microinjection of Glu into the dorsomotor nucleus of the vagus (DMV) and the ambiguus nucleus (AN), where the vagus nerve originates, produced marked bradycardia. Many of these cardiovascular responses were accompanied by increased, or decreased parasympathetic PNA. In six animals, sympathetic renal nerve activity (RNA) and PNA also increased simultaneously during the pressor response. The present study also examines the connection between the DMV–AN and the sacral intermediolateral column (IML), where parasympathetic preganglionic neurons (PGNs) of the pelvic nerve located. Biotinylated dextran amine (BDA), an anterograde tracer, was iontophoretically injected into the DMV or AN. No labelled terminal or neuron was detected in the sacral IML, but labelled terminals were observed in the bilateral LC, and also in the bilateral sides of the FTG, FTL, RVLM, DM, and CVLM. These results suggest that neurons of the DMV and/or AN may indirectly regulate the sacral parasympathetic PGNs through the LC for supraspinal control of the pelvic nerve. Furthermore, these results also suggest the coexistence of multiple autonomic integrating mechanisms of different kinds within various cardiovascular areas of the pontomedulla.

Key Words: glutamate, pelvic nerve activities, pressor areas, sacral parasympathetic system

Introduction

The importance of supraspinal structures in the neural control of micturition was first identified by Barrington in his pioneer research (2). The existence and location of a neural structure essential for micturition and reflex urinary bladder (UB) activity in various species of animals was later demonstrated in the region of the locus coeruleus (LC) and its complex (13, 21, 22, 25, 31). The LC structure also contains a dense population of noradrenergic neurons, which may be important for sympathetic integration of cardiovascular functions (6, 16, 27).

The pelvic nerve, which originates from the sacral spinal cord, is the major parasympathetic nerve serving the pelvic organs, and provides parasympathetic output to integrate the functions of the pelvic viscera, including

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the UB (15, 28). With the application to the pelvic nerve of horseradish peroxidase (HRP), this retrograde axonal transport agent was identified in the intermediolateral column (IML) of the sacral spinal cord, which corresponds to those of the thoraco–lumbar IML of the sympathetic system (4, 12–15, 23, 29, 30). Using electrophysiological techniques to stimulate the pelvic nerve and record the evoked responses from the parabrachial nucleus, Noto and his colleagues have also demonstrated the existence of a supraspinal reflex pathway to the UB. They also showed that at various sites in the dorsal tegmentum, this pathway can modulate pelvic nerve activity (PNA) (32).

Electrical or chemical activation of neurons in the LC produces a very active vasomotor response, as well as contraction and voiding of the UB (8, 32). Not only the LC, but many brainstem areas that integrate cardiovascular functions also modulate a variety of UB functions in cats, suggesting that the sacral parasympathetic system is activated simultaneously (8). These areas include the gigantocellular tegmental field (FTG), the lateral tegmental field (FTL), the rostral ventrolateral medulla (RVLM), the dorsomedial medulla (DM), and the caudal ventrolateral medulla (CVLM). The cardiovascular responses induced from the above structures are accompanied with different patterns of sympathetic nerve firing (9). Therefore, the present study examined whether the above-mentioned cardiovascular reactive areas are involved in modulating the PNA.

The parasympathetic systems are separated into cranial and sacral parts. Little is known, however, about the communication between these two parts. Of the cranial parasympathetic nerves, the vagus nerve has been correlated closely with cardiovascular integration. The parasympathetic preganglionic fibers of the vagus originate from the dorsal motor nucleus of the vagus (DMV) and the ambiguus nucleus (AN) in the medulla (1, 19, 24). Stimulation of the DMV or the AN by monosodium L-glutamate (Glu) produces marked bradycardia and changes in the motility of the gastrointestinal tract. The latter effect is more apparent during stimulation of the DMV than of the AN (17). Therefore, the present study examines the connection between the parasympathetic preganglionic neurons of the DMV–AN and the PGNs of the sacral IML region.

Materials and Methods

General Procedures

Fifty cats of either sex, weighing 2.4–3.2 kg, were anesthetized with a mixture of urethane (400 mg/kg) and α-chloralose (40 mg/kg) administered intraperitoneally (i.p.), and paralyzed with gallamine triethiodide (2 mg/kg per 30 min, intravenously [i.v.]). The rectal temperature was maintained at 37.0 ± 0.5 °C with an autoregulated heating pad. The trachea was cannulated and artificially ventilated with spontaneously-breathed O_2-enriched room air to maintain the end-tidal CO_2 at approximately 4%. The left femoral vein and artery were cannulated with a polyethylene tube. The former was used for drug administration and the latter for monitoring systemic arterial blood pressure (SAP), mean SAP (MSAP) and heart rate (HR). All recordings were made with a Gould ES-1000 polygraph.

Recording the Urinary Bladder Motility

An abdominal midline incision was made to expose the UB and urethra. The motility of the UB was monitored with a balloon system connected to a pressure transducer (Gould, model 13-4615-50). The balloon was placed into the bladder lumen through an incision in the fundus of the UB and inflated with saline, usually 1–2 ml, to keep the UB at a constant and optimal volume to ensure a sensitive detection of changes in UB motility and PNA. The latter procedure is important because the UB responses to brain stimulation are affected by intravesical pressure (15). At high intravesical pressure, relaxation is intensified and contraction is inhibited; at low intravesical pressure, contraction is intensified and relaxation is inhibited. Only at optimal intermediate intravesical pressure, can both the contraction and relaxation of the UB that accompanies the changes in PNA be observed simultaneously with the cardiovascular responses during brain stimulation. Stimulation, either electrical or chemical, was usually applied during the interval between rhythmic contraction of UB. In some instances stimulation was applied during the course of contraction to demonstrate the inhibition. Magnitude of contraction was computered by measuring the area (mm^3) of the contraction curve before and after stimulation.

Recording the Nerve Activity

The unilateral pelvic nerve lies in close proximity to the detrusor muscle. After dissection of the urethra,

the nerve was isolated from the surrounding connective tissue, cut at the distal end, and desheathed using an operating microscope. It was then placed in a bipolar platinum electrode with a conventional electrophysiological setup, consisting of a differential amplifier (bandpass: 10–3k Hz) to amplify and record the efferent activity of the whole-bundle nerve. Nerve activity was rectified and integrated using an integrator (Gould model 13-4615-70) with a reset time of 5 s. Nerve signals were monitored with an oscilloscope (Tektronix 5113) and stored on a tape recorder (Neuro Data DR-890) for later analysis. Sympathetic renal nerve activity (RNA) was also recorded in six cats, as previously described (6).

Brain Stimulation

The head of the animal was fixed in a David-Kopf stereotaxic apparatus. After occipital craniotomy, the brainstem was exposed and the cardiovascular points at various brain loci were approached stereotaxically according to their coordinates. Both electrical stimulation and Glu microinjection were delivered through a two-barrel glass micropipette, pulled to an outside-tip diameter of approximately 20–30 μm, which was inserted into the brainstem nuclei at an angle of 34°. One barrel was filled with 3 M NaCl, in which a platinum wire was inserted as an electrode for electrical stimulation, and the other was filled with Glu (0.1 M) dissolved in artificial cerebrospinal fluid containing 1% pontamine sky blue, pH 7.4 (Sigma) (10). The reactive points were identified first by electrical stimulation, and then by microinjection of Glu after the electrically induced response subsided (at least 3 min) (7). Electrical stimulation was applied as rectangular pulses delivered through a constant-current unit connected to a stimulator (Grass S-88). Each brain point was stimulated for 15 s with a train of pulses of 0.5 ms, 80 Hz at 50 μA. If a positive response was recorded, then Glu was injected by nitrogen gas through a pressure-regulated pneumatic pump (Pneumatic Pressure System, Model PPS-2, Medical Systems Greenvale, NY) over an interval of 5–10 s.

Identification of Stimulating Points

At the end of each experiment, the animal was sacrificed with an i.v. overdose of sodium pentobarbital. The brain was removed and fixed in 4% formaldehyde in saline overnight at 4 °C, and serial sections of 50 μm thickness were transversely cut using a cryostat (Reichert-Jung, 2800 Frigocut E). One set of sections was left blank for gross identification of the Glu-injection site by staining with pontamine sky blue. The other was counterstained with cresyl violet for detailed histology.

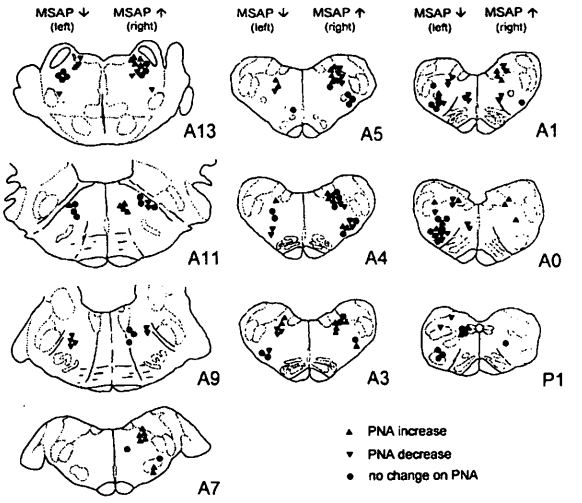

Fig. 1. Points in pons and medulla evoking changes of PNA and SAP. Changes of cardiovascular parameters and PNA consequent to Glu stimulation (30-50 nl, 0.1 M) were mapped on the lower brainstem from A13 (13 mm rostral to the obex) to P1 (1 mm caudal to the obex). Points porduced increase of MSAP (↑) were mapped on the right side, while decrease of MSAP (↓) on the left side. ▲ indicates increase; ▼ decrease; ● no change of PNA. Note that points showing increase in PNA are concentrated at the LC of the pons, and DM and DMV of the dorsal medulla. Points showing no change of PNA are concentrated at the ventral medulla; RVLM and CVLM.

Data Analysis

Percentage changes in SAP, HR, and nerve activity in response to Glu stimulation were calculated as the maximum value of the change stimulated by Glu divided by the control value. Data for comparison were analyzed by one-way ANOVA and correlation methods. The difference was considered statistically significant at $p < 0.05$. All values are presented as the mean±standard error of the mean (SEM).

Anterograde Tracing with Biotinylated Dextran Amine (BDA)

Cats of either sex, weighing 2.4–3.2 kg, were anesthetized with a mixture of urethane (400 mg/kg) and α-chloralose (40 mg/kg) administrated i.p. The

brainstem was exposed for stimulation of the cardiovascular areas. Biotinylated dextran amine (BDA, 10% solution in saline; Molecular Probes), an anterograde tracer, was iontophoretically injected (5–7 μA positive current, 0.1 Hz, for 25 min) into the DMV or AN (5). Five weeks after the pretreatment, cats were sacrificed under deep anesthesia with sodium pentobarbital (40 mg/kg), i.p., and perfused transcardially with 2000 ml Ringer solution, following by 2000 ml fixative solution containing 4% paraformaldehyde and 1.25% glutaraldehyde in phosphate-buffered saline (PBS, 0.1 M, pH 7.4) at 4 °C. The brain and spinal cord were removed immediately after perfusion, and placed in 30% sucrose in 0.1 M PBS overnight at 4 °C. The tissue was cut into sections of 40 μm thickness using a cryostat. An avidin-biotin-HRP complex (ABC) was used to visualize the BDA-containing fibers (18). The sections were washed in 0.5% H_2O_2 solution for 30 min, and then incubated in 0.1 M PBS with 0.25% Triton X-100 and 2% bovine serum albumin (BSA) for 1 h. Sections were washed twice with 0.1 M PBS, then incubated for 2 h in PBS containing the ABC complex (1:80) and 2% BSA. Sections were washed twice with 0.1 M PBS and twice with Tris buffer (0.05 M), and then treated with a nickel-enhanced diaminobenzidine (DAB) solution (consisting of 0.2% nickel ammonium sulfate, 0.05% DAB, and 0.003% H_2O_2 in 0.05 M Tris buffer) to yield black products (5, 36). Sections were then counterstained with neutral red.

Results

Stimulation of the cardiovascular reactive areas in the pons and medulla produced not only cardiovascular effects, but also facilitation (↑), inhibition (↓), or no change (~) in PNA. Tables 1–3 summarize these changes and the stimulation points are mapped in Figure 1. Changes in PNA and UB motility were recorded during electrical and Glu stimulation in various areas of the pons and medulla. Many points produced UB contraction simultaneously with increases in PNA during stimulation (Figs. 2, 3). In contrast, relaxation of the UB usually occurred simultaneously with PNA decrease.

Brain Stimulation Produces Increase in SAP

A slightly higher proportion of points produced an increase in PNA (PNA↑/ total stimulated points) during Glu stimulation of the DM (35/49; 71%) and LC (8/14; 57%). Figure 2A shows an example of Glu microinjection into the LC, producing SAP increases accompanied by increases in RNA and PNA. The onset of PNA increase was simultaneous with UB contraction. Similar changes were observed when Glu was microinjected into the DM (Fig. 2B), with an increase in PNA. A small proportion (7/95; 7%) of points in the DM produced pressor responses simultaneously with a decrease in PNA. The proportion of points that did not show significant change in PNA (~) in the DM (10/49; 20%) was slightly higher than in the FTG (9/13; 69%) or the RVLM (11/19; 58%). The only significant correlation between the SAP and PNA in the various brain structures was found in the LC (Table 4).

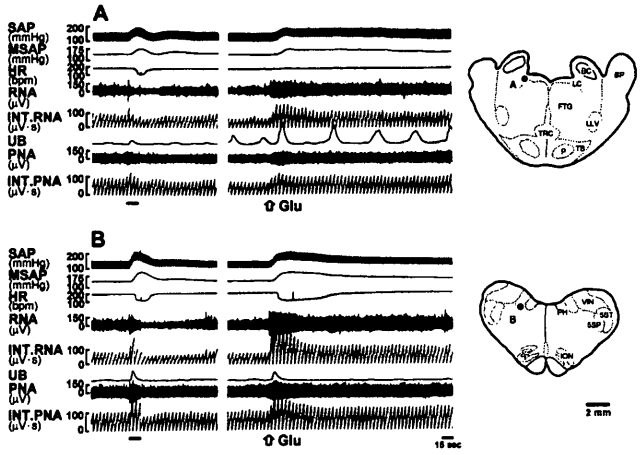

Fig. 2. Microinjection of Glu induced an increase of SAP concomitant with increases of RNA and PNA in LC (A) and DM (B). Stimulations were made at two points (solid circle): LC (A, 12 mm rostral to the obex), and DM (B, 4 mm rostral to the obex). Both electrical (50 μA) and Glu (0.1 M, 30 nl) stimulations produced an increase of SAP accompanied with increases of RNA (a sympathetic nerve) and PNA (a parasympathetic nerve) and the UB contraction. In this and the following figures, arrow (⇧) shows the time of starting Glu microinjection. Abbreviations: INT.RNA, integrated RNA; INT.PNA, integrated PNA; LC, locus coeruleus; BC, brachial conjunctiva; SON, superior olivary nucleus; TB, trapezoid body; VMN, medial vestibular nucleus; VIN, inferior vestibular nucleus; PH, nucleus of praepositus hypoglossi; 5sp, aliminar spinal trigeminal nucleus; 5st, spinal trigeminal tract; ION, inferior olivary nucleus.

Brain Stimulation Produces Decrease in SAP

In the depressor areas of the FTL, NTS, and CVLM, the change in PNA was variable (Table 2). The proportion of points producing no change in PNA was higher in the CVLM (16/33; 48%) than in the FTL or the NTS. However, the correlation between the depressor response and the change in PNA was not significant (Table 4).

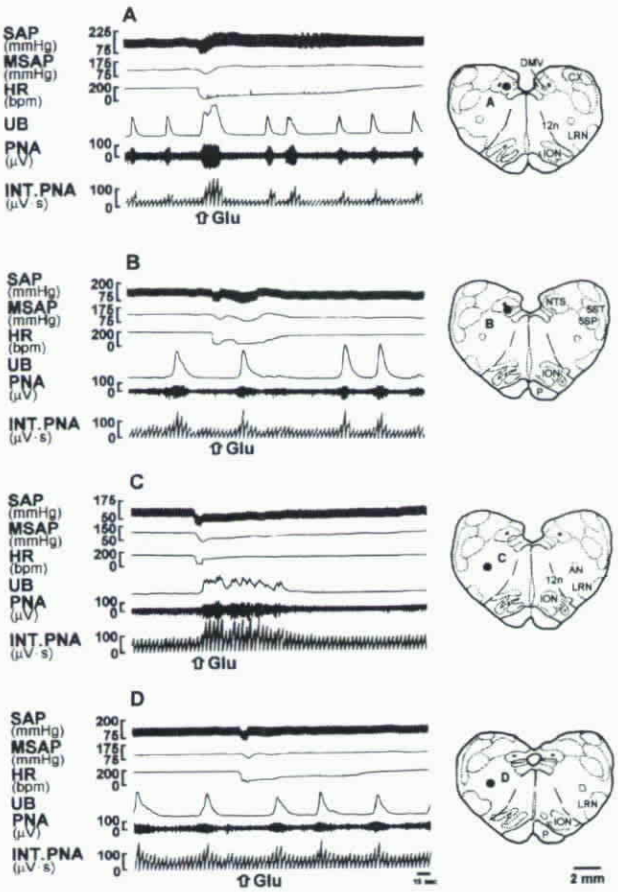

Fig. 3. Glu activation of vagal areas around the dorsomotor nucleus of vagus (DMV) or the ambiguus nucleus (AN) may or may not produce changes of PNA. Microinjection of Glu produced marked bradycardia in the DMV (points A and B) and in the AN (points C and D). In DMV, the bradycardia was concomitantly with an increase of PNA (A, at obex level), or accompanied with no PNA change (B). In AN, similar bradycardia induced by Glu occurred concomitantly with an increase of PNA at point C, but accompanied without PNA change at point D (1 mm caudal to obex). Abbreviations: PH, nucleus of praepositus hypoglossi; 5sp, aliminar spinal trigeminal nucleus; 5st, spinal trigeminal tract; ION, inferior olivary nucleus.

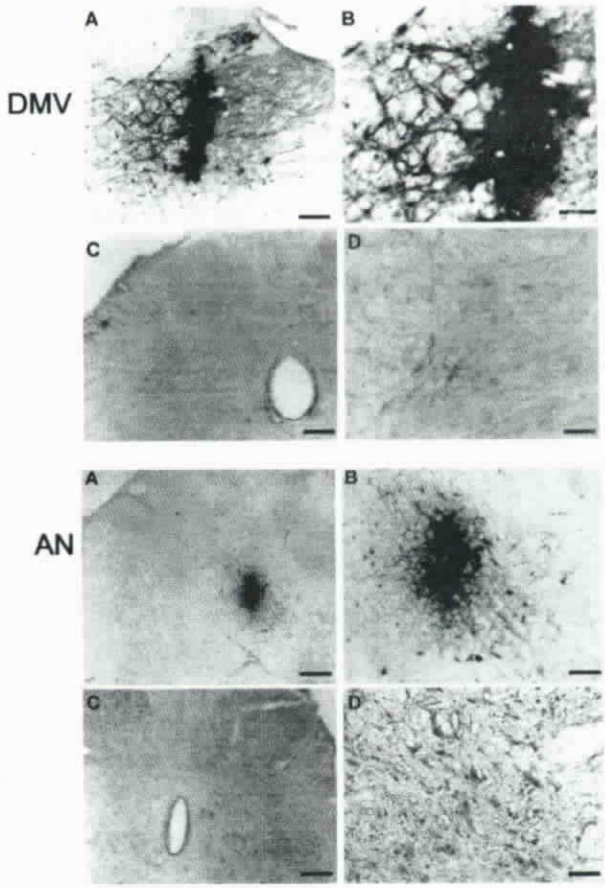

Fig. 4. No labelled terminals in the sacral IML was observed following iontophoretical injection of BDA into DMV or AN of the right medulla. DMV: A. The injection site in DMV, calibration bar = 200 μm. B. High power magnification taken from the same site of the A, calibration bar = 100 μm. C. Calibration bar = 50 μm. No terminals staining for BDA in the sacral IML. D. Calibration bar = 20 μm. High power magnification taken from the same site of the C. AN: A. The injection site in AN, calibration bar = 500 μm. B. High power magnification taken from the same site of the A, calibration bar = 200 μm. C. No terminals staining for BDA in the sacral IML, calibration bar = 50 μm. D. High power magnification taken from the same site of the C, calibration bar = 20 μm.

Brain Stimulation Produces Vagal Bradycardia

Microinjection of Glu into the DMV or the AN produced marked bradycardia (Fig. 3). In either the DMV (Fig. 3A, B) or the AN (Fig. 3C, D), Glu-induced bradycardia was accompanied by either an increase or no change in PNA. The incidence of PNA increase in the DMV (31/48; 64%) was slightly higher than in the AN (37/78; 47%). Furthermore, the AN also showed a high proportion of stimulating points (24/78; 31%) that were not associated with significant change in PNA. The correlation between the vagal response and the change in PNA was analyzed and is presented in Table 3. A positive correlation exists between bradycardia and PNA increase in both the DMV ($R = 0.377$, $P = 0.008$, $N = 48$) and the AN ($R = 0.147$, $P = 0.198$, $N = 78$), but only that in the DMV is significant ($p < 0.05$) (Table 4). These data suggest that the DMV and AN, both nuclei of the cranial parasympathetic portion, have some connections with the sacral parasympathetic pelvic nerve.

Anatomical Pathway Tracking

Three animals were treated with an iontophoretical injection of the anterograde tracer, BDA, into the DMV. BDA was injected into the AN in another three animals. No labelled neurons were observed in the sacral IML region in either case, one month after injection (Fig. 4).

Table 1. Changes of PNA Concomitant with an Increase of MSAP Consequent to Glu Stimulation on Various Brainstem Areas

Pressor Responses

	PNA↑	PNA↓	PNA~
LC(N=14)			
MSAP(%)	26.1 ± 4.3		18.3 ± 3.5
HR(%)	-21.4 ± 9.6		-3.3 ± 2.1
PNA(%)	36.6 ± 8.1		-0.3 ± 2.0
n/N	8/14		6/14
FTG(N=13)			
MSAP(%)	29.5 ± 6.9		22.7 ± 4.5
HR(%)	-14.8 ± 8.0		-17.0 ± 7.2
PNA(%)	44.0 ± 11.9		-0.3 ± 0.3
n/N	4/13		9/13
RVLM(N=19)			
MSAP(%)	46.2 ± 10.3	44.0 ± 11.9	37.8 ± 5.2
HR(%)	-17.6 ± 8.3	-21.3 ± 13.7	-21.6 ± 6.7
PNA(%)	35.4 ± 15.9	-30.3 ± 14.7	1.7 ± 1.3
n/N	5/19	3/19	11/19
DM(N=49)			
MSAP(%)	40.6 ± 5.3	44.0 ± 7.0	40.8 ± 4.4
HR(%)	-32.5 ± 5.0	-34.3 ± 9.2	-23.6 ± 6.3
PNA(%)	45.4 ± 8.0	-32.3 ± 5.0	-0.4 ± 1.2
n/N	35/49	4/49	10/49

Values are mean±SEM. Response of PNA is classified into 3 types: ↑, increase; ↓, decrease; ~, no change. N, total stimulated points. n, number of stimulated points responded to stimulation.

Table 2. Changes of PNA Concomitant with a Decrease of MSAP Consequent to Glu Stimulation on Various Areas

Depressor Responses

	PNA ↑	PNA ↓	PNA ~
FTL (N=13)			
MSAP (%)	-13.7 ± 2.0	-19.6 ± 3.5	-18.4 ± 3.1
HR (%)	-16.0 ± 6.0	-12.2 ± 4.9	-20.4 ± 8.5
PNA (%)	24.3 ± 4.6	-18.4 ± 3.8	-1.0 ± 1.0
n/N	3/13	5/13	5/13
NTS (N=7)			
MSAP (%)	-23.3 ± 7.6	-23.3 ± 2.8	
HR (%)	-26.3 ± 6.9	-8.3 ± 6.6	
PNA (%)	27.5 ± 4.5	-31.7 ± 18.6	
n/N	4/7	3/7	
CVLM (N=33)			
MSAP (%)	-40.0 ± 6.4	-33.5 ± 4.6	-41.9 ± 4.1
HR (%)	-26.5 ± 4.7	-23.8 ± 3.9	-29.6 ± 4.9
PNA (%)	58.4 ± 15.9	-17.4 ± 2.8	0.9 ± 0.8
n/N	9/33	8/33	16/33

Values are mean±SEM. N, total stimulated points. n, number of stimulated points responded to stimulation.

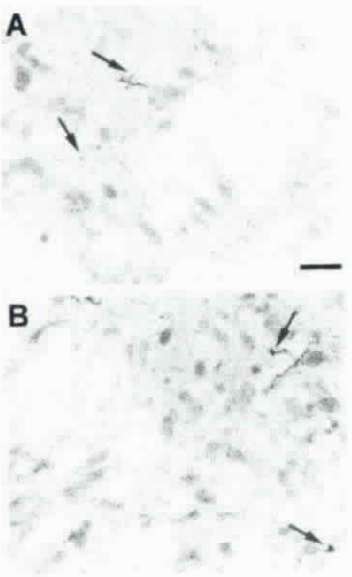

Fig. 5. Labelled terminals (arrows) were observed in the LC following iontophoretical injection of BDA into the DMV or AN of the right medulla. A. Anterograde terminals from DMV (the same injection site of Fig. 4) were observed in LC. B. Anterograde terminals from AN (the same injection site of Fig. 5) were observed in LC. Calibration bar = 50 μm.

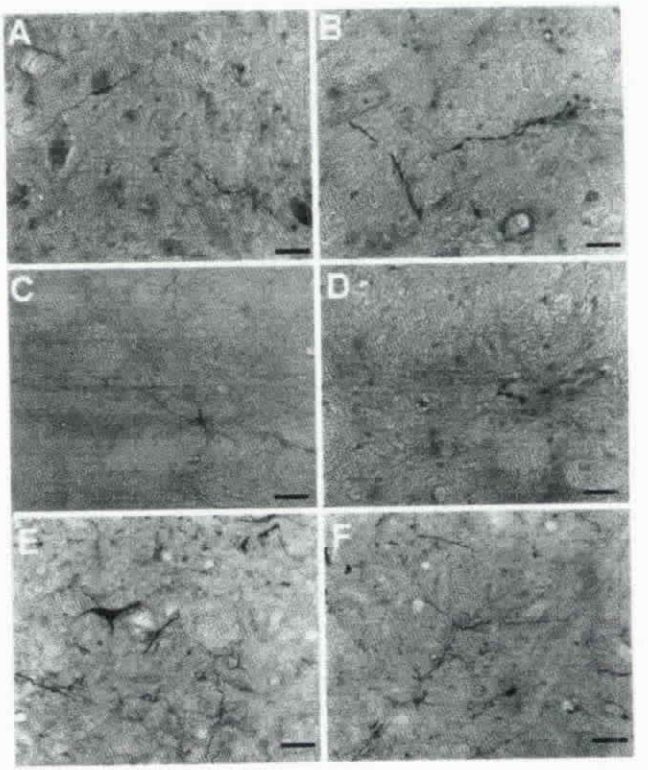

Fig.6. Labelled terminals were observed in the LC, FTG and FTL areas following unilateral injection of BDA into the AN of the right medulla. The injection site of AN was the same as shown in Fig. 5. Labelled terminals were shown on the ipsilateral LC in A, FTG in C, and FTL in E. Labelled terminals were shown on the contralateral LC in B, FTG in D and FTL in F. Note that high density of labelled terminals were observed in FTL. Labelled neurons were also observed in the ipsilateral FTL. However, only a few labelled terminals were observed in FTG. A, B and D. Calibration bar = 20 μm, C, E and F. Calibration bar = 50 μm.

However, labelled terminals were observed in the LC following iontophoretical injection of BDA into the DMV or the AN (Fig. 5). Furthermore, after iontophoretical injections of BDA into the AN, labelled terminals were also observed in the ipsilateral and contralateral pontine areas (LC, FTG and FTL) (Fig. 6), and medullary areas (RVLM, DM and CVLM) (Fig. 7).

Discussion

Microinjection of Glu into the cardiovascular-reactive areas of the LC, FTG, RVLM, and DM produced pressor responses; into the FTL, NTS, and CVLM produced depressor responses; and into the DMV and AN produced marked bradycardia. These Glu-induced cardiovascular responses were often accompanied by changes in PNA. Areas showing an increase in PNA were found mainly in the dorsal parts of the pons and medulla. Areas showing a decrease in PNA were variable, whereas areas with no accompanying PNA changes were mainly found in the ventral part of the medulla.

Activation of the above-mentioned pressor areas often produced a simultaneous increase in the parasympathetic PNA, and an increase in the sympathetic RNA. Our previous studies have shown that on many occasions, increases in sympathetic vertebral nerve activity and RNA occurred simultaneously during the pressor response that followed Glu activation of the LC, FTG, RVLM, and DM, but the regulation of these different sympathetic nerve activities was separate (9, 35). As a result, when different brain mechanisms are activated, various combinations of changes in SAP and the activity of different nerves can occur. A similar concept was implied by studies of the activities of the cardiac sympathetic nerve and the renal nerve (26, 33). Okada and Ninomiya (33) reported that a wide range of changes in cardiac sympathetic nerve activity and renal nerve activity can be produced by electrical stimulation of the hypothalamus in anesthetized rabbits. Meckler and Weaver (26) reported that after the supraspinal control was removed by high cervical cord transection in unanaesthetized cats, RNA decreased only 3% relative to the control, whereas the activity of the splanchnic and cardiac sympathetic nerves decreased about 50%. Their observations also imply that different neurons are responsible for the control of cardiac nerve activity and RNA. These results are consistent with our previous studies describing the coexistence of multiple integrating

Table 3. Changes of PNA Concomitant with a Marked Bradycardia Following Microinjection of Glu into the DMV and AN

Vagal Responses

	PNA ↑	PNA ↓	PNA ~
DMV (N=48)			
MSAP (%)	-1.6 ± 3.0	-9.1 ± 5.9	3.8 ± 3.9
HR (%)	-43.0 ± 3.2	-40.4 ± 3.8	-40.7 ± 5.4
PNA (%)	54.0 ± 8.4	-26.4 ± 5.7	1.2 ± 2.2
n/N	31/48	8/48	9/48
AN (N=78)			
MSAP (%)	-23.4 ± 3.7	-10.7 ± 5.4	-22.7 ± 3.7
HR (%)	-52.1 ± 2.7	-44.8 ± 3.3	-53.8 ± 3.3
PNA (%)	54.4 ± 9.4	-26.2 ± 2.4	-0.4 ± 0.9
n/N	37/78	17/78	24/78

Values are mean±SEM. N, total stimulated points. n, number of stimulated points responded to stimulation.

Table 4. Correlation between the Change of PNA and MSAP Increase Resulting from Glu Stimulation of the Pressor and Depressor Areas; between the PNA Response and the HR Decrease Consequent to Glu Activation of the Vagal Areas

	Pressor areas MSAP vs PNA				Depressor areas MSAP vs PNA			Vagal areas HR vs PNA	
Area	**LC***	**FTG**	**RVLM**	**DM**	**FTL**	**NTS**	**CVLM**	**DMV***	**AN**
R	0.582	0.323	0.107	0.388	0.328	-0.235	0.203	0.377	0.147
P	0.029	0.281	0.664	0.127	0.274	0.612	0.257	0.008	0.198
N	14	13	19	49	13	7	33	48	78

Asterisk (*) shows that the correlation of change between MSAP and PNA or between HR and PNA is significant with the p value less than 0.05 by Pearson correlation. R: correlation coefficient; N: the total number of stimulated points.

mechanisms within various cardiovascular areas, that are not only limited to autonomic functions, but other functions as well, such as the spinal reflex (38) and the motility of the UB (8). A similar situation may exist in the areas responsible for supraspinal control of the sacral parasympathetic pelvic nerve, where the control mechanism is diversely distributed throughout the neural axis of the brainstem, including the sympathetic pressor areas.

The increase in SAP is mainly the result of the increase in sympathetic outflow (11). The LC structure contains a dense population of noradrenergic neurons which may be important for sympathetic integration of cardiovascular functions (6, 9), and are also important for integration of micturition by increasing PNA and decreasing pudendal nerve activity (8, 13, 20, 25, 27, 31). In this study, we analyzed the correlation coefficient between the changes in PNA and SAP in the sympathetic pressor areas of the pons and medulla. We found that only the increase in SAP induced from the LC is significantly positively correlated with an increase in PNA. In the other pressor or depressor areas, the correlation between SAP and PNA was not obvious. Various changes in PNA, *i.e.*, increase, decrease, or even no change, were observed concomitantly with similar pressor responses induced in the cardiovascular areas of the pons and medulla. This may suggest that the mechanism for cardiovascular integration in the above-mentioned brain loci is separate from the mechanism for pelvic visceral integration.

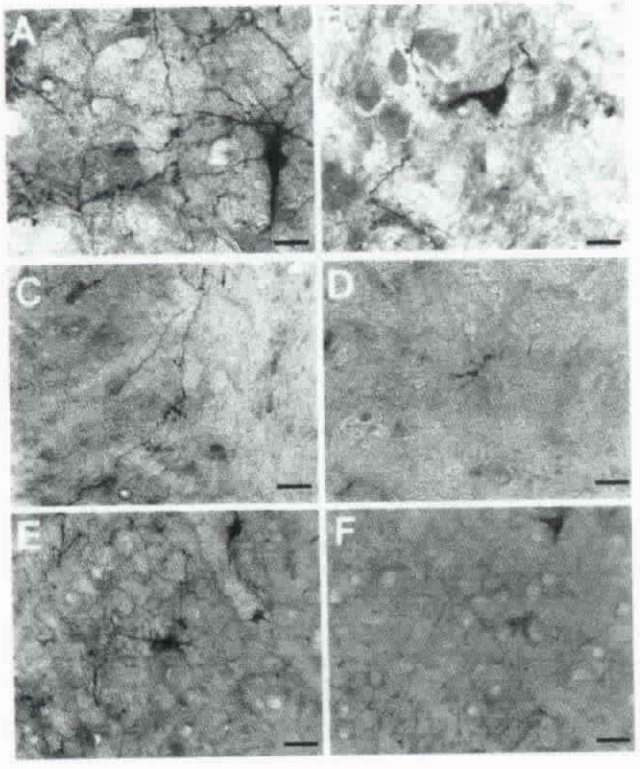

Fig.7. Labelled terminals were observed in the RVLM, DM and CVLM areas following unilateral injection of BDA into the AN of the right medulla. The injection site of AN was the same as shown in Fig. 5. Labelled terminals were shown on the ipsilateral RVLM in A, DM in C, and CVLM in E. Labelled terminals were shown on the contralateral RVLM in B, DM in D and CVLM in F. Note that high density of labelled terminals and neurons were observed in bilateral RVLM and CVLM. However, only a few labelled terminals were observed in the contralateral DM. A, B and D. Calibration bar = 20 μm. C, E, and F. Calibration bar = 50 μm.

The vagus nerve is the main inhibitory source affecting heart rate and myocardial contraction. It supplies motor fibers that control the mucous glands and the muscles of the pharynx, esophagus, stomach, and the rest of the gastrointestinal (GI) tract up to the region of the descending colon (3). The remaining lower GI tract and various pelvic organs, *i.e.*, the colon, UB, uterus, etc., are innervated by the sacral parasympathetic fibers of the pelvic nerve. In the present study, we chose the response of the increases of PNA and UB contraction to indicate the action of sacral parasympathetic system. We found that stimulation of neurons located in DMV and AN induced decrease of HR and increases of PNA and UB contraction. This indicates that functions of the sacral parasympathetic system also can be regulated by DMV and AN. Our results show that simultaneous changes in HR and PNA occur more often during Glu activation of the vagal preganglionic DMV and AN. In particular, simultaneous PNA increase and HR decrease were positively correlated during DMV activation. This suggests that some connections may exist between the cranial parasympathetic DMV and the sacral parasympathetic system. However, the detail connections between DMV and/or AN with the sacral parasympathetic system need further investigation.

In the present study, the labelled anterograde terminals and retrograde neurons were examined by iontophoretical injection of BDA into the DMV or AN. This tracer can be transported over a long distance along the axon to reveal fine details of the terminal structures. The reaction of BDA is faster and simpler than that of *Phaseolus vulgaris* leucoagglutinin (PHA-L) (5, 34). The reaction product of BDA is permanent and compatible with many retrogradely transported fluorescent tracers (37). Unfortunately, in the present study, one month after the administration of BDA which was based on the speed of its transport (5), the terminals in the spinal cord were poorly labelled. In addition, the parasympathetic sacral IML was electrically stimulated in order to record evoked potential in the DMV or AN in several animals. Unfortunately, no evoked potential was recorded from either nucleus. This may suggest that there is no direct connection between the vagal preganglionic areas and the sacral IML region. On the other hand, BDA products were observed in the LC when this tracer was injected in either the DMV or AN, suggesting that fibers from the DMV and AN neurons directly innervate the LC. Therefore, it is not impossible that through this indirect pathway, the DMV or AN integrate the sacral parasympathetic PGNs through the LC. Furthermore, terminals and neurons labelled by injecting BDA into the AN were also visible in the above-mentioned areas that integrate the cardiovascular responses, suggesting the DMV and AN, the vagal PGNs, may functionally connect with these cardiovascular areas.

Taken together, our results demonstrate the coexistence of cardiovascular-reactive neurons and PNA-reactive neurons in the same area of the pontomedulla. Areas in the dorsal part of the pons and medulla, especially the LC, are responsible for the increase in PNA. In particular, Glu stimulation of the PGNs of the vagus nerves, *i.e.*, the DMV and AN, produces increases in PNA and UB contraction. The connection from the DMV and AN to the sacral parasympathetic PGNs is probably multi-synaptic.

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