

Mechanisms Underlying the Cardioinhibitory and Pressor Responses Elicited from the Medullary Neurons in the Gigantocellular Tegmental Field of Cats

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Abstract

A stimulation of the gigantocellular tegmental field (FTG) in the medulla oblongata often increases systemic arterial blood pressure (SAP) and decreases heart rate (HR). We investigated if the cardioinhibitory/depressor areas, including the nucleus ambiguus (NA), the dorsal motor nucleus of vagus (DMV) and the caudal ventrolateral medulla (CVLM), underlied the functional expression of FTG neurons in regulating cardiovascular responses. In 73 chloralose-urethane anesthetized cats, the HR, SAP and vertebral nerve activity (VNA) were recorded. Neurons in the FTG, NA, DMV and CVLM were stimulated by microinjection of sodium glutamate (25 mM Glu, 70 nl). To study if the NA, DMV, and CVLM relayed the cardioinhibitory messages from the FTG, 24 mM kainic acid (KA, 100 nl) was used as an excitotoxic agent to lesion neurons in the NA, DMV or CVLM. We found that the cardioinhibition induced by FTG stimulation was significantly reduced by KA lesioning of the ipsilateral NA or DMV. Subsequently, a bilateral KA lesion of NA or DMV abolished the cardioinhibitory responses of FTG. Compared to the consequence of KA lesion of the DMV, only a smaller bradycardia was induced by FTG stimulation after KA lesion of the NA. The pressor response induced by Glu stimulation of the FTG was reduced by the KA lesion of the CVLM. Such an effect was dominant ipsilaterally. Our findings suggested that both NA and DMV mediated the cardioinhibitory responses of FTG. The pressor message from the FTG neurons might be partly working *via* a disinhibitory mechanism through the depressor neurons located in the CVLM.

Key Words: gigantocellular tegmental field, caudal ventrolateral medulla, nucleus ambiguus, dorsal motor nucleus of the vagus, vertebral nerve activity

Introduction

Numerous physiological studies in cat implicate neurons of the gigantocellular tegmental field (FTG, defined in Bermans 1968, 4) in controlling the sleep-waking cycle (23), saccadic eye movements (13), and cardiovascular functions (28, 29, 31, 43). The FTG is

a large longitudinal zone in the brainstem, extending from the pons into the medulla oblongata (37). Elisevich *et al.* (20) also found the descending projections from the FTG to the paramedian reticular nucleus, which may participate in sympathoinhibition (8, 27, 48) or affect somatic motoneuronal excitability in the spinal cord (40). The FTG neurons also serve

as a relay for baroreflex-mediated sympathoinhibition (28). We have previously shown that activation of FTG neurons increases systemic arterial blood pressure (SAP) and decreases heart rate (HR) (28). However, the neural mechanisms mediating these cardiovascular responses were not known.

Various nuclei in the medulla oblongata are essential for regulating the circulatory parameters including the cardiac rhythm (6, 9, 17, 18). The vagal motor nuclei consist of two discrete nuclei, i.e., the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus (DMV) (27). Although both nuclei regulate HR (26, 27, 32, 33), the majority of preganglionic parasympathetic fibers terminating in heart originates from the NA (27). In addition to a direct cardiac inhibition, the NA might inhibit sympathetic outflow from the adjacent C₁ area in the RVLM (32, 33). Machado and Brody (33) reported that destruction of cell bodies in NA could alter the inhibitory influence on sympathetic vasomotor tone. Adjacent to the NA, the CVLM neurons also play a role more for sympathoinhibition. Destruction of CVLM neurons increases blood pressure, causes sympathoexcitation, and abolishes sympathetic baroreceptor reflex (10, 15, 46). Aicher and Reis (2) concluded that the CVLM depressor area was a tonically active sympathoinhibitory region and involved in cardiovascular integration under physiological condition (26).

Excitotoxic cell death is commonly induced experimentally by the administration of kainic acid (KA) (11, 14, 36). Stimulation of the FTG produced cardioinhibitory and hypertensive responses; hence, the aim of the present study was using KA lesioning techniques to determine whether the cardioinhibitory responses of FTG were mediated by the vagal motor nuclei. We also examined whether the FTG-pressor response required a disinhibition of neurons in the CVLM.

Materials and Methods

A total of 73 cats, of either sex, weighing 2.5 - 3.5 kg, were anesthetized intraperitoneally with a mixture of urethane (400 mg/kg) and α -chloralose (40 mg/kg). After the trachea was intubated, the cat was artificially ventilated with the end tidal CO₂ maintained at approximately 4%. During experiments, the cats were paralyzed with gallamine triethiodide (2 mg/kg/30 min). The femoral artery and vein were cannulated for measuring SAP and drug administration, respectively. The mean SAP (MSAP), and HR were taken and displayed on a Gould ES 1000 polygraph (Gould, Cleveland, OH, USA). The rectal temperature was kept at 37 $\pm$ 1 °C by a homeostatic blanket (Harvard Apparatus, Inc, Holliston, MA, USA).

The head of the cat was fixed in a David-Kopf

stereotaxic instrument (Tujunga, CA, USA). After removal of the occipital and the parietal bones, stimulations of the brain were made through a three-barrel micropipette with a tip diameter of 30-50 μ m (24, 25). The micropipette was mounted on an electrode carrier and inserted into the brain at an angle of 34°. One barrel of the micropipette, containing physiological saline inserted inside with a piece of silver wire, was used for electrical stimulation by delivering a 15 s train of rectangular current pulses (50 - 100 μ A and, 0.5 ms pulse duration at 80 Hz). Another barrel was filled with Glu (25 mM in artificial CSF with 0.4% pontamine sky blue, pH 7.4) for chemical stimulation (70 nl), while the third pipette was filled with KA (24 mM in artificial CSF with 0.4 % pontamine sky blue, pH 7.4) for chemical lesion (200 nl). The other two pipettes were connected via two separate PE-50 tubings, to a pneumatic pump (Medical System, BH-2, Greenvale, NY, USA) for microinjection. The chemical injection site and stimulation area were verified by histological examination. A spread of pontamine sky blue in a diameter of 0.15 mm corresponded to a microinjection of 70 nl, while 0.22 mm for 200 nl.

Neural Recording

The left vertebral sympathetic nerve was exposed and its activities were recorded as described previously (26). In brief, the nerve was dissected free from the surrounding connective tissues, desheathed, cut distally, and placed on a bipolar platinum electrode under mineral oil. The efferent whole nerve activities were amplified (bandpass: 10 - 3 k Hz), rectified, and integrated by an integrator (Gould 13-4615-70, Gould, Cleveland, OH, USA) with a reset time of 5 s. Signals were monitored with an oscilloscope (Tektronix 5113, Tucker, Dallas, TX, USA) and stored on a tape recorder (Neuro Data DR-886, New York, NY, USA) for later analysis. At the end of each experiment, the cat was sacrificed by intravenous injection of saturated KCl. The noise level of neural recording was determined 10 min after animal was sacrificed. The true nerve activity was then obtained by subtracting the noise level from the integrated signals (27).

Histology

The brain was removed and immersed in 10 % formalin solution for 2 - 3 days. Frozen sections in 50 μ m thickness were prepared by a cryostat microtome (Reichert-Jung, 2800 Frigocut, Heidelberg, Germany) and stained with cresyl violet. Sites of chemical stimulation and lesion were reconstructed from sections containing the electrode tracks and marks of the pontamine sky blue.

Data Analysis

Percent changes of SAP and perturbation of nerve activity following stimulation of the sympathoexcitatory or sympathoinhibitory sites were calculated by dividing the value of maximum change with the control value: $(\text{response value} - \text{control value}) / (\text{control value}) \times 100\%$. The control value of nerve activity was derived by averaging the integrated nerve activities for a period of 30 s before stimulation. The largest deviation from the control level within 30 s after stimulation was considered as the value of maximum change, which was positive during excitation and negative during inhibition of the nerve activity. Data were presented as mean $\pm$ standard error of the mean. *P* value less than 0.05 calculated from Student's *t*-test was considered statistically significant.

Results

Electrical stimulation of the FTG increased SAP and vertebral nerve activity (VNA) but decreased HR. Microinjection of 25 mM Glu (70 nl) into the electrical-stimulated sites further confirmed that these responses were due to an activation of FTG neurons rather than the fibers of passage. To explore the connections between FTG and other cardiovascular-regulating sites in the brainstem, the effects of KA lesions of discrete areas in the brainstem on the responses elicited by activating FTG neurons were examined as follows.

Effects of Lesioning the NA on the FTG-Induced Cardioinhibition

Effects of lesioning the NA with KA on the Glu-induced cardioinhibitory responses of the FTG were studied in 25 cats. In 10 cats, one side of the NA that is ipsilateral to the FTG stimulation was lesioned. In another 8 cats, the contralateral side of NA was lesioned. In the other 7 cats, bilateral lesions of the NA were made. A representative example is illustrated in Fig. 1. Within a few seconds after Glu injection into the FTG, the HR exhibited a rapid decrease, from 179 to 125 beat/min (Table 1) and gradually recovered 10 min after the Glu injection. Lesioning the contralateral (right) NA, in contrast, decreased the resting HR from 178 to 101 beat/min, an effect lasting for more than 60 min. In either case, the resting HR was not further decreased when bilateral NA was lesioned (Table 1).

Before NA lesion, microinjection of Glu into the FTG produced a fall of HR by -59%. After bilateral NA was lesioned, the slowing of HR induced by FTG activation was not significant (Table 1). The effects of ipsilateral and contralateral lesion of NA were also compared. When ipsilateral or contralateral

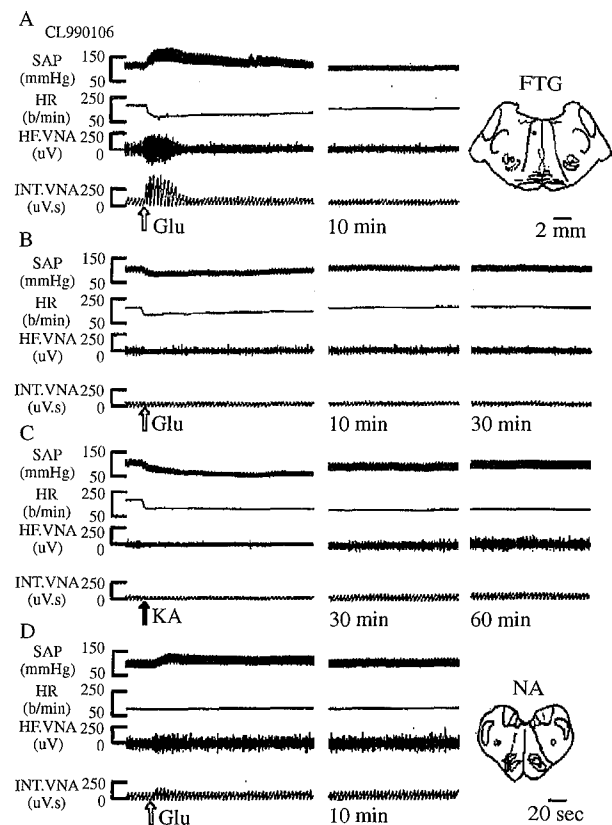


Fig. 1. Cardioinhibitory responses of the FTG induced by Glu were markedly reduced after lesioning the ipsilateral NA.

A. Microinjection of Glu (0.25 M, 70 nl) into the left FTG decreased HR, but increased SAP and VNA. B. Glu (0.25 M, 70 nl) stimulation of the left NA resulted in pronounced cardioinhibition and decrease in SAP but associated with a slight change in the VNA. C. The initial excitatory effect of KA (24 mM, 50 nl) produced similar responses when it was microinjected during lesioning the NA. D. Two hrs after NA lesioning, the same dose of Glu microinjected into the same point in the FTG produced no cardioinhibitory responses. Instead, the SAP slightly increased. Abbreviations: SAP: systemic arterial blood pressure; HR: heart rate; HF.VNA: high frequency VNA; INT.VNA, integrate vertebral sympathetic nerve activity.

NA was lesioned, the FTG induced-cardioinhibitory response was significantly attenuated (by -46% or -34%), but concomitant with an enhanced VNA. Thus, unilateral lesion of the NA, particularly on the ipsilateral (left) side, was sufficient to reduce Glu-induced cardioinhibitory responses from the FTG. After bilateral lesions of the NA Glu-induced cardioinhibitory effect of the FTG was almost eliminated.

Effects of Lesioning the DMV on the FTG-Induced Cardioinhibition

The effects of DMV lesioning on the Glu-induced cardioinhibitory responses of FTG in 25 cats are summarized in Table 2 and illustrated in Fig. 2. Among these 25 cats, 9 were subjected to DMV

Table 1. Effects of NA lesioning on cardioinhibition and decrease in SAP induced by Glu stimulation in FTG.

	MSAP			HR			Change of VNA			
	control stimulation response (mmHg) (%)			control stimulation response (bpm) (%)			control stimulation response (Volts ² /Hz) (%)			
Ipsilateral leasioning										
A	100.5±12.4	135.6±18.8	35.6±23.2	178.5±23.2	98.2±52.5	-46.3±26.3	0.011±0.006	0.039±0.021	71.4±6.5	
B	85.6±10.1	101.2±15.3	18.3±11.3*	125.0±35.1	125.0±35.1	0.0±0.0*	0.011±0.006	0.015±0.008	22.9±21.5*	
(n=10)										
Contralateral leasioning										
A	100.6±8.8	125.0±9.7	24.6±8.3	178.1±25.0	116.3±39.0	-34.4±22.1	0.006±0.004	0.038±0.028	81.5±5.0	
B	83.1±12.5	95.6±14.7	15.1±6.1*	101.3±27.1	101.3±27.1	0.0±0.0*	0.006±0.004	0.013±0.008	51.3±6.3*	
(n=8)										
Bilateral leasioning										
A	103.1±14.7	141.4±19.4	38.3±17.3	165.7±21.9	70.3±45.9	-58.6±22.5				
B	80.0±19.1	95.7±28.2	18.5±12.3*	93.6±10.3	93.6±10.3	0.0±0.0*				
(n=7)										

n=number of cats; mean ± SEM; *: Statistically significant by Student's *t*-test; *P*<0.05;

MSAP: mean systemic arterial pressure; HR: heart rate; VNA: vertebral nerve activity; KA: kainic acid; N: Anucleus ambiguous; FTG: gigantocellular tegmental field

A: before NA lesioning by KA; B: after NA lesioning by KA

Table 2. Effects of DMV lesioning on cardioinhibition and decrease in SAP induced by Glu stimulation in FTG.

	MSAP			HR			Change of VNA		
	control stimulation response (mmHg) (%)			control stimulation response (bpm) (%)			control stimulation response (Volts ² /Hz) (%)		
<i>A. Ipsilateral lesioning group</i>									
Ipsilateral lesioning									
A	106.7±16.8	134.9±27.0	26.1±11.3	187.2±11.3	90.0±33.0	-51.5±18.9	0.007±0.002	0.023±0.007	66.8±14.1
B	85.0±16.7	95.3±16.0	12.7±6.9*	165.0±28.7	165.0±28.7	0.0±0.0*	0.007±0.002	0.010±0.002	31.6±16.2*
(n=9)									
After medulla bisection									
C	90.0±26.2	95.0±26.2	11.9±2.4#	187.5±22.8	187.5±22.8	0.0±0.0#			
(n=4)									
<i>B. Contralateral lesioning group</i>									
Contralateral lesioning									
A	103.1±15.0	130.5±25.3	25.7±7.4	189.4±13.8	96.3±31.2	-48.7±17.1	0.008±0.004	0.029±0.019	71.4±5.8
B	83.9±22.7	121.9±31.0	46.9±19.8*	167.5±32.1	121.9±33.3	-26.9±14.6*	0.008±0.004	0.011±0.007	19.6±16.1*
(N=8)									
After medulla bisection									
C	76.3±8.2	91.3±8.2	20.0±5.7#	190.0±25.5	162.5±28.6	-14.9±5.1#			
(n=4)									

n=number of cats; mean ± SEM; *: Statistically significant by Student's *t*-test; *P*<0.05; #: Statistically nonsignificant by Student's *t*-test for B and C; *P*>0.05; MSAP: mean systemic arterial pressure; HR: heart rate; VNA: vertebral nerve activity;

KA: kainic acid; DMV: dorsal motor nucleus of the vagus; FTG: gigantocellular tegmental field

A: before DMV lesioning by KA; B: after DMV lesioning by KA; C: after medulla bisection

lesion, first on the same (left) side, then followed by contralateral (right) side of FTG. In another eight cats, the process of DMV lesioning was made on the

contralateral side and then the ipsilateral. In the other eight animals, bilateral lesions were made promptly. Similar to that of the NA, microinjection of KA into

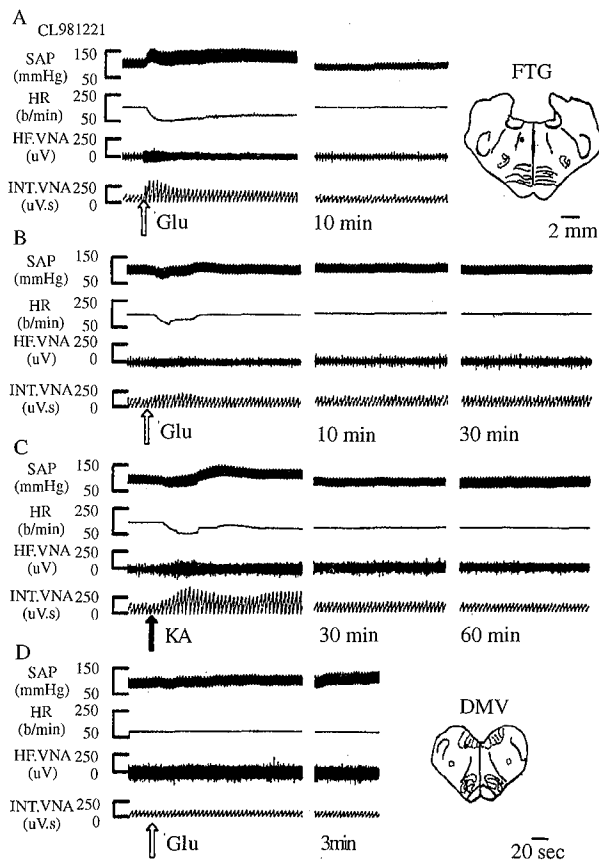


Fig. 2. The cardioinhibitory responses of the FTG induced by Glu were markedly reduced after lesioning the ipsilateral DMV.

A. Microinjection of Glu (0.25 M, 70 nl) into the left FTG resulted in cardioinhibition and increase in SAP with little change in VAN. B. The ipsilateral (left) DMV was explored first by microinjection of Glu (0.25 M, 70 nl) and then lesioned by KA (24 mM, 50 nl) in C. D. Two hrs after DMV lesioning, a same dose of Glu in the same point of FTG did not produce cardioinhibitory responses.

the DMV produced a more apparent fall of HR than application of Glu into the same area. Lesioning the left DMV substantially decreased the resting HR from 187 to 165 beat/min. Lesioning the right DMV decreased the resting HR from 189 to 168 beat/min. Therefore, the effects of decreasing the resting HR was more pronounced after lesioning the left than the right DMV (Table 2). KA lesion of the DMV produced a less prominent bradycardia than that of the NA (reduction of HR in range for DMV and NA lesion: -21~-22 and 55~77 beat/min, respectively).

Bilateral lesions of the DMV consistently abolished the cardioinhibitory responses elicited by Glu stimulation of the FTG (Table 2). When ipsilateral DMV was lesioned, the cardioinhibitory responses elicited by FTG stimulation were significantly reduced (decrease in HR before and after lesion: -97 and 0 beat / min, respectively, a drop of ~52%). After the DMV

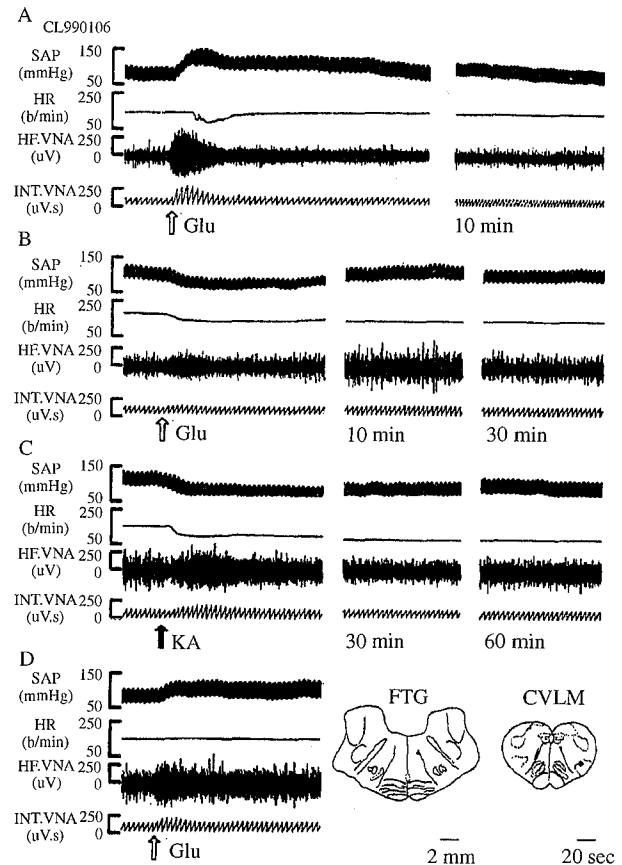


Fig. 3. Hypertensive responses of the FTG induced by Glu were markedly reduced after lesioning the ipsilateral CVLM.

A. Microinjection of Glu (0.25 M, 70 nl) into the left FTG increased SAP and VNA, but decreased HR. B. Glu (0.25 M, 70 nl) stimulation of the right CVLM markedly decreased SAP, HR and briefly and slightly decreased VNA. C. Similar responses were produced when KA (24 mM, 50 nl) was microinjected during the process of lesioning the CVLM. D. Two hrs after CVLM lesioning, a same dose of Glu into the same point of FTG elicited no cardioinhibitory response. Instead, SAP increased.

was lesioned, FTG-induced cardioinhibition was reduced from -93 to -46 beat / min (a drop of HR from -49% to -27%). Thus, unilateral lesion, particularly on the ipsilateral (left) side, was sufficient to significantly attenuate FTG-induced cardioinhibitory responses.

Effects of Lesioning the CVLM on the FTG-Induced Pressor Response

Effects of lesioning the CVLM with KA on the pressor responses elicited by Glu stimulation of the FTG were studied in 23 cats. In 10 cats, the ipsilateral CVLM was lesioned. In another 7 cats, contralateral CVLM was lesioned. In the other 6 cats, bilateral CVLM was lesioned. The effects of lesion are illustrated in Table 3 and Fig. 3. Lesioning the left CVLM decreased the resting SAP from 106 to 80 mmHg.

Table 3. Effects of CVLM lesioning on hypertensive and decrease in HR induced by Glu stimulation in FTG.

	MSAP			HR			Change of VNA		
	control	stimulation	response	control	stimulation	response	control	stimulation	response
	(mmHg)	(%)		(bpm)	(%)		(Volts ² /Hz)	(%)	
A	105.5±11.3	137.2±11.2	30.9±12.5	177.5±24.2	106.0±30.6	-37.5±22.6	0.004±0.001	0.017±0.002	78.8±3.3
B	80.0±18.4	91.8±21.7	16.1±13.7*	134.7±43.8	134.7±43.8	0.0±0.0*	0.004±0.001	0.005±0.002	22.3±19.6*
(n=10)									
Contralateral lesioning									
A	101.9±9.6	127.4±12.9	25.5±12.1	178.6±29.4	138.0±41.6	-23.8±17.6	0.003±0.001	0.014±0.004	82.6±6.4
B	82.9±22.7	99.0±27.2	19.7±11.5	136.4±29.8	136.4±29.8	0.0±0.0*	0.003±0.001	0.004±0.002	37.0±29.3*
(n=7)									
Bilateral lesioning									
A	100.8±8.9	140.3±9.8	40.1±14.7	185.8±18.8	135.8±34.7	-25.8±20.3			
B	91.3±9.5	106.3±13.7	16.7±10.3*	130.8±19.2	130.8±19.2	0.0±0.0*			
(n=6)									

n=number of cats; mean ± SEM; *: Statistically significant by Student's *t*-test; *P* < 0.05; MSAP: mean systemic arterial pressure; HR: heart rate; VNA: vertebral nerve activity; KA: kainic acid; CVLM: caudal ventrolateral medulla; FTG: gigantocellular tegmental field

A: before CVLM lesioning by KA; B: after CVLM lesioning by KA

Lesioning the right CVLM decreased the resting SAP from 102 to 83 mmHg. Bilateral CVLM lesions caused a decrease of SAP from 101 to 91 mmHg (Table 3).

When the CVLM was intact, the pressor responses induced by Glu stimulation of the FTG was +26~+31%. After CVLM lesioning, the same Glu activation in FTG also induced pressor action. However, regardless of which side (ipsilateral or contralateral) of the CVLM was lesioned first, subsequent activation of FTG reduced a similar pressor response (by +16~+20%). However, unilateral lesioning, particularly on the ipsilateral (left) side (by 15%) dominant than contralateral (right) side (by 6%), caused a sufficient attenuation of the Glu-induced pressor responses. After bilateral CVLM were lesioned, the Glu-induced pressor responses of the FTG were decreased (from +40 to +17%) (Table 3). VNA did not change, as compared to a 5 % increase before CVLM was lesioned (Fig. 3).

Discussion

We have demonstrated here that KA lesion of the DMV and NA in the lower brainstem abolished the cardioinhibitory responses elicited by activation of FTG neurons. This observation indicated the existence of a direct or indirect neural pathway mediating the cardioinhibitory messages from the FTG to the NA or DMV. Our observations also suggested that the cardioinhibitory message was mainly propagated ipsilaterally *via* the pathway from the FTG to

the NA.

Glu excitotoxicity plays a key role in inducing neuronal cell death. Activation of Glu receptors causes an influx of sodium and calcium ions. The high intracellular levels of calcium then initiate signaling cascades within susceptible neurons that cause neuronal death through undefined sequences of events (12). Ca^{2+} plays an important role in regulating a number of neuronal processes. In ischemia and hypoglycemia, $[\text{Ca}^{2+}]$ levels within the extracellular fluid of the brain are drastically reduced (19, 22). Various authors have reported that neural stimulation decreases external calcium *in vivo* and *in vitro* (45). Mafra *et al.* (34) report that an important role of extracellular $[\text{Ca}^{2+}]$ in maintaining the L-Glu re-uptake mechanism in the mammalian CNS. Excitotoxic cell degeneration is induced by the administration of KA, an agonist of the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainite class of Glu receptors (11). Therefore, in present studies, the activity of neurons in the NA, DMV, or CVLM was supposed to be decreased after KA lesion (Fig. 1, 2 and 3).

Stimulation of neurons in the FTG elicited cardioinhibitory response, an increase of VNA, and pressor response (Fig. 1, 2, 3, A). A stimulation of the FTG has been shown to produce both excitatory and inhibitory effects on spinal motoneurons in the cat (7, 37, 39-41). Thus, FTG neurons may be involved in both somatic and visceral motor controls. It is intriguing that the cardioinhibitory effect of FTG is reduced or even abolished after NA or DMV lesion.

This observation indicated that the FTG-induced cardioinhibitory effects might be mediated through mechanisms contained in the NA and DMV (Fig. 1, 2, D; Table 1, 2). After the NA or DMV was lesioned, the resting HR was decreased (Table 1, 2). It could be due to the long-lasting effects of KA in inducing excitotoxic neurodegeneration of brain tissue that required 4 min to 24 h after local or systemic injections (5, 11, 14, 21, 36, 47). However, this complication did not mask the functional illustration of the cardioinhibitory responses of FTG, which was likely to be produced by activating neurons in the NA or DMV (Fig. 1D, 2D).

The NA may be related to autonomic control of both HR and arterial blood pressure (33). Parasympathetic cardioinhibitory preganglionic neurons have been shown to be located primarily in the ventrolateral NA (27). McKittrick and Calaresu (35) reported that there is an inhibitory connection between the NA and RVLM. The present results confirmed that stimulation of neurons in NA caused cardioinhibitory response and decrease of VNA (Fig. 1B). Sympathoexcitatory neurons, which innervate sympathetic preganglionic neurons in the intermediolateral cell column (IML) of the spinal cord, are located in the RVLM; including the region of the lateral paragigantocellular nucleus (6). Our data showed that after the NA was lesioned, the FTG-induced decrease of HR and VNA response was reversed. Lesion of the NA eliminated hypertension produced by Glu-induced activation of neurons of the FTG. Therefore, the cardioinhibitory effects of FTG may be mediated through an activation of NA, thereby inhibiting the RVLM. The FTG is involved not only in HR control through NA but also in central regulation of arterial pressure. Besides, the reduction of the resting HR after the NA lesion was more pronounced than that after DMV lesion (Table 1, 2). This observation indicates that the effect of NA on cardioinhibitory response is more important than DMV (27).

The gigantocellular nucleus lies in the medial portions of the reticular formation at the mesencephalon and upper pons. Neurons here have large soma and their fibers project to the spinal cord through the reticulospinal tracts. Stimulation of the FTG causes wakefulness. The FTG neurons receive baroreceptor input and are subject to inhibition by activating NA/DMV. Therefore, our data suggest that the cardioinhibitory pathway from the FTG to the NA/DMV may play an important role in mediating changes of HR from wakefulness to sleep.

The reduction of the resting MSAP and HR, but not VNA, after either ipsilateral or contralateral side of the DMV lesion implies the lesion affecting the cardioinhibitory but not sympathetic neurons in FTG (Table 2). Ipsilateral DMV lesion greatly eliminated

the Glu induced-cardioinhibition and reduced the pressor effects of FTG stimulation. Contralateral DMV lesion reduced the Glu induced-cardioinhibitory effects but the pressor responses induced by FTG stimulation remained unaltered. It is likely that the cardioinhibitory messages propagate more predominantly in ipsilateral than contralateral side of the DMV, but the pressor messages propagate more dominant on the contralateral than ipsilateral side of DMV (Table 2). However, after midline bisection of the medulla oblongata, either ipsilateral or contralateral lesion of the DMV did not attenuate the pressor and HR effects of FTG stimulation. This controversial observation suggested an essential midline-crossing projecting fiber tracts in carrying commands from the FTG to the DMV.

Parasympathetic cardioinhibitory and gastrointestinal preganglionic neurons have been shown to be located in the DMV (27). Approximately 20% of the parasympathetic preganglionic cells were located in the DMV, with the other 80% located in NA (27). Lesioning the NA or DMV by KA reduced FTG-induced bradycard. In the present study, we also showed that the reduction after the lesion of NA, however, was more pronounced than that after DMV lesion. Therefore, the NA might play a more important role than the DMV in cardioinhibitory regulation. Ipsilateral DMV lesion greatly eliminated Glu induced-cardioinhibitory responses and reduced the pressor effects of the FTG stimulation. Contralateral DMV lesion reduced the cardioinhibitory induced by FTG stimulation but the elicited pressor responses remained unaltered. Either ipsilateral or contralateral lesion of the NA greatly reduced or eliminated the cardioinhibitory responses induced by Glu stimulation of the FTG. Ipsilateral or contralateral lesioning of the NA significantly attenuated the pressor effects of FTG stimulation. Taken together, these findings indicated that the pressor responses induced from the FTG depended largely on the neural structures of the ipsilateral DMV, while the cardioinhibitory responses induced from the FTG depended on the integrity of the DMV and NA on both sides.

Gamma-aminobutyric acid (GABA)-containing neurons in CVLM cause the inhibitory outflow to the RVLM and tonically inhibit their pacemaker characteristics (42, 44). We found that administration of KA into the depressor area of CVLM reduced FTG-elicited increases in SAP and sympathetic VNA. Neurons of the CVLM contain non-NMDA receptor (38). Therefore, blockade of non-NMDA receptors in the CVLM might alter the level of Glu, which in turn modulates the release of other neurotransmitters, like GABA, and thereby resulting in tonic inhibition. Blockade of non-NMDA receptors in the depressor CVLM led to an increase of SAP and decrease of

VNA. This observation indicated endogenously excitatory inputs to the inhibitory CVLM neurons (10). A major question that remains unknown is whether the gigantocellular pressor area is functionally related to the control of medullary sympathoinhibitory regions. A major baroreflex pathway is relayed *via* the nucleus of the solitary tract (NTS)→CVLM→RVLM→intermediolateral cell column (IML), which includes an excitatory projection from the NTS to the CVLM (3, 16, 17) and an inhibitory projection from the CVLM to reticulospinal neurons in the RVLM (1, 30). In the present studies, the Glu-induced pressor and VNA responses of FTG were intensely reduced by lesioning the CVLM. These results support the model that the hypertensive action induced by Glu microinjections into the FTG is mediated by inhibiting the CVLM. The pressor message from the FTG neurons might be partly working *via* a disinhibitory mechanism through the depressor neurons located in the CVLM.

In summary, findings of the present experiment showed that the bradycardiac responses resulted from Glu-stimulation of the FTG were mediated through excitation of the vagal motor neurons in the NA and/or the DMV. The FTG modulated sympathetic outflow and in turn, the vasomotor tone, through its projections to the CVLM. Through which, the FTG exerted an indirectly disinhibitory effect on the sympathetic premotor neurons in the RVLM, and thus, caused a pressor response.

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