Localization of Ascending Inotropic and Chronotropic Pathways in the Cat

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SUMMARY We studied the locations and organization of spinal pathways controlling the cardiac vagi in three groups of cats. In all animals, β-adrenergic blockade was induced and the L4 spinal segment was exposed. The right carotid sinus nerves (RCSN) and right peroneal nerves (RPN) were sectioned and the central ends were prepared for stimulation. In one group of cats, hearts were paced and indices of ventricular contractility were monitored. In another group heart rate and blood pressure were recorded and cardiac pacing was not induced. RCSN stimulation produced negative inotropic and chronotropic responses. These responses were attenuated by simultaneous RPN stimulation. Unilateral spinal lesions at L4 placed in the area of the dorsolateral sulcus (DLS) attenuated, whereas such bilateral lesions abolished the effects of RPN stimulation. The data suggest that afferent fibers entering one side of the spinal cord excite a bilateral ascending pathway that inhibits the cardiac vagi. A third group of cats then was studied to determine whether the left and right limbs of the pathway affect bilateral cardiac vagal preganglionic somata or only the complementary ipsilateral cardiac vagus. These animals were instrumented for monitoring either heart rate or contractility and the left vagus was sectioned. Thus, functional cardiac innervation was provided solely by the right vagus. RCSN stimulation produced negative inotropic and chronotropic responses which were attenuated by RPN stimulation. A right DLS lesion at L4 abolished the effects of RPN stimulation. These data suggest that the spinal ascending afferent pathway affects principally the ipsilateral cardiac vagal preganglionic somata.

SINCE the original work by Kerr and Alexander (1964), studies have been performed to identify spinal pathways involved in cardiovascular control. Descending pressor (Foreman and Wurster, 1973), descending depressor (Ilert and Seller, 1969), ascending pressor, and ascending depressor (Chung and Wurster, 1976) pathways were localized bilaterally in the dorsolateral funiculi (DLF), ventrolateral funiculi (VLF), dorsolateral sulci, and DLF, respectively. Since most studies were performed on vagotomized animals, the roles of the pathways in cardiac vagal control were not determined (Ilert and Seller, 1969; Foreman and Wurster, 1973; Chung and Wurster, 1976).

The existence of ascending pathways affecting vagal control of the heart have been described (Iriuchijima and Kumada, 1963, 1964; Quest and Geber, 1972). Bilateral sciatic nerve stimulation produces tachycardia and abolishes the bradycardia in response to carotid sinus nerve stimulation or physiological baroreceptor activation in cats with β-adrenergic blockade (Quest and Geber, 1972). Electrical activation of the cut central ends of various peripheral nerves produces evoked responses in single units or slips of the cardiac vagal branches (Iriuchijima and Kumada, 1963, 1964).

Cardiac vagal preganglionic somata are organized according to physiological function in the cat. Somata of the dorsal motor nucleus of the vagus (DMN) affect myocardial contractility whereas cell bodies of the nucleus ambiguus (NA) are involved in heart rate (HR) control (Geis and Wurster, 1980b). Neither the existence nor the location of a spinal pathway modulating cardiac contractility by way of DMN somata has been described. The existence and location of a spinal pathway affecting NA somata has not been determined. The present study was designed to demonstrate the existence, location, and organization of the ascending pathway affecting vagal control of cardiac contractility and the pathway affecting vagal control of HR.

Methods

General Preparation

Sixty-six cats (2.3-4.2 kg) of either sex were anesthetized with chloroform followed by α-chloralose (40-45 mg/kg, iv). The animals were placed in one of three groups for localizing the ascending chronotropic pathway, for localizing the ascending inotropic pathway, and for determining the organization of the two pathways. In each cat, femoral arterial and venous catheters were inserted and a tracheostomy was performed. The right peroneal nerve was cut and the aortic depressor and carotid sinus nerves were sectioned bilaterally to denervate the baroreceptors. The animal was secured with a cranial holding and spinal clamping device. Spinal cord segments L2-L5 were exposed and the central
cut ends of the right peroneal and right carotid sinus nerves were placed on bipolar stimulating electrodes. The nerves and spinal cord were bathed with warm mineral oil throughout the experiment.

Each cat was artificially ventilated and end-expiratory %CO₂ was monitored (Beckman model LB1) and maintained at 4.0-4.5%. Rectal temperature was held at 37.0 ± 0.2°C by a heating pad. Blood pressure was measured with a Statham P23Db pressure transducer and HR was monitored by a cardiotachometer. Tracings were displayed on an oscillograph (Grass model 7).

β-Adrenergic blockade was induced with propranolol. HR responses to isoproterenol (0.5 μg/kg, iv) were noted before and after propranolol administration. The absence of tachycardia in response to isoproterenol after propranolol injection indicated β-adrenergic blockade. A dose of 1.0 mg/kg of propranolol iv was sufficient to abolish the effects of the β-agonist in all animals.

Localization of Spinal Pathways

The ascending chronotropic pathway was localized in 20 cats. In these animals, only HR and blood pressure were monitored. Another 20 animals were used to identify the ascending inotropic pathway. In these cats, HR, blood pressure, ventricular contractility, and ventricular end-diastolic pressures were monitored. Ribs 2-5 were removed on the right side and the pericardium was incised. Stainless steel electrodes were inserted into the right ventricular myocardium and hearts were paced at the spontaneous rate demonstrated after β-adrenergic blockade. Pacing stimuli (4.0 V, 1.0 msec) were delivered from a stimulus isolation unit (Grass model 4678) connected to a stimulator (Grass model S48).

A Walton-Brodie strain gauge arch was sutured to the right ventricle and the output was displayed on the oscillograph. The output was calibrated in grams and termed strain gauge force (SGF). Catheters were inserted into the left and right ventricular chambers by cardiac puncture and pressures were monitored with P23Db pressure transducers. The right intraventricular pressure was recorded on an oscillograph (Grass model 7) while the left intraventricular pressure was amplified by a d.c. carrier amplifier (Honeywell model 130-2C) and simultaneously displayed on an oscilloscope (Tektronics model 565) and the oscillograph. The left ventricular system connected to the oscilloscope had a natural frequency of 100 Hz and a damping factor of 0.4. Oscilloscope preamplifier gains were adjusted for accurate determination of end-diastolic pressures.

SGF and maximal rate of change of left intraventricular pressure (Max dP/dt) were used as indices of ventricular contractility. Continuous photographs of the left intraventricular pressure tracing on the oscilloscope were taken by an oscilloscope camera (Grass C4). Max dP/dt was measured usually from photographic enlargements of high speed recordings. The maximal slope was determined by taking several measurements from each heart beat. To ascertain stable measurements, we analyzed several heart beats before and during stimulation until we obtained consistent values.

We localized the ascending inotropic and chronotropic pathways by noting cardiac responses to peroneal nerve stimulation before and after making spinal lesions with a scalpel at L4. In initial experiments, cardiac parameters did not change with right peroneal nerve stimulation. However, right carotid sinus nerve stimulation produced cardiovascular responses which were attenuated by simultaneous stimulation of the right peroneal nerve. Therefore, we localized the pathways by comparing the responses to individual and simultaneous stimulation of the nerves before and after making spinal lesions.

Stimulus intensities for peroneal nerve activation were determined by recording compound action potentials from the central nervous system while stimulating the peripheral end in 10 cats. Three times the threshold intensity for C fiber activation was used (1.0 msec, 50 Hz). Stimulation parameters sufficient to produce maximal bradycardia were used for activation of the right carotid sinus nerve. A maximal intensity of 4.0 V (1.0 msec, 50 Hz) was used to avoid producing cardiovascular responses by activating adjacent nerves (Kunze, 1972). To further eliminate this possibility, at the end of the experiment the carotid sinus nerve again was stimulated after the nerve central to the electrode had been crushed. In all experiments the responses were abolished by nerve destruction.

The nerves were stimulated before and after making a lesion in the right dorsolateral sulcus areas (DLS) and after making a left DLS lesion at L4. In 10 animals used for localizing the ascending inotropic pathway, stimuli were repeated after placing bilateral lesions in the DLF and the VLF. These lesions were made prior to DLS ablation.

The division of the autonomic nervous system mediating the responses to nerve stimulation was determined in three cats instrumented for monitoring HR with spontaneous beating hearts, and in three cats instrumented for measuring myocardial contractility with cardiac pacing. β-Adrenergic blockade was induced. Cardiac parameters to individual stimulation of the carotid sinus nerve and peroneal nerve and simultaneous stimulation of both nerves were noted before and after bilateral vagotomy. Spinal lesions were not performed in these cats.

Organization of the Pathways

Twenty cats were used to determine whether the pathways project bilaterally or unilaterally to cardiac vagal nuclei. A left vagotomy was performed at the beginning of each experiment. HR and blood pressure were monitored in 10 of the cats and
another 10 animals were instrumented for measuring HR, blood pressure, Max dP/dt, SGF, and end-diastolic pressures. After instrumentation, responses were noted during individual and simultaneous stimulation of the carotid sinus and peroneal nerves. The stimuli were repeated before and after placing a lesion in the right DLS at L4.

**Data Analysis**

At the end of each experiment, the cat was perfused transcardially and fixed, and spinal cord segment L4 was removed. Ten-μm serial sections were cut and stained according to the method of Kluver and Barrera (1953), and lesion sites were verified histologically.

Pre- and postlesion control parameters were compared by analysis of variance. Analysis of variance and the Scheffe test were used for comparing pre- and postlesion responses to nerve stimulation. Probability (P) and variance ratio (F) values less than 0.05 were considered statistically significant (Gilbert, 1976). Data are expressed throughout as means ± SEM.

**Results**

Control parameters were not affected significantly by previous carotid sinus nerve or simultaneous carotid sinus and peroneal nerve stimulation before or after making spinal cord lesions (F > 0.05). Therefore, the data are expressed as the change from control during nerve stimulation. The average control HR and mean arterial blood pressure (MABP) in cats without cardiac pacing were 158.4 ± 7.3 beats/min and 125.6 ± 10.7 mm Hg, respectively. Control HR, MABP, Max dP/dt, SGF, left ventricular end-diastolic pressure (LVEDP), and right ventricular end-diastolic pressure (RVEDP) in animals with paced hearts were 160.3 ± 8.4 beats/min, 132.9 ± 9.4 mm Hg, 3256.8 ± 98.5 mm Hg/sec, 15.6 ± 2.4 g, 14.6 ± 3.2 mm Hg, and 12.6 ± 2.7 mm Hg, respectively.

Carotid sinus nerve stimulation decreased HR and MABP (P < 0.05) in cats without cardiac pacing (Fig. 1). Max dP/dt, SGF, and MABP decreased (P < 0.05) and LVEDP and RVEDP did not change (P > 0.05) during carotid sinus nerve stimulation in animals with cardiac pacing (Fig. 2). All responses were attenuated significantly (P < 0.05) by simultaneous peroneal nerve stimulation (Figs. 1 and 2). After a lesion in the DLS at L4 had been made, carotid sinus nerve stimulation produced responses which were not significantly different (P > 0.05) from corresponding prelesion responses (Figs. 1 and 2). After the lesion, peroneal nerve stimulation still significantly attenuated the responses to carotid sinus nerve stimulation (P < 0.05). However, postlesion decreases in HR and MABP in cats without pacing (Fig. 1) and decreases in Max dP/dt, SGF, and MABP in cats with paced hearts (Fig. 2) were significantly greater (P < 0.05) than corresponding decreases before the lesion during peroneal nerve stimulation.

Subsequent lesions of the left DLS (now a bilateral DLS lesion existed) completely abolished per-
Oneal nerve attenuation of the decreases in cardiac function produced by carotid sinus nerve stimulation (Figs. 1 and 2). Responses to carotid sinus nerve stimulation after making bilateral lesions were not significantly different ($P > 0.05$) from responses before or after making a right DLS lesion. After bilateral lesions, the responses to simultaneous carotid sinus nerve and peroneal nerve stimulation were not significantly different from the responses produced by carotid sinus nerve stimulation before or after making a unilateral spinal lesion ($P > 0.05$).

L4 spinal lesions placed at sites other than the DLS had no effect on control parameters or the responses to nerve stimulation. Carotid sinus nerve stimulation produced decreases in HR and MABP in cats with unpaced hearts and decreases in Max $dP/dt$, SGF, and MABP in animals with cardiac pacing. These responses were not significantly different ($P > 0.05$) after bilateral lesions in the DLF or VLF. The inhibition of the responses to carotid sinus nerve stimulation by simultaneous peroneal nerve stimulation was not significantly affected by the lesions ($P > 0.05$).

The L4 spinal lesions that abolished the peroneal attenuation of cardiac inhibition by carotid sinus nerve stimulation are shown in Figure 3. The lesions were about 1.0 mm in depth in the DLS region extending slightly into the posterior columns and DLF. The lesion sites for interrupting peroneal-induced inhibition of cardiac chronotropic and inotropic responses were similar.

In the experiments performed on left vagotomized cats, right carotid sinus nerve stimulation decreased HR and MABP ($P < 0.05$) in cats without cardiac pacing (Fig. 4) and decreased Max $dP/dt$, SGF, and MABP ($P < 0.05$) but did not change LVEDP and RVEDP ($P > 0.05$) in animals with paced hearts (Fig. 5). These responses were significantly attenuated ($P < 0.05$) by simultaneous peroneal nerve stimulation (Figs. 4 and 5). A right DLS lesion did not affect the responses to carotid sinus nerve stimulation ($P > 0.05$) but abolished the effect of simultaneous peroneal nerve stimulation. After the lesion, the changes in HR and MABP in cats without pacing and the changes in Max $dP/dt$,
FIGURE 6  Lesion sites for determining the medullary projections of the spinal pathways. A illustrates lesions made in cats used for studying the chronotropic pathway. B illustrates lesions made in cats used for studying the inotropic pathway. Blackened areas illustrate the sites ablated.

SGF, and MABP in cats with paced hearts during stimulation of both nerves were not significantly different (P > 0.05) from the changes produced by carotid sinus nerve stimulation before or after the lesion. Response to carotid sinus nerve stimulation were not affected by the lesion (P > 0.05).

The lesions placed in cats with left vagotomy are illustrated in Figure 6. The lesions for HR and MABP studies (Fig. 6A) and Max dP/dt and SGF studies (Fig. 6B) were similar. Lesions extended approximately 1.0 mm in depth in the region of the DLS.

Six cats were instrumented to compare HR or contractility responses to nerve stimulation before and after bilateral vagotomy. Prevagotomy responses were similar to those illustrated for "intact" cats in Figures 1 and 2. Right carotid sinus nerve stimulation produced decreases in HR in cats without cardiac pacing. Decreases in contractility were produced in cats with cardiac pacing. The effects of carotid sinus nerve stimulation were attenuated by simultaneous stimulation of the right peroneal nerve. The lesion studies of the present investigation localized a spinal pathway of the somatoparasympathetic reflex bilaterally in the dorsolateral sulci at L4. Right peroneal nerve stimulation significantly inhibited (P < 0.05) the negative inotropic and chronotropic responses to right carotid sinus nerve stimulation (Figs. 1 and 2). After a right DLS lesion, the peroneal nerve still significantly (P < 0.05) inhibited the effects of carotid sinus nerve stimulation. However, the inhibition after the lesion was significantly less (P < 0.05) than before the lesion. These data indicate the lesion ablated a portion of the pathway. After a second lesion in the

Discussion

In the present experiments, right carotid sinus nerve stimulation produced decreases in HR and myocardial contractility. The responses were attenuated by simultaneous stimulation of the right peroneal nerve. Lesions placed bilaterally in the area of the DLS abolished the effects of right peroneal nerve stimulation.
left DLS, the responses to stimulating both nerves were not different (P > 0.05) from the responses to carotid sinus nerve stimulation alone. Therefore, the second lesion interrupted the remaining fibers of the pathway.

Cardiac vagal preganglionic somata are located ipsilateral to the vagus nerve carrying their axons (Geis and Wurster, 1980a). In the present study, experiments were performed to determine whether the left and right limbs of the ascending pathways affect ipsilateral or bilateral cardiac vagal preganglionic somata. β-Adrenergic blockade was produced in a series of cats and the left vagus was sectioned. Thus, the right vagus was the only efferent nerve capable of modulating cardiac function. Since right peroneal nerve stimulation activated the pathway bilaterally (Figs. 1 and 2), peroneal stimulation would continue to modulate the cardiac vagus after the right limb of the pathway was sectioned if medullary descussation occurs. However, a right DLS lesion abolished the inhibition of the negative chronotropic and inotropic responses to right carotid sinus nerve stimulation by right peroneal nerve stimulation (Figs. 4 and 5). Therefore, the intact left limb of the pathway did not affect the right cardiac vagus.

Cardiac vagal preganglionic somata have been localized in the DMN and the NA in cats (Geis and Wurster, 1980a). The two populations of somata are organized according to physiological function. Electrical stimulation of the DMN produces negative inotropic responses, whereas NA stimulation affects HR (Geis and Wurster, 1980b). DLS lesions interrupted HR and inotropic responses to peroneal nerve stimulation. These data suggest that a relatively discrete bundle of fibers ascends in the spinal cord and projects to both DMN and NA somata.

The data provided by Iriuchijima and Kumada (1963, 1964) and Quest and Gebber (1972) suggest that the pathway regulates tonic vagal control of the heart. Peripheral nerve stimulation produces tachycardia and inhibition of spontaneous activity of cardiac vagal branches. The data from the present study conflict with these results. Peroneal nerve stimulation did not produce significant changes in HR, Max dP/dt, or SGF and interruption of the pathway did not affect control parameters. The conflicting results may be due to the different animal preparations.

Quest and Gebber stimulated the sciatic nerves, whereas the peroneal nerve was activated in the present study. Both nerves contain group I, II, III, and IV afferent fibers (Johansson, 1962). However, the sciatic nerve is composed of more fibers than is the peroneal nerve (Crouch, 1969). Furthermore, Quest and Gebber stimulated bilaterally, whereas unilateral activation was used in the present study. Therefore, spatial summation may be necessary for altering spontaneous cardiac vagal preganglionic activity. However, Quest and Gebber showed the data from a single animal in their report and did not provide the average responses of all animals studied. In the present study, some cats demonstrated tachycardia during peroneal nerve stimulation. Yet, pooled data from all cats indicated no significant difference from control (P > 0.05) during peroneal nerve stimulation.

The conflicting results between the present work and the investigation of Iriuchijima and Kumada may be due to species variations or the interpretation of electrophysiological data. Nerve activity does not necessarily reflect the functional response of the organ innervated.

The locations of the pathways (Figs. 3 and 6) compare well with the location of ascending spinal pathways involved in blood pressure regulation (Ranson and Billingsley, 1916a, 1916b; Johansson, 1962; Coote and Downman, 1966). Chung and Wurster (1976) used a similar animal preparation to localize the ascending pressor pathway. The blood pressure data of the present study compare well with their results. Unilateral stimulation of the peroneal nerve produced a pressor response which was attenuated significantly by a right DLS lesion. The response to peroneal nerve stimulation was converted to a slight depressor response after bilateral DLS lesions. Thus, the fibers of the ascending pressor pathway and the pathway involved in cardiac vagal control may be identical.

The pathway controlling the cardiac vagus may be identical to the tract of Lissau which ascends in the spinal cord at the apex of the dorsal horn (Brodal, 1969; Truex and Carpenter, 1969). Furthermore, since the lesions extended into the DLF and posterior columns, the pathway may be a component of classical posterior columns and spinocerebellar pathways (Brodal, 1969; Truex and Carpenter, 1969; Webster, 1977).

Speculation regarding baroreceptor function and differential control of the heart can be made from the results of the present investigation. Studies have demonstrated progressive decreases in baroreceptor reflex sensitivity with increasing exercise levels (Bevegard et al., 1966; Bristow et al., 1971). The slope of the regression line relating HR to blood pressure decreases as moderate exercise increases (Pickering et al., 1971; McRichie et al., 1976). The reflex bradycardia in resting conscious dogs in response to methoxamine-induced hypertension is abolished during severe exercise (McRitchie et al., 1976). Studies carried out by Gebber (1970) and Gebber and Snyder (1970) suggest that activated central suprabulbar centers depress the baroreceptor reflex during exercise. The somato-parasympathetic reflex may regulate baroreceptor activation of vagal preganglionic neurons also. Conceivably, impulses elicited from exercising limbs and muscles could reflexly activate the ascending spinal pathways and inhibit the baroreceptor-induced activation of cardiac vagal neurons.
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