Cardiac Responses during Stimulation of the Dorsal Motor Nucleus and Nucleus Ambiguus in the Cat

G. STEVEN GEIS AND ROBERT D. WURSTER

SUMMARY Studies were performed to determine the role of the dorsal motor nucleus (DMN) and nucleus ambiguus (NA) in cardiac control in cats. Heart rate (HR), mean arterial blood pressure (MABP), ventricular contractility, left ventricular end-diastolic pressure (LVEDP), and right ventricular end-diastolic pressure (RVEDP) were monitored in anesthetized, paralyzed and β-blocked animals. The rate of rise of the rising limb of the left ventricular pressure curve (dP/dt) and the output of a strain gauge sutured to the right ventricle (SGF) served as indices of ventricular contractility. Pacing electrodes were inserted into the ventricular myocardium and stimulating electrodes were stereotaxically placed in the DMN and NA. The nuclei were stimulated with and without cardiac pacing, before and after ipsilateral vagotomy. DMN stimulation produced decreases in MABP, dP/dt, and SGF, no HR change, and increases in LVEDP and RVEDP. HR and MABP decreased and dP/dt, SGF, LVEDP, and RVEDP increased with NA stimulation. The responses to NA stimulation were abolished by ventricular pacing. The responses to stimulating either nucleus were abolished by ipsilateral vagotomy. The decreases in dP/dt and SGF with DMN activation were not secondary to preload decreases since LVEDP and RVEDP increased during stimulation. However, since the dP/dt and SGF changes during NA stimulation were abolished by cardiac pacing, the responses were secondary to bradycardia. The data suggest cardiac vagal preganglionic somata are organized according to physiological function. Cell bodies of the DMN control ventricular contractility whereas NA somata are involved in HR control.

DIFFERENTIAL cardiac control refers to the anatomical organization of neural structures for controlling specific cardiac functions. Central and peripheral sympathetic and peripheral parasympathetic structures demonstrate this phenomenon. Activation of the hypothalamus (Peiss, 1962), vasomotor center (Chai and Wang, 1962), stellate ganglion, or ventral roots (Randall et al., 1957; Randall and Rohse, 1956) on the right side produces increases in heart rate, whereas stimulation of corresponding left structures has minor chronotropic effects. Regional cardiac inotropic responses result from specific cardiac nerve stimulation (Randall and Armour, 1977). Right vagal activation produces negative chronotropic responses while left vagal stimulation affects AV nodal conduction (Cohn and Lewis, 1913; Hamlin and Smith, 1968). Resting heart rate decreases after a lesion is made in the right descending pressor pathway but is not affected by cutting the left pathway (Wurster, 1977).

Cardiac vagal preganglionic somata may be organized for differential cardiac control. The cell bodies are located in three distinct medullary regions: the dorsal motor motor nucleus of the vagus (DMN), the nucleus ambiguus (NA), and an intermediate zone (IZ) between the DMN and NA (Geis and Wurster, 1978). Certain morphological characteristics of the three cell body populations differ. Malone (1913) suggested that morphological variations of neurons reflect differences in neuronal function.

The present investigation was designed to determine the functional significance of the location of cell bodies in the DMN and NA. The nuclei were stimulated electrically while cardiac parameters were monitored. The IZ was excluded from the study due to the paucity and scattered distribution of cell bodies in this region (Geis and Wurster, 1978).

Methods

Twenty cats (2.3–4.0 kg) were preanesthetized with chloroform followed by general anesthesia with α-chloralose (40–45 mg/kg, iv). Femoral arterial and venous catheters were inserted. A tracheotomy was performed and the aortic depressor and carotid sinus nerves were sectioned bilaterally to denervate the baroreceptors. Each cat was then secured in a Horsley-Clarke stereotactic device, paralyzed with decamethonium bromide (C10) (0.03 mg/kg, iv), and artificially ventilated. β-Blockade was induced with propranolol (0.1 mg/kg, iv). An occipital craniotomy was performed. Blood pressure and heart rate were monitored with a Statham P23Db pressure transducer and a cardiotochome-
ter, respectively. End-expiratory %CO₂ was monitored and maintained at 4.0-4.5% (Beckman LB1). Rectal temperature was maintained at 37.0 ± 0.2°C by a heating pad.

The right second through fifth ribs were then removed. The pericardium was incised and stainless steel pacing electrodes were inserted into the right ventricular myocardium. Stimuli for pacing (4.0 V, 1.0 msec) were delivered from a stimulus isolation unit (Grass model SI 4678) connected to a stimulator (Grass model S48). Pacing rates were adjusted to the spontaneous heart rate demonstrated after β-blockade.

Strain gauge output and dP/dt were used as indices of ventricular contractility. Catheters were inserted into the left and right ventricular chambers by cardiac puncture. Ventricular pressures were monitored by P23Db pressure transducers. The right intraventricular pressure was recorded by means of an oscillograph (Grass model 7) while the left intraventricular pressure was amplified by a DC carrier amplifier (Honeywell model 130-2C) and simultaneously displayed on an oscilloscope (Tektronics model 565) and the oscillograph. We measured dP/dt as the rate of rise of the rising limb of the left intraventricular pressure curve. The ventricular system connected to the oscilloscope had a natural frequency of 100 Hz and a damping factor of 0.4. Oscillograph preamplifier gains were adjusted for accurate determination of end-diastolic pressures.

A Walton-Brodie strain gauge arch was sutured to the right ventricle. Strain gauge output was displayed on the oscillograph. Outputs were calibrated in grams. The values were not interpreted as absolute measures of cardiac force. Strain gauge output will be referred to as strain gauge force (SGF) in the remaining discussion.

After surgical instrumentation, the left dorsal motor nucleus (DMN) and nucleus ambiguus (NA) were stimulated in 10 cats and the right nuclei were activated in another 10 animals with bipolar electrodes (David Kopf, SNE-100; 100 μm, exposed tip length; 100 μm, electrode tip diameter). The electrode tip was stereotaxically (Snider and Niemer, 1961) inserted into each nucleus at three different medullary planes including the obex level, 1.5 mm rostral to the obex and 1.5 mm caudal to the obex. In 10 cats, sites 0.5 mm medial and lateral to the DMN and NA also were stimulated in each of the three medullary planes. A constant current (Grass model 1A), isolated (Grass model SIU 5) stimulus (Grass S44) of 20 seconds duration (0.1–0.15 mA, 1.0 msec, 50 Hz) was delivered at each site. Each site was randomly stimulated three times with cardiac pacing and three times without cardiac pacing. The ipsilateral vagus then was sectioned and each site was stimulated again three times with and without cardiac pacing.

Changes in afterload can produce secondary, non-neurally mediated alterations in heart rate and contractility (Falsetti et al., 1971; Ross and Braunwald, 1964). Therefore, the changes in cardiac function during brainstem stimulations may be passive responses to neurally induced blood pressure changes. To exclude this possibility, bilateral vagotomies were performed in 10 cats and the responses to injections of phenylephrine (30 μg/kg, iv) and histamine (2.5 μg/kg, iv) were recorded.

Each animal was perfused with 0.9% saline followed by 10% formalin fixative. The brainstem was removed and stored overnight in a solution of 10% formalin and sodium nitroprusside. Ten-micron serial sections were cut and stained with cresyl violet. The stimulation sites were identified by the blue stain resulting from the reaction of iron deposits from the electrode tip with sodium nitroprusside.

Data were organized into groups corresponding to the nucleus stimulated. Thus, NA and DMN groups were formed. For each group, control parameters and the responses to stimulating the left and right sides were compared by the unpaired t-test. Analysis of variance was used to compare control parameters and the responses to stimulating the three medullary planes for each group. For each group, comparisons were made between prevagotomy controls and responses, pre- and postvagotomy controls and responses. Cardiovascular responses to each drug were compared with controls by paired t-test. Variance (F) and probability values less than 0.05 were considered statistically significant (Gilbert, 1976).

Results

For each nucleus the data corresponding to the two sides of the brain stem were pooled, since control parameters and the responses to stimulating the left and right sides were not significantly different (P > 0.05). Since analysis of variance indicated no significant differences (F > 0.05) in control parameters and the responses to stimulating each nucleus in the different medullary planes, the data associated with the three planes were pooled.

DMN stimulation produced significant (P < 0.05) decreases in dP/dt, SGF, and mean arterial blood pressure (MABP) (Figs. 1 and 2). The stimulus did not affect heart rate (HR) (P > 0.05) but produced significant increases in left ventricular end-diastolic pressure (LVEDP) and right ventricular end-diastolic pressure (RVEDP) (P < 0.05).
Ipsilateral vagotomy did not affect control parameters (P > 0.05) but abolished the responses to DMN stimulation (P > 0.05). Neither control parameters nor the responses to DMN stimulation were affected by cardiac pacing (P > 0.05).

Intact cats demonstrated significant decreases (P < 0.05) in MABP and HR but significant increases (P < 0.05) in dP/dt, SGF, LVEDP, and RVEDP during NA stimulation (Figs. 3 and 4). Cardiac pacing did not affect control parameters (P > 0.05) but abolished the responses to stimulation (P > 0.05). Ipsilateral vagotomy had no effect on control parameters (P > 0.05). NA stimulation produced no significant changes (P > 0.05) in parameters after vagotomy. Stimulation of sites 1.0 mm medial and lateral to the DMN or NA had no affect on cardiac function.

The electrode tracts in each of the three medullary planes penetrated are illustrated in Figure 5.
RESPONSES TO STIMULATING THE DMN AND NA/Geis and Wurster

Figure 5  The three planes of electrode penetration from a representative experiment (bottom = 1.5 mm caudal to obex, middle = obex level, top = 1.5 mm rostral to obex). Filled circles indicate sites of the electrode tip at which changes in cardiac function were produced. Unfilled circles indicate sites of the electrode tip at which no changes in cardiac function were elicited. NA = nucleus ambiguus, LRN = lateral reticular nucleus, ION = inferior olivary nucleus, N XII = hypoglossal nucleus, DMN = dorsal motor nucleus, NS = nucleus solitarius.

Discussion

Cardiac vagal preganglionic somata in the DMN are capable of controlling ventricular contractility while NA somata can affect heart rate. Stimulation of the DMN produced decreases in the indices of ventricular contractility (dP/dt and SGF) but no HR changes (Figs. 1 and 2). NA stimulation resulted in HR decreases (Figs. 3 and 4). The responses to stimulating either nucleus were abolished by ipsilateral vagotomy.

Hamlin and Smith (1968) and Cohn and Lewis (1913) reported that right vagal stimulation inhibits the SA node while left vagal stimulation affects AV nodal conduction. In the present study the cardiac responses to stimulating corresponding brainstem sites on the left and right sides were not significantly different. The differences between our results and the reports by Hamlin and Smith and by Cohn and Lewis are not necessarily contradictory. Cardiac responses to vagal stimulation depend on the level at which the nerve is activated. Randall and Armour (1977) reported that the branches to the SA node on the right vagus diverge from the main trunk at sites more caudal than corresponding branches of the left vagus. Thus, stimulating the right vagus may affect SA nodal firing while activating the left vagus at a corresponding level may affect AV nodal conduction, since the major branches to the SA node are not within the main trunk at that level. The sites of our stimulations may have been at a level where the composition of somata with axons traveling to the SA node were similar on the left and right sides. Furthermore, Hamlin and Cohn and Lewis used dogs for their studies, whereas cats were studied in the present investigation. Thus, species variations may be responsible for the different results.

Contractility is an intrinsic myocardial property (Falsetti et al., 1971; Ross and Braunwald, 1964). SGF and dP/dt are measures of contractile force which depend on preload, afterload, and contractility. The changes in dP/dt and SGF with DMN stimulation were not secondary responses to changes in preload and afterload. LVEDP and RVEDP increased during stimulation (Figs. 1 and 2). Decreases in contractile force cannot result from preload increases unless the heart functions on the descending limb of the Frank-Starling curve (Ross and Braunwald, 1964; Sarnoff and Mitchell, 1962). Aortic pressure changes with DMN stimulation did not produce the changes in dP/dt and SGF. Bilaterally vagotomized cats demonstrated no changes (P > 0.05) in the indices of ventricular contractility with drug-induced blood pressure alterations.

Contractile force changes produced by NA stimulation were secondary to HR decreases. Bradycardia increases diastolic filling time, end-diastolic pressure and, ultimately, contractile force (Hamlin, 1977). LVEDP and RVEDP increased during NA stimulation (Figs. 3 and 4). Cardiac pacing abolished all responses to NA stimulation. Therefore,

a representative experiment. Since cresyl violet stains cell bodies, various nuclei were apparent. Electrode tips were located in the DMN or NA when changes in cardiac function were elicited.

In bilaterally vagotomized cats, phenylephrine and histamine produced significant increases (P < 0.05) and decreases (P < 0.05) in MABP, respectively. The other cardiovascular parameters did not change significantly (P > 0.05) after administration of either drug.
the changes in dP/dt, SGF, LVEDP, and RVEDP were mechanical alterations produced by bradycardia.

Anatomical studies suggest parasympathetic preganglionic somata develop a viscerotopic organization. Vagal branches to different viscera were sectioned (Mitchell and Warwick, 1955) and the locations of the resulting chromatolytic neurons were compared. Cell bodies innervating a given organ were arranged in relatively distinct groups. The studies are limited since the entire brain stems were not thoroughly examined. However, the possibility of viscerotopic organization may be significant to the present study.

Somata of the DMN and NA may demonstrate cardiotopic organization. DMN cell bodies at the obex level may control ventricular contractility while more rostral somata may affect HR. NA somata at the obex may regulate HR while somata of the rostral NA may affect ventricular contractility. The responses to stimulating each nucleus at the obex level, 1.5 mm rostral to the obex and 1.5 mm caudal to the obex were not significantly different ($F > 0.05$). Histological evidence indicated that cardiac vagal preganglionic somata are located in these medullary planes (Geis and Wurster, 1978). Thus, the data suggest the responses illustrated in Figures 2 and 4 characterize myocardial control by all somata of the DMN and NA, respectively.

However, the stimulating electrodes may have been activating axons or interneurons of a cardiac vagal reflex. This is possible for four reasons. First, histological data indicate the electrode tips were for activating central neural structures with minimal current spread (Willkus and Peiss, 1963). Third, stimulating 0.5 mm medial and lateral to the DMN and NA had no effect on cardiac parameters. Fourth, all responses were abolished by vagotomy ipsilateral to the stimulating electrode.

Medullary reflex pathways affecting the cardiac vagus are bilateral. The bradycardia in response to unilateral carotid sinus nerve stimulation is diminished by unilateral vagotomy and completely abolished by bilateral vagotomy (Quest and Gebber, 1972). Carotid sinus chemoreceptor pathways project bilaterally as do aortic baroreceptor and aortic chemoreceptor pathways (Kunze, 1972). The nucleus tractus solitarius lies immediately dorsolateral to the DMN and contains the second-order neurons of the baroreceptor and chemoreceptor reflexes (Code et al., 1936; Cottle, 1964). Calaresu and Pearce (1965) produced bradycardia by unilateral electrical stimulation of the nucleus tractus solitarius. The responses were abolished only after bilateral vagotomy. Thus, if the stimuli in the present study were activating known afferent autonomic pathways, the responses would be abolished only after bilateral vagotomy. However, ipsilateral vagotomy abolished the responses to DMN and NA stimulation in our studies. Therefore, electrical currents were activating vagal preganglionic neurons and not afferent component of cardiac vagal reflexes.

The extent of vagal innervation of the ventricles is controversial. Anatomical data suggest minimal or no innervation by parasympathetic preganglionic axons (Cullis and Tribe, 1913; Nonidez, 1939). However, physiological studies have supported parasympathetic ventricular innervation. DeGeest et al. (1965) stimulated the vagi while monitoring ventricular contractility. Stimulation of either vagus produced decreased left ventricular systolic pressure when HR, coronary perfusion pressure, and end-diastolic volume were kept constant. The reduction ranged from 7 to 34%, depending on the stimulation frequency, and the responses were abolished by atropine. Daggett et al. (1967) also demonstrated a negative inotropic effect on the left ventricle by vagal stimulation in a right heart bypass preparation in which aortic pressure, cardiac output, and heart rate were held constant. Negative inotropic effects of vagal stimulation on ventricular myocardium in the dog have been confirmed by several groups (Harmon and Reeves, 1968; Levy et al., 1966). The results of the present investigation confirm the capacity of the cardiac vagus to regulate ventricular contractility.

The data also provide physiological evidence confirming the anatomical location of cardiac vagal preganglionic somata in the DMN and NA in the cat (Geis and Wurster, 1978). However, the results conflict with reports by Calaresu and Pearce (1965) and Gunn et al. (1968). Cardiac responses to stimulation of a variety of brain stem sites were monitored. Short latency bradycardia was produced by NA activation. The authors concluded that cardiac vagal preganglionic somata are located in the NA. However, changes in HR alone do not necessarily reflect cardiac innervation. Parasympathetic nerve fibers also control cardiac inotropic and dromotropic activity (Randall and Armour, 1977). As shown by the present study, cardiac vagal preganglionic somata are organized according to physiological function. Since Calaresu and Pearce and Gunn et al. recorded only HR, the effects of DMN stimulation were not apparent.

Randall and Armour (1977) suggested that the nervous system may have the capacity to differentially regulate cardiac function. Stimulation of specific peripheral nerves produced specific cardiac responses. These data suggested that under one set of circumstances the nervous system may regulate cardiac output by HR control whereas under another set of circumstances the nervous system might regulate cardiac output by means of ventricular contractility. The anatomic substrate for differential cardiac control at the central level has been described (Geis and Wurster, 1978). The present study provides physiological evidence in confirmation of the anatomical report.
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