Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM

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Campos, R. R., and R. M. McAllen. Cardiac inotropic, chronotropic, and dromotropic actions of subretrofacial neurons of cat RVLM. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1102–R1111, 1999.—The cardiac actions of microinjecting sodium glutamate (0.5–2 nmol) among sympathetic premotor neurons of the subretrofacial nucleus in the rostral ventrolateral medulla (RVLM) were studied in chloralose-anesthetized cats after bilateral vagotomy, sinoaortic denervation, adrenalectomy, and α1-receptor blockade. Glutamate microinjections increased heart rate by 25.9 ± 1.8 beats/min (17.5%), systolic rate of rise in left ventricular pressure (LVdP/dt) by 1,443 ± 110 mmHg/s (119%), and arterial blood pressure by 26.9 ± 1.7 mmHg (50%), whereas they shortened the electrocardiogram P–R interval in 85 of 103 cases by 7.5 ± 1.2 ms (11.4%), triggering junctional rhythms on five occasions. The increase in LVdP/dt usually led the rise in blood pressure, and its magnitude greatly exceeded any increase attributable to changes in heart rate, diastolic filling, or afterload. Right-sided microinjections caused significantly greater tachycardias than did left-sided microinjections, but only left-sided microinjections triggered junctional rhythms (5 of 52 vs. 0 of 51; P < 0.05), whereas microinjections on either side raised LVdP/dt equally. Subretrofacial neurons thus drive positive chronotropic, inotropic, and dromotropic actions via the cardiac sympathetic nerves, whereas subsets among them preferentially control different aspects of cardiac function.

blood pressure; brain stem; rostral ventrolateral medulla; autonomic nervous system

THE SUBRETROFACIAL NUCLEUS in the rostral ventrolateral medulla (RVLM) is known to contain a population of neurons that sends axonal projections to the sympathetic preganglionic neurons in the spinal cord and whose activity is critical for the maintenance of vasomotor tone (8–10). When activated experimentally in anesthetized cats, these sympathetic premotor neurons drive the neural supplies to blood vessels, adrenal medulla, and heart but apparently not to other targets (32).

Finer-resolution functional mapping of the region with microinjections of sodium glutamate to activate cell bodies has revealed that subsets of this sympathetic-excitatory neuron group are more restricted in their physiological actions. Cell groups within this population may preferentially or selectively drive the vasomotor supply to a single tissue, such as muscle, kidney, gut, or skin (11, 12, 27, 35, 36). A further subset has now been found to provide strong drive to the inferior cardiac nerve (6).

We have only limited information about what this important neural pathway does to the heart. The present study was therefore undertaken to define its full range of cardiac control by systematically studying the chronotropic, inotropic, and dromotropic effects of activating subretrofacial neurons. Preliminary accounts of parts of this work have been presented in abstract form (5, 33).

METHODS

Animals and anesthesia. Experiments were performed on 12 cats (8 male, 4 female; 3.1–5.1 kg), which were anesthetized with α-chloralose (70 mg/kg iv) after premedication with ketamine hydrochloride (11 mg/kg im). Additional doses of 2–10 mg pentobarbital sodium were given intravenously during the course of experiments (see Control and preliminary experiments). General anesthesia was maintained throughout the experiment at a level sufficient to suppress withdrawal reflexes. No paralyzing agent was given. All experiments conformed to National Health and Medical Research Council of Australia guidelines and were approved by the Animal Experimental Ethics Committee of the Howard Florey Institute.

Preparation. Animals were given a tracheostomy and ventilated artificially throughout the experiment with oxygen-enriched air. Ventilation was adjusted to suppress spontaneous respiratory efforts while maintaining end-tidal CO2 levels at ~3.5–4%. A bilateral pneumothorax was made to reduce the effects of positive pressure ventilation on cardiac filling. Rectal temperature was maintained at ~37–38°C by means of a servo-controlled electric blanket. The left femoral vein was catheterized for intravenous administration of drugs. A polyethylene catheter attached to a transducer was inserted into the left femoral artery to measure arterial pressure. A second 1.5 mm diameter polyethylene catheter attached to a transducer was inserted via the left carotid artery and manipulated until its tip lay in the left ventricle (assessed by the pressure waveform). Catheters were filled with heparinized saline (50 U/ml), and care was taken to remove all air bubbles. The ventricular catheter was kept as short as possible.

The animal was mounted supine in a stereotaxic frame, and the ventral surface of the medulla was exposed as described previously (37), by removing the pharynx and larynx and opening the base of the skull to ~5 mm lateral to the midline, extending from the atlantooccipital membrane up to the level of the tympanic bullae. The dura was opened and retracted clear of this area.

The left and right adrenal glands were exposed retroperitoneally and excised after careful ligation of their blood vessels. Both cervical vagosympathetic trunks and both sinus nerves were then sectioned. All cats were given prazosin (1 mg/kg iv) to block the effects of sympathetic vasoconstrictor drive and thus minimize increases in blood pressure after the activation...
of subretrofacial neurons. A second dose of half that amount was given 3–4 h later in some animals.

Experimental procedure. Glass micropipettes were pulled from 1-mm diameter capillary tubing, and their tips were broken back to a diameter of 10–15 µm. They were filled with 0.1 M monosodium glutamate in normal saline. One such pipette was mounted in a micromanipulator and inserted vertically into sites 0.7 mm deep to the ventral surface of the medulla. Pulses of pressurized air, controlled by switching a solenoid valve, were used to make 5- to 20-nl microinjections of glutamate into each site, the volume monitored by observing the meniscus through a dissecting microscope with a calibrated graticule. On five occasions, a larger volume was injected in error, and these results were discarded. Over the course of the experiment, glutamate microinjections were made into 8–14 sites on each side of the medulla, moving the pipette horizontally each time by 0.2–0.3 mm. Injections were aimed at the subretrofacial nucleus (within the region 3.5–4.5 mm lateral to the midline, 1–3 mm caudal to the trapezoid body), tracking along it to cover the whole responsive region on each side (6). Injections into the left and right sides of the medulla were alternated. The intervals between injections were determined by the return of the measured parameters to a steady state (typically 5–10 min).

At the end of the experiment on two cats, and in one other cat that had been prepared in the same way but received no medullary glutamate injections, a pacing electrode pair attached to a polyethylene catheter was inserted down the right external jugular vein to the region of the right atrium. Square stimulus pulses of 1- or 5-ms duration were applied through these electrodes at ~3 Hz at a voltage that was increased until it stimulated the heart. Guided by the electrocardiogram, the stimulus voltage and location were adjusted to ensure reliable pacing of the atrium without direct stimulation of the ventricles. The heart was then paced at a range of rates between resting heart rate and the upper limit for 1:1 pacing (~230 beats/min). Each paced rate was held for 10–20 s to allow left ventricular pressure measurements to be made in the steady state.

Also at the end of the experiment on three cats (2 of which were also used for pacing), the left and right cardiac sympathetic nerves were sequentially stimulated. One stellate ganglion at a time was exposed retropleurally by removing the sympathetic nerves were sequentially stimulated. One stellate ganglion at a time was exposed retropleurally by removing the ganglion at a time was exposed retropleurally by removing the sympathetic chain, over a pair of platinum wire hooks. Other peripheral ends were placed, together with that of the cut sympathetic chain, over a pair of platinum wire hooks. Other nerve branches of the stellate ganglion apart from the inferior cardiac nerve were crushed, and the area was covered with liquid paraffin. Square pulses of 0.2-ms duration were delivered between the hook electrodes, and the voltage was adjusted to be supramaximal for heart rate changes. Repetitive stimuli at frequencies ranging from 0.5 to 20 Hz (generally in an ascending sequence) were then delivered to the electrodes. Each frequency was held for a period of 20–40 s to allow steady-state responses to be measured. The process was then repeated for the cardiac nerves on the other side.

Recording and measurements. The electrocardiogram was recorded between two 19-gauge hypodermic needles inserted subcutaneously either side of the thorax. The signal was amplified, filtered (band pass 1–100 Hz), and recorded along with the arterial and left ventricular pressure signals on magnetic tape. These signals were also recorded on computer, either online or from tape playback, using the CED 1401 Plus interface and Spike2 program (Cambridge Electronic Design, Cambridge, UK) and digitized at frequencies of 500, 100, and 1,000 Hz, respectively. Each computer record included a control period of ~1 min before a glutamate injection and 2–4 min after the injection. A quasi-differentiated left ventricular pressure signal was displayed for information at the time of the experiment, but was not used for the analysis described here.

Analysis was performed by custom-written routines in the Spike2 program. The rate of rise of left ventricular pressure (LVdP/dt) was derived from the left ventricular pressure signal by the gradient of a line of best fit through five consecutive data points (i.e., 5 ms) during the rapid pressure rise of the isovolumetric contraction phase. This measurement was triggered by a threshold pressure, the level of which was optimized in each record to follow the steepest part of the slope during both the control and response period.

Although this method would have underestimated the true peak value of LVdP/dt, it nevertheless provided a robust measure of sympathetic inotropic action (see RESULTS) and had the advantage of being relatively unaffected by small fluctuations and noise on the ventricular pressure signal. On one occasion a clot was found to have progressively dampened the ventricular pressure trace during the course of the experiment, so those measurements of LVdP/dt were discarded from the analysis.

End-diastolic ventricular pressure was calculated from the line of best fit of five points taken at 10-ms intervals, triggered at a set time before the R wave of the electrocardiogram. An instrument fault caused a slow baseline drift in the transducer signal; accordingly, measurements of end-diastolic ventricular pressure were taken with reference to the mean control value at the start of each record and presented as changes from that value. The gain was stable, however, so measurements of LVdP/dt were unaffected.

Heart rate was calculated by computer from the upswing of the ventricular pressure trace. The peak detection algorithm in the Spike2 program was used to identify the R wave (or on some occasions the S wave when this proved more reliable) of the electrocardiogram. The P wave was then detected by the same algorithm, with the added constraint that the peak should occur within a specified interval before each R (or S) wave. Those time limits were set manually after inspection of the trace. The P–R interval was then measured from the times between the respective identified peaks. In the cases where the S wave was detected, an appropriate value in each case (10–20 ms) was subtracted from the P–S interval to give the P–R interval. In one experiment, the quality of the electrocardiogram was too poor for the P wave to be detected reliably, so those data were discarded.

Analysis. To exclude cases where the microinjection missed the responsive area, an arbitrary threshold criterion was imposed. Only records in which either LVdP/dt increased by at least 20% or heart rate increased by at least 5% were considered to be positive responses and analyzed further (see RESULTS). Subsequent analysis was done in two stages. First, the responses to individual stimuli were assessed for significant changes from the prestimulus baseline. This was done by the cumulative sum test (22), taking at least 30 s of control period and applying $P < 0.01$ as the criterion for significance (35). Changes in heart rate, blood pressure, LVdP/dt, and the P–R interval were assessed this way, and the maximum change in each parameter was calculated. End-diastolic left ventricular pressure was also measured, and its value at the time of the peak response in LVdP/dt was recorded. Additionally, the beat-by-beat values of both end-diastolic ventricular pressure and LVdP/dt were collected from the control periods before each stimulus.

Second, data were collected for grouped analysis. An automated routine measured the mean values of heart rate, blood
pressure, LVdP/dt, and P–R interval over 10 heartbeats taken every 15 s throughout the record (the first 3 being prestimulus control values). These data were grouped separately for left- and right-sided glutamate injections in each animal. The mean control values of heart rate, blood pressure, and LVdP/dt were not normally distributed across animals. Accordingly, a nonparametric procedure (Mann-Whitney rank sum test) was used to test for left-right differences in responses. A left-right difference in incidence of brain stem stimulation causing a junctional rhythm was assessed by the χ² test. Where appropriate, correlations between measurements were assessed by least-squares linear regression. The criterion for significance in all these tests was taken as P < 0.05.

RESULTS

Control and preliminary experiments. To validate the method of LVdP/dt measurement and establish a reference against which to compare the effects of brain stimulation in the present preparation, the left and right cardiac sympathetic nerves were separately stimulated at frequencies between 0.5 and 20 Hz in three animals at the end of the experiment. As expected, these stimuli caused substantial increases in both heart rate and LVdP/dt, with differences in relative emphasis between the effects of left and right nerve stimuli (Fig. 1). The P–R interval in the electrocardiogram decreased with both left and right nerve stimulation (data not shown).

In one cat, stimulation of the left nerves at higher frequencies set up a junctional rhythm, which was associated with other electrocardiogram changes and a progressive diminution of LVdP/dt (Fig. 1, ringed points) that reversed on cessation of the stimulus.

Also in three animals (2 the same as in pacing experiment, 1 other) the heart was paced via the right atrium at a range of frequencies between 150 and 231 beats/min. This served as a control for the direct effects of tachycardia on measurements of LVdP/dt (16, 43) and P–R interval (18). Tachycardic pacing caused a significant increase in LVdP/dt in one animal but no significant change in the other two. The slope of the relation was 3.8 ± 2.9 mmHg/s for each beat per minute (mean of 3 animals ± SE). Pacing at faster heart rates also caused a significant increase in the P–R interval in all three cats, the slope for the relation being 0.2 ± 0.05 ms for each beat per minute. Blood pressure did not change until the highest pacing rates, when it fell by 5 mmHg in two of the three animals.

Preliminary brain stimulation experiments were performed on three cats. In these vagotomized, adrenalectomized, barodenervated animals, the baseline heart rate was high (225 ± 8 beats/min), and whereas glutamate injections into the region of the subretrofacial nucleus produced strong increases in LVdP/dt (data not shown), they caused little or no tachycardia. In the following eight experiments, baseline heart rate was lowered to ~150 beats/min and maintained there with intermittent doses of intravenous pentobarbital sodium (see METHODS). This procedure unmasked large tachycardias in response to medullary glutamate injections.

Figure 2 shows the strong relation between resting heart period and the maximum shortening of heart period obtained in response to medullary glutamate injections in each animal (r² = 0.95, P < 0.001). The regression line of this relation points to a minimum achievable pulse interval of ~250 ms (a rate of 240 beats/min). Accordingly, the following analysis has used only data from the eight animals in which baseline heart rate allowed enough of a margin for a significant tachycardia to be expressed.

Effects of sodium glutamate microinjections into the ventrolateral medulla. In the eight cats, 117 of 156 microinjections into the region of the subretrofacial nucleus in the ventrolateral medulla (61 on the left side and 56 on the right) produced a positive response from the heart, which was defined arbitrarily as an increase of at least 20% in LVdP/dt or a 5% increase in heart rate.
Positive responses were always obtained from a contiguous area on each side of the medulla, within which all injections were effective, and which corresponded closely to the region previously shown to evoke inferior cardiac nerve activity (6). Every positive response included a significant increase in LVdP/dt, whereas 113 of 117 responses also included a significant increase in heart rate.

The mean increase in heart rate was 25.9 ± 1.8 beats/min (n = 117), a 17.5% increase over the baseline value of 148.1 ± 5.2 beats/min (8 animals). The mean increase in LVdP/dt was 1,443 ± 110 mmHg/s (n = 100), a 119% increase over the baseline value of 1,216 ± 93 mmHg/s (7 animals). The P–R interval in the electrocardiogram decreased significantly in 85 of 103 occasions, by a mean of 7.5 ± 1.2 ms (11.4%), from a baseline value of 66 ± 1.8 ms (7 animals). In these prazosin-treated animals, resting mean arterial blood pressure was 53.3 ± 3.0 mmHg (n = 8), but it increased in response to medullary stimulation by 26.9 ± 1.7 mmHg (+50%, n = 117).

Figure 3 shows a representative example of a positive response to glutamate microinjection into the ventrolateral medulla. The changes in all parameters began within 2–3 s of the start of the injection and reached their peaks after 15–60 s. Thereafter, blood pressure and LVdP/dt returned to baseline levels over the next 3–5 min. The changes in heart rate and P–R interval lasted somewhat longer, taking 5–10 min to return to baseline.

Contractility. Two procedures were used to determine whether the measured increases in LVdP/dt were caused by sympathetic inotropic drive or were attributable to other factors. To test for the effect of diastolic filling, the influence on LVdP/dt of changes in end-diastolic ventricular pressure was estimated. To measure this action, the spontaneous beat-to-beat variations in end-diastolic left ventricular pressure that occurred during the control period before each stimulus were plotted against the accompanying changes in LVdP/dt. Figure 4 shows the results of this procedure for an individual experiment and for the grouped data from seven experiments. In every case, the peak value attained during the response to medullary glutamate injection represented a very large upward shift in LVdP/dt from the control ventricular function relation (Fig. 4).

The second procedure aimed to control for the direct effects of tachycardia on LVdP/dt (see Control and preliminary experiments). Figure 5 shows the mean increases in LVdP/dt caused by glutamate injections into the left and right sides of the medulla in each of seven animals plotted against the accompanying tachycardia. Comparison of these data with the effects of pacing (shown with regression line on the same graph) shows that the increases in LVdP/dt were substantially greater than any that could be attributed to the tachycardia.

The relationship between changes in LVdP/dt and blood pressure was also studied in some detail. Medullary stimuli generally caused a brisker rise in LVdP/dt than in blood pressure, although the falling phases of their respective responses ran closely parallel. These features may be seen in the examples shown in Fig. 6, where the values of LVdP/dt and blood pressure at intervals throughout the response are plotted against each other. Data are shown both for a single glutamate microinjection and for the mean of all effective responses in that animal. The lead of LVdP/dt over blood pressure during the rising phase is shown by the hysteresis in these plots, whereas the parallel decline of these two measurements is reflected in the straightness of the lines joining the points from that phase. The peak blood pressure attained during any one response was also closely correlated with the peak rise in LVdP/dt. In each of seven animals, the r2 values for this relation were 0.34, 0.65, 0.83, 0.77, 0.94, 0.81, and 0.89 (P < 0.02 in all cases).

Dromotropic responses. Shortening of the P–R interval of the electrocardiogram was a less consistent feature of the response to ventrolateral medullary glutamate injections than was the increase in LVdP/dt or the tachycardia. In 11 of 103 cases, the P–R interval did not change significantly, whereas in a further seven cases it actually increased by 4.4 ± 0.7 ms. In the remaining 85 cases, however, the P–R interval short-
ened significantly in response to the medullary glutamate injection. Figure 7A shows an example. A shortened P–R interval was always accompanied by a tachycardia, but in virtually every case it accounted for only a small proportion of the reduced heart period.

Figure 7B shows a more extreme case, where the P wave progressively approached and then disappeared into the QRS complex, indicating that a junctional rhythm had been established. Temporary junctional rhythms of this type were seen in response to five glutamate injections in two animals.

Differences between responses to left- and right-sided stimuli. Figure 8 presents the grouped data from eight experiments (7 each in the cases of \( \Delta L_{\text{VP}}/\text{dt} \) and P–R interval), showing mean responses to left- and right-sided glutamate injections at times from 30 s before to 180 s after the stimulus. Left- and right-sided injections caused similar changes in blood pressure and \( \Delta L_{\text{VP}}/\text{dt} \), but chronotropic responses in particular were significantly asymmetric (\( P < 0.05 \) for times between 15 and 105 s after the stimulus; Mann-Whitney). Every animal showed stronger tachycardias in response to right-sided rather than left-sided glutamate injections, although both sides were always able to evoke a significant increase in heart rate. The degree of left-right asymmetry varied markedly between animals, as shown in Fig. 9, in which the mean tachycardias after left- and right-sided injections in each cat are compared. The mean right-to-left response ratio ranged from 1.3 to 16.5 in different cats.

In contrast, shortening of the P–R interval appeared to be more strongly influenced by neurons on the left side of the medulla, although the separation between mean responses to left- and right-sided stimuli in the grouped data (Fig. 8) was not statistically significant. However, all five cases of junctional rhythm were evoked by left-sided glutamate injections, a significant difference in incidence compared with none evoked from the right (\( P < 0.05 \); \( \chi^2 \) test).

Fig. 4. Ventricular function relations. A: data from individual responses in 1 animal to left (○) and right (●) sided medullary glutamate injections. Peak increase in \( \Delta L_{\text{VP}}/\text{dt} \) of each response is plotted against change in end-diastolic left ventricular pressure (\( \Delta \text{EDVP} \)) at time of response, compared with mean prestimulus control value. Small dots represent beat-to-beat values taken from corresponding prestimulus control periods. Least-squares regression line is drawn through these points, and its intersection with vertical axis gives ordinate zero. B: data from 7 experiments plotted as in A. Mean responses from left (○)- and right (●)-sided medullary glutamate injections are shown for each animal. For clarity, only regression lines of beat-to-beat control relations are shown.

Fig. 5. Effect of heart rate on \( \Delta L_{\text{VP}}/\text{dt} \). Small dots show data from pacing experiments, in which \( \Delta L_{\text{VP}}/\text{dt} \) is plotted against increase in heart rate caused by pacing (\( \Delta \text{HR} \)). Fifteen data points were taken from 3 animals, and least-squares regression line is shown. Large symbols show mean increases in \( L_{\text{VP}}/\text{dt} \) caused by left (○) and right (●)-sided medullary glutamate microinjections in 7 animals plotted against accompanying tachycardia.

Fig. 6. Time course of relation between \( L_{\text{VP}}/\text{dt} \) and blood pressure. Relation between \( L_{\text{VP}}/\text{dt} \) and arterial blood pressure is shown at different time points taken at 15 s intervals during individual response to medullary glutamate injection (○), and for the mean of all responses shown by that animal (●). Note hysteresis caused by relative lead of increase in \( L_{\text{VP}}/\text{dt} \) over increase in blood pressure during first 15–45 s of response. Time sequence of data points indicated by arrow.
The lateral asymmetry of heart rate compared with other responses is further illustrated in Fig. 10, in which the tachycardia has been plotted against the increase in $LVdP/dt$ for 100 individual responses in 7 animals. The shape of the line fitting the responses to right-sided stimuli in this plot echoes that of the line fitting responses to right cardiac sympathetic nerve stimulation (shown with reversed axes in Fig. 1). It appears that both the right cardiac nerves and the right subretrofacial nucleus first preferentially increase heart rate; however, only with stronger stimuli, when heart rate saturates, do they cause a substantial increase in $LVdP/dt$.

**DISCUSSION**

Sympathetic premotor neurons in the RVLM of the cat form a relatively tight cell column, identifiable as the subretrofacial nucleus (8, 9, 31, 45). This premotor cell group is a major source of descending vasomotor drive (9, 10) and includes topographically arranged subsets that preferentially or selectively control the vasomotor supplies to different tissues (11, 12, 27, 34–36, 40).

The role of this nucleus in cardiac sympathetic control has hitherto been less well-defined. It is known, however, that the inferior cardiac nerve may be excited by activating neurons within a restricted region of the ventrolateral medulla, encompassing the subretrofacial nucleus (6, 32). It is also clear that this premotor neuron population is not identical to the one that drives muscle vasoconstriction, although their anatomic overlap is substantial (6). The neurons are centered rostral to most muscle vasoconstrictor premotor neurons and (by inference) caudal and lateral to most renal sympathetic and cutaneous vasoconstrictor premotor neurons, respectively (6, 36). The present experiments were undertaken to investigate how much, and in what way, those subretrofacial neurons may influence heart function.

Several lines of evidence led us to expect that neurons in the RVLM affect heart function via its sympathetic nerve supply. First, viral tracing studies indicate...
that neurons in this region are connected polysynaptically to the heart (46, 47). Second, heart rate falls when neurons within diffusion distance of the ventral surface of the medulla at this level are chemically inactivated (20, 37). Third, microinjections of excitant amino acids into this nuclear region affect heart rate, although in a complex and variable manner: a vagal bradycardia often masks an underlying tachycardia, which may be a result of the effects of released catecholamines, direct sympathetic drive to the heart, or both (32). Fourth, applying neuroexcitant substances such as leptazol or the serotonin2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane to the ventral medullary surface over this area causes an increase in contractile force (4, 28).

Finally, electrical stimulation within the ventrolateral medulla of anesthetized cats has been reported to increase both heart rate and pulse pressure (7, 41).

The present experiments have confirmed that neurons in a restricted region of the ventrolateral medulla, presumably sympathetic premotor neurons, may influence both heart rate and LVdP/dt via the cardiac sympathetic nerves, and they have shown that the latter action cannot be explained by any change in diastolic filling or heart rate. The effectiveness of small injections of glutamate shows that the cell bodies of the neurons responsible for these actions were close to the injection site (11, 19, 26). Furthermore, it has been demonstrated for the first time that these neurons can act via sympathetic pathways to regulate atrioventricular conduction. Finally, it was found that those neurons on the left and right sides of the medulla differ in their actions on the heart.

No index of cardiac contractility in vivo is perfect, but within one animal, a change in LVdP/dt may be considered to be a reasonably reliable indicator of a change in inotropic state (39, 49). The major potential confounding factors are any variation in filling pressure, which might act via the Frank-Starling law to increase contractility, and any action that a reduced pulse interval might exert by mobilizing intracellular calcium (43). Both of these factors were found to be small in comparison to the large primary effects of medullary stimulation on LVdP/dt.

We did not explicitly control for the effects of increased arterial pressure (afterload) on the inotropic state of the left ventricle, although we attempted to minimize it by using prazosin to block the vasomotor actions of subretrofacial neurons. Opinions are divided about the importance of this effect in vivo, but all agree that it is not large. Furnival et al. (16), for example, measured a mean increase of 120 mmHg/s in dog heart LVdP/dt for each 10-mmHg increase in blood pressure. Elzinga et al. (13), however, concluded from their own data on cat and dog hearts as well as from a critical review of the literature that any influence of blood pressure on LVdP/dt is either very small or made up of opposing actions that cancel within the physiological range. Even applying the higher estimate, the secondary effects of blood pressure on the present measurements of LVdP/dt in the present experiments would only have been ~300 mmHg/s. We conclude that the changes in blood pressure are unlikely to have had more than a minor impact on the large increases in LVdP/dt obtained in response to medullary glutamate injections.

A further relevant point is that the animals in the present experiments had been treated with the α-adrenergic blocker prazosin. It is therefore likely that the principal cause of the rise in blood pressure after medullary stimuli was a rise in cardiac output, resulting from neural inotropic drive (vagal and adrenal pathways were removed, and tachycardic pacing did not increase blood pressure). In other words, the rise in blood pressure was probably largely a consequence of the rise in cardiac contractility. The observation that
the rise in blood pressure lagged behind the rise in LVdP/dt (Fig. 6) further supports this view.

An incidental observation made during this study was that little tachycardia could be evoked by stimulation of medullary neurons unless resting heart rate was below 200 beats/min (Fig. 1). This agrees with old observations that there is a limit to which heart rate may be raised by reflexes or stimulation of the cardiac sympathetic nerves (48); yet if resting heart rate is lowered (e.g., by stimulating the peripheral cut end of the cervical vagus), previously ineffective stimuli may again raise heart rate via the cardiac sympathetic nerves (21). In the present experiments, supplementary pentobarbital sodium was used to the same effect. The heart rate ceiling is evidently a peripheral phenomenon caused by the inability of the effector to respond, and it closely matches the maximum rate attainable by pacing.

An important finding of this study is that there is lateral specialization among sympathetic premotor pathways in their control of different aspects of cardiac function. It is known that left and right cardiac sympathetic nerves differ in their range of actions on the heart, and this has been correlated with their innervation territories (2, 18, 24, 38, 42). The present results imply that the sinoatrial node is driven principally by subretrofacial neurons on the right side of the medulla, just as it is by the right cardiac sympathetic nerves, whereas the atrioventricular node appears to be controlled more strongly by subretrofacial neurons on the left, as it is by the left cardiac sympathetic nerves. Left ventricular function may be accessed by subretrofacial and sympathetic neurons on both sides, but those on the right side need to be activated relatively strongly (see Figs. 1 and 10).

From the anatomic standpoint, the present results presumably mean that right-sided premotor neurons in the medulla mostly drive right-sided preganglionic neurons in the spinal cord. This suggestion that the descending pathway to the cardiac sympathetic nerves is uncrossed receives support from other observations. Foreman and Wurster (15) found an almost exclusively ipsilateral effect on the T2 sympathetic outflow (a substantial proportion of which supplies the heart) after electrical stimulation of descending tracts in the cervical cord, suggesting that the pathway does not decussate below that level. It is also known that most subretrofacial neurons send their descending axons ipsilaterally into the cervical cord (1, 31), indicating that the descending pathway does not decussate above that level.

From the physiological standpoint, these lateral differences imply that particular subsets of subretrofacial neurons are functionally specialized to control the rate, force, or conduction of the heart. This might be because each premotor neuron of the cardiac supply selectively drives but one of those functions (those with chronotropic actions being more numerous on the right, etc.). Alternatively, a premotor neuron’s actions could be merely preferential rather than exclusive. In either case, though, the actions of each subset must presumably be mediated by synaptic contacts on preganglionic neurons with at least as great a degree of functional specialization. On the basis of many studies of the pre- and postganglionic neurons supplying a variety of targets, Jänig and McLachlan (23) propose that each individual sympathetic neuron belongs to one of a limited number of functional classes, which are dedicated to control single target functions. In the case of the cardiac supply, vagal preganglionic neurons that are believed to have a selective negative dromotropic action have been identified in the cat (29), and single sympathetic fibers with a presumed positive chronotropic action have been identified in the dog (25). The latter are believed to constitute a private line whereby atrial stretch receptors reflexly cause a purely sympathetically mediated cardioacceleration, unaccompanied by any vagal component or inotropic drive (17). The present data are compatible with the view that sympathetic premotor neurons in the subretrofacial nucleus show the same degree of functional specificity (36). But they cannot disprove the alternative possibility that individual premotor neurons have preferential, rather than exclusive, actions on each class of cardiac sympathetic neuron.

Finally, from the viewpoint of cardiovascular control, even if the drive exerted by individual subretrofacial neurons is only preferential, collectively they still provide a basis from which the brain may independently control different aspects of cardiac function. Such pathways could be independently engaged in both reflex and centripally driven responses in which independent action is required.

Perspectives

The present study is not the first to find lateral preferences in cardiac sympathetic actions evoked from the brain, but earlier studies used electrical stimulation, which precludes their results being tied to any particular premotor cell group (19). Most also concentrated on other brain areas such as the hypothalamus (14) or dorsal medulla (7), whereas cardiac sympathetic actions evoked from the ventrolateral medulla were attributed to descending fiber tracts (7, 41). But in view of the present findings, and by analogy with vasomotor pathways (37), it seems likely that cardiac sympathetic actions evoked by electrical stimulation of the hypothalamus (44), and perhaps also other areas of the brain, would be relayed synaptically by subretrofacial neurons.

It may also be the case that another well-documented phenomenon, centrally triggered cardiac arrhythmia, is relayed through the subretrofacial neurons studied in this paper. A full range of clinically relevant dysrhythmias, up to and including ventricular fibrillation, may be triggered by stimulation within the brain (30). Those effects depend on raised sympathetic drive, the vagal influence being generally protective. Attempts to localize this effect, too, have used electrical stimulation and concentrated on the hypothalamus (3, 30). So it is entirely possible that the arrhythmogenic actions of electrical hypothalamic stimulation are relayed by the
subretrofacial neurons identified here. The new finding that functional rhythms may be evoked, entirely via the cardiac sympathetic pathway, by chemically stimulating subretrofacial neurons, shows that these medullary premotor neurons do indeed have arrhythmogenic capabilities.

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