Vasodepressor Neurons in Medulla Alter Cardiac Contractility and Cardiac Output

Guy Drolet, John Chalmers, and William Blessing

We injected neuroexcitatory and neuroinhibitory agents into the depressor region of the caudal ventrolateral medulla of anesthetized rabbits and determined the effect on arterial pressure, myocardial contractility, cardiac output, and plasma catecholamines and neuropeptide Y. Brief excitation of the sympathoinhibitory neurons with medullary injection of L-glutamate increased arterial pressure, peripheral vascular resistance, and myocardial contractility. Cardiac output was unaffected. Prolonged inhibition of the sympathoinhibitory neurons with medullary injection of muscimol increased arterial pressure, peripheral vascular resistance, and myocardial contractility. There was a progressive fall in cardiac output. These changes were accompanied by an increase in plasma neuropeptide Y and plasma norepinephrine, but no change in plasma epinephrine. Our findings indicate that the sympathoinhibitory vasomotor neurons in the caudal ventrolateral medulla tonically suppress the activity of sympathetic preganglionic neurons controlling myocardial contractility as well as peripheral vasomotor tone. Dysfunction of these medullary neurons could underly some forms of experimental hypertension. (Hypertension 1993;21:210-215)

KEY WORDS • medulla oblongata • cardiac output • myocardial contraction • rabbit studies

Arterial blood pressure increases after interference with the function of sympathoinhibitory vasomotor neurons located in the caudal ventrolateral medulla oblongata (CVLM). Hypertension in the spontaneously hypertensive rat (SHR) may be related to impaired function of these neurons, located between the nucleus ambiguus and the lateral reticular nucleus in the general region containing the A1 norepinephrine neurons. Electrolytic lesions of the caudal ventrolateral medulla alter cardiac output and myocardial contractility. However, the lesioning procedure affects fibers of passage and, in addition, significantly increases plasma vasopressin, an effect now recognized to reflect an excitatory effect on the A1 cells. When pharmacological agents with a purely inhibitory effect on neuronal cell body function are injected into the CVLM, they increase sympathetic vasomotor tone without increasing plasma vasopressin. Regional blood flow is differentially affected by neurons in the caudal medulla, but so far there is little information concerning how excitation or inhibition of neuronal function in the caudal medulla affects myocardial contractility and cardiac output. The present study examines this question in the anesthetized rabbit.

From the Departments of Medicine and Physiology (J.C., W.B.), Centre for Neuroscience, Flinders University of South Australia, Bedford Park, Australia, and the Centre de Recherche du CHUL (G.D.), Université Laval, Unité de Recherche sur l'Hypertension, Ste-Foy, Canada.

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Address for correspondence: Dr W.W. Blessing, Department of Medicine, Flinders Medical Centre, Bedford Park 5042, SA Australia.

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Surgical Preparation

Experiments were performed on 18 male New Zealand White rabbits (2.5-3.0 kg, Hillside Rabbit Stud, New South Wales, Australia). All experiments were approved according to the guidelines set by the Flinders University Animal Ethics Committee. In some animals (n=9) anesthetized with thiopentone (40 mg/kg i.v.) and 1% halothane in oxygen and mechanically ventilated via an endotracheal tube, a right thoracotomy was performed, and a Doppler ultrasound flow transducer (4 mm i.d.) was placed around the ascending aorta just distal to the coronary arteries. In some rabbits (n=6), pacing leads were sewn onto the epicardial surface of the right atrium. These leads, and insulated wires connected to the transducer crystals, were positioned subcutaneously for later retrieval. Cardiovascular experiments were performed after a recovery period of at least 1 week.

On the day of the experiment, each rabbit was anesthetized with urethane (1.5 g/kg infused into a marginal ear vein over 30 minutes, Sigma Chemical Co., St. Louis, Mo.). The trachea was cannulated, and the animal was paralyzed with pancuronium bromide (0.5 mg/kg i.v. initially, with supplemental doses as necessary) and artificially ventilated with oxygen-enriched air, using a rodent ventilator (model 681, Harvard Apparatus, South Natick, Mass.). The end-expiratory CO2 was monitored (Datex Normocap CO2 monitor, Helsinki, Finland) and maintained at 35-40 mm Hg. Body temperature was monitored by a rectal thermistor probe and maintained at 38-39°C by a heating pad. A polyethylene catheter was inserted into the left femoral artery for recording arterial pressure (AP), sampling arterial blood for blood gas and pH analysis, and for...
Neck flexion was adjusted so that the dorsal surface of the membrane, and removal of the edges of the occipital bone.

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Measurement of Circulatory Variables

A strain gauge transducer (model 231D, Statham Division, Gould Inc., Oxnard, Calif.) connected to a polygraph (model 7, Grass Instrument Co., Quincy, Mass.) was used to record AP. Mean AP was obtained by filtering the phasic signal. Heart rate (HR) was computed with a Grass 7P4F tachograph triggered by the phasic arterial signal. Left ventricular pressure was measured by connecting the specially fabricated catheter to a Millar catheter micromanometer (Millar Inc, Houston, Tex.). This fluid-filled catheter transducer system has a natural resonant frequency of 100 Hz and damping coefficient of 0.6 and is suitable for measuring left ventricular pressure and rate of change of left ventricular pressure (LV dP/dt).11 derived from the left ventricular pressure signal by a Tektronik AM 501 operational amplifier (Tektronix Inc, Beaverton, Ore.) connected as a differentiator. The LV dP/dt signal was calibrated directly by substituting a triangular wave of known slope into the differentiator. Cardiac output was measured by a Doppler ultrasonic flowmeter (Baker Institute, Melbourne, Australia) with the sensitivity adjusted so that the phasic flow signal was zero at end diastole. This system is known to give reliable measurement of cardiac output in kilohertz of Doppler shift in rabbits.14 Total peripheral resistance was calculated in "units" by dividing the mean AP in millimeters of mercury by the cardiac output in kilohertz. HR was recorded on a beat-to-beat basis by a cardiostamper (Beckman Instruments Inc., Schiller Park, Ill.) triggered by the left ventricular pulse on phasic Doppler flow.

Continuous records of phasic and mean AP, HR, high and low gain left ventricular pressure, LV dP/dt, and phasic and mean aortic flow (cardiac output) were displayed on a Gould 2800 biomedical recording system (Gould Inc., Instruments Division, Cleveland, Ohio) or on a Grass model 7 polygraph.

In one rabbit AP, HR, and LV dP/dt changes in response to CVLM injection of l-glutamate and muscimol were measured after cardiac vagal blockade with methylscopolamine (50 µg/kg i.v. and 25 µg/kg every 15 minutes).

Plasma Catecholamines and Neuropeptide Y

Arterial blood samples (1.5 ml) were taken into heparinized tubes from the femoral catheter 5 minutes before and 15 and 30 minutes after injection of muscimol (1 nmol) into the CVLM. The volume withdrawn was replaced by an equal volume of heparinized saline (0.9%). The samples were stored on ice before centrifugation at 3,000 rpm for 10 minutes. Plasma supernatant was then stored at −80°C until assay. Plasma catecholamines (norepinephrine and epinephrine) were measured using high-performance liquid chromatography with electrochemical detection with a sensitivity of less than 20 pg/ml.15 Plasma NPY was measured by radioimmunoassay,16 and the limit of sensitivity for this assay was 3.0 pg per tube.

Statistical Analysis

All data are expressed as mean±SEM. Statistical analysis was performed on a Macintosh II computer programmed with the statistical package STATVIEW II. Dose–response curves were analyzed by linear regression and by analysis of variance with repeated measures. After injection of muscimol into the medulla, serial temporal changes in cardiovascular variables, plasma NPY, and plasma catecholamines were analyzed by polynomial regression and by analyses of variance with repeated measures. First- or second-order polynomial regression relations were chosen according to the sig-
nificance of the residual variance. Post hoc comparisons were assessed using Fisher’s protected “t” tests.

Results

Effect of Chemical Excitation of Neurons in the CVLM

Excitation of neuronal function by a unilateral injection of L-glutamate (0.1–10 nmol) into the CVLM produced cardiovascular changes that commenced within seconds of the injection. The maximum effect was observed within approximately 1 minute, and the baselines returned to preinjection values after approximately 5 minutes. L-Glutamate caused dose-dependent decreases in AP, HR, peripheral vascular resistance, and myocardial contractility (Figure 2). There was no significant dose–response fall in cardiac output. However, the highest (10 nmol) dose of L-glutamate significantly reduced cardiac output by 0.11 ±0.04 kHz (n=7, p<0.05), albeit by a small amount (approximately 10% of the resting cardiac output). These responses were observed within 1 minute of the L-glutamate injection. In some rabbits (n=6), HR was held constant during L-glutamate injection by atrial pacing at a rate just above resting levels. Unilateral injection of L-glutamate (10 nmol) in these animals still caused a decrease in AP, not significantly different from the fall in AP in un paced rabbits (−36±2 unpaced, −31±4 mm Hg paced, n=6, paired t test, p>0.05). Similarly, the highest dose of L-glutamate still significantly reduced cardiac output by 0.12±0.04 kHz (n=6, p<0.05), a reduction not significantly different from the decrease in cardiac output after injection of 10 nmol L-glutamate into the CVLM in un paced rabbits. After muscarinic blockade with intravenous methylscopolamine, injection of L-glutamate into the CVLM caused falls in AP, vascular resistance, and myocardial contractility of similar magnitude to those observed without vagal blockade. There was no change in HR or cardiac output.

Effect of Chemical Inhibition of Neurons in the CVLM

Inhibition of neuronal function by bilateral injection of GABA (1–100 nmol) into the CVLM altered cardiovascular function, commencing within 1 minute and reaching a maximum after approximately 5 minutes. Parameters returned to preinjection values after approximately 10 minutes. We observed dose-dependent increases in AP, peripheral vascular resistance, and myocardial contractility (Figure 2). Cardiac output and HR were not significantly altered.

After bilateral injections of muscimol (1 nmol) into the CVLM, there was a progressive marked increase in AP, commencing within 1 minute (Figure 3). During the first 15–20 minutes the increase in AP was accompanied by a parallel increase in peripheral vascular resistance and myocardial contractility. During the next 10 minutes these parameters decreased a little, but they were still significantly elevated 30 minutes after the muscimol injection. There was no significant change in HR. Cardiac output began to decrease within 1 minute of the muscimol injection and continued to fall during the 30-minute observation period (Figure 3). After muscarinic blockade with intravenous methylscopolamine, injection of muscimol into the CVLM caused increases in AP, vascular resistance, and myocardial contractility of similar magnitude to those observed without vagal blockade. Cardiac output progressively decreased with no change in HR.

Plasma NPY was substantially increased 15 and 30 minutes after injection of muscimol, and plasma norepinephrine was significantly elevated 15 minutes after injection of muscimol. Plasma epinephrine was not significantly affected by the muscimol injection. These results are summarized in Table 1.
Arterial blood gases and pH were measured before injection of muscimol and at the 15-minute postinjection time point. There were no significant changes of pH, PCO₂, or PO₂ after the injection of muscimol (control: pH 7.40±0.02, PCO₂ 39±2 mm Hg, PO₂ 318±32; after muscimol: pH 7.39±0.02, PCO₂ 40±2 mm Hg, PO₂ 324±41, n=6, all p>0.05).

Discussion

Our results confirm the previous observation of a dose-related fall in AP after injection of L-glutamate into the CVLM and a dose-related rise in AP after similar injections of GABA and muscimol, confirming the presence of a population of inhibitory cardiovascular neurons in the CVLM. The present study elucidates the peripheral mechanisms of these changes. When the inhibitory neurons are activated, there is a fall in AP and HR accompanied by a fall in myocardial contractility; there is a simultaneous fall in total peripheral vascular resistance so that cardiac output is not changed. Our pacing studies and the experiment with muscarinic blockade show that vagal effects do not play a significant role in the AP and contractility effects induced by injection of L-glutamate into the CVLM. This agrees with previous observations. When the inhibitory neurons are themselves inhibited by GABA, there is a dose-related increase in AP, vascular resistance, and myocardial contractility without a change in HR. The increases in peripheral resistance and myocardial contractility were balanced so that cardiac output remained constant. Muscimol is a high affinity agonist at GABA-A receptors, with a long duration of action because the agent is not taken up by GABAergic nerve terminals. Muscimol also increased AP, peripheral vascular resistance, and myocardial contractility without a change in HR. The increase in peripheral vascular resistance was greater than the increase in myocardial contractility, and a progressive decrease in cardiac output was observed.

We have previously demonstrated that injection of muscimol (1 nmol) into the CVLM entirely prevents the cardiovascular actions of similar injections of L-glutamate as well as blocking certain cardiovascular reflexes for at least 30 minutes. The cardiovascular changes observed during the first 15 minutes are likely to be a primary effect of the muscimol-induced loss of the normal, tonically active, CVLM inhibition of cardiovascular function. Arterial blood PO₂, PCO₂, and pH were normal at this time. Plasma epinephrine was not significantly increased during the muscimol-induced increase in peripheral resistance and myocardial contractility. We consider it most likely that the increases were mediated via increases in the sympathetic input to the heart and peripheral vessels. The increases were not prevented by muscarinic vagal cardiac blockade. Direct recordings from renal, splanchnic, and lumbar sympathetic nerves have demonstrated reduction in activity

![Figure 3. Line graphs show cardiovascular parameters during the 30 minutes after bilateral injection of muscimol (1 nmol) into the caudal ventrolateral medulla. Top three curves were fitted by second-order polynomial regression and the bottom two by linear regression.](http://hyper.ahajournals.org/doi/abs/10.1161/01.HYP.90.5.213?journalCode=hyp)

| Table 1. Plasma Neuropeptide Y and Catecholamines After Injection of Muscimol Into Caudal Ventrolateral Medulla Oblongata |
|-----------------|-----------------|-----------------|-----------------|
|                  | Control         | 15 min          | 30 min          |
| NPY (pg/ml)      | 1,559±258*      | 2,228±349       | 2,859±386       |
| (8)              |                 |                 |                 |
| Norepinephrine   | 426±114         | 1,523±450†      | 967±169         |
| (7)              |                 |                 |                 |
| Epinephrine      | 124±57          | 238±76          | 213±143         |
| (6)              |                 |                 |                 |

NPY, neuropeptide Y. Plasma concentrations of NPY, norepinephrine, and epinephrine 15 and 30 minutes after bilateral injection of muscimol (1 nmol) into the caudal ventrolateral medulla. Values are mean±SEM. Numbers in parentheses refer to number of animals.

*Significant linear regression, p<0.05.
†Significantly greater than control value, analysis of variance with repeated measures and Fisher’s protected “t” test, p<0.05.
with excitation of CVLM neurons and increase in nerve activity with inhibition of CVLM neurons. Similarly, the increases in plasma NPY and plasma norepinephrine observed after muscimol injections presumably reflect increases in peripheral sympathetic vasomotor tone.

There remains the possibility that the GABA- and muscimol-induced increases in myocardial contractility were not sympathetically mediated but occurred in response to the increase in peripheral resistance (increase in afterload) or possibly to some change in venous return (change in preload). However, studies have established that peak dP/dt is a robust measure of myocardial contractility unaffected by increases in afterload. Similarly, changes in preload cannot explain the observed increases in myocardial contractility. Cardiac output must, over time, be equal to venous return. In our present study there was a progressive fall in cardiac output, with no change in cardiac rate. Venous return would therefore have progressively decreased after the muscimol injection, reducing left ventricular preload. This reduction would, if anything, decrease myocardial contractility.

Only one study has measured changes in regional vascular resistance after alteration of neuronal function in the CVLM in anesthetized animals. Willette et al demonstrated in rats that mesenteric, renal, and hind limb vascular resistances all increased with inhibition of CVLM neuronal function. Blood flow in all these beds was reduced, consistent with our finding of a general fall in cardiac output after injection of muscimol into the CVLM. Maeda et al showed that cerebral blood flow decreases during hypotension induced by activation of depressor neurons in the CVLM. Cardiovascular function has been monitored after recovery from anesthesia in rabbits with electrolytic lesions made in the CVLM. These animals also exhibited a marked increase in peripheral vascular resistance and myocardial contractility together with a fall in cardiac output. Cardiac failure and florid pulmonary edema were often observed in these animals. The syndrome was related to an increase in plasma vasopressin as well as an increase in peripheral sympathetic activity. The changes in plasma vasopressin were related to irritative effects of the electrolytic lesions. Inhibiting CVLM neurons with muscimol does not increase plasma vasopressin, and therefore this hormone is not involved in the cardiovascular changes observed after muscimol or GABA injections in the present experiments.

The A1 catecholamine neurons are probably responsible for the vasopressin changes after alteration of neuronal function in the CVLM. However, evidence indicates that the depressor neurons do not belong to the A1 cell population even though the two groups of neurons are located in a similar region of the medulla oblongata. The depressor neurons appear to act by a direct projection to the rostral medulla, inhibiting the sympathoexcitatory neurons by an action on GABA receptors, and the A1 cells probably do not project to this region. Experimental increase of AP in the unanesthetized rabbit induces expression of the c-fos protein in a group of neurons, presumably the depressor neurons, in the CVLM, and very few of these cells belong to the A1 group.

It is not yet known whether specific inhibitory CVLM neurons, possibly located in specific subregions, can alter vasomotor tone in specific sympathetic vascular beds, such as occurs in the case of sympathoexcitatory neurons in the rostral ventrolateral medulla. The answer to this question will bear on the overall question of the physiological role of the CVLM inhibitory vasomotor neurons. There is a growing consensus that they may constitute the central inhibitory link between baroreceptor inputs to the nucleus tractus solitarius and the sympathetic premotor cells in the rostral ventrolateral medulla. Activation of baroreceptors, such as occurs with a rise in AP, causes a reflex, neurally mediated reduction in myocardial contractility.

In conclusion, our present study indicates that the CVLM contains neurons whose activity tonically inhibits myocardial contractility as well as peripheral vascular resistance. Malfunction of these inhibitory neurons might be relevant to some forms of hypertension in humans.

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References


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