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 reduced 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.

Methods

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. Verification of the injection site was always 100 nl and was monitored by observing the movement of the fluid-air meniscus in the barrel of the pipette. Each injection took 2 seconds. Each pipette was calibrated in 100-nl steps. The volume injected was always 100 nl and was monitored by observing the movement of the fluid-air meniscus in the barrel of the pipette. Each injection took 2 seconds. Each pipette was left in place for 1 minute after an injection and then withdrawn from the medulla. Subsequent injections at the same site were made by reinserting the pipette at the same coordinates. Neuroactive agents were dissolved in Ringert's solution. At the end of the experiment 100 nl pontamine blue was injected at the same site for histological verification of the injection site. Because the medulla was directly visualized, the injection sites were always observed to be within the vasodepressor area of the caudal ventrolateral medulla (Figure 1).

To excite neuronal function in the CVLM we unilaterally injected 0.1–10 nmol monosodium L-glutamate (Sigma). To inhibit neuronal function we bilaterally injected 1–100 nmol γ-aminobutyric acid (GABA, Sigma) or 1 nmol muscimol hydrochloride (Sigma).

Measurement of Circulatory Variables
A strain gauge transducer (model 23ID, 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). Dose-response curves were analyzed by linear 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 unpaced 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 unpaced 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. Plasma 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.
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**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.

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, PCO$_2$, or PO$_2$ after the injection of muscimol (control: pH 7.40±0.02, PCO$_2$ 39±2 mm Hg, PO$_2$ 318±32; after muscimol: pH 7.39±0.02, PCO$_2$ 40±2 mm Hg, PO$_2$ 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 PO$_2$, PCO$_2$, 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.

**TABLE 1. Plasma Neuropeptide Y and Catecholamines After Injection of Muscimol Into Caudal Ventrolateral Medulla Oblongata**

<table>
<thead>
<tr>
<th>Plasma NPY or catecholamine</th>
<th>Control</th>
<th>15 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY (pg/ml)</td>
<td>1,559±258*</td>
<td>2,228±349</td>
<td>2,859±386</td>
</tr>
<tr>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>426±114</td>
<td>1,523±450†</td>
<td>967±169</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>124±57</td>
<td>238±76</td>
<td>213±143</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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. 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.

Acknowledgments

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References


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