Influence of rostral ventrolateral medulla on renal sympathetic baroreflex in conscious rabbits

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Mayorov, Dmitry N., and Geoffrey A. Head Influence of rostral ventrolateral medulla on renal sympathetic baroreflex in conscious rabbits. Am J Physiol Regulatory Integrative Comp Physiol 280: R577–R587, 2001.—Previous studies with anesthetized animals have shown that the pressor region of the rostral ventrolateral medulla (RVLM) is a critical site in vasomotor control. The aim of this study was to develop, in conscious rabbits, a technique for microinjecting into the RVLM and to determine the influence of this area on renal sympathetic nerve activity (RSNA) and arterial pressure (AP) using local injections of glutamate, rilmenidine, ANG II and sarile. Rabbits were implanted with guide canulas for bilateral microinjections into the RVLM (n = 7) or into the intermediate ventrolateral medulla (IVLM, n = 6) and an electrode for measuring RSNA. After 7 days of recovery, injections of glutamate (10 and 20 nmol) into the RVLM increased RSNA by 81 and 88% and AP by 17 and 25 mmHg, respectively. Infusion of glutamate (2 nmol/min) into the RVLM increased AP by 15 mmHg and the RSNA baroreflex range by 38%. By contrast, injection of the imidazoline receptor agonist rilmenidine (4 nmol) into the RVLM decreased AP by 8 mmHg and the RSNA baroreflex range by 37%. Injections of rilmenidine into the IVLM did not alter AP or RSNA. Surprisingly, treatments with ANG II (4 pmol/min) or the ANG II receptor antagonist sarile (500 pmol) into the RVLM did not affect the resting or baroreflex parameters. Infusion of ANG II (4 pmol/min) into the fourth ventricle increased AP and facilitated the RSNA baroreflex. Our results show that agents administered via a novel microinjecting system for conscious rabbits can selectively modulate neuronal activity in circumscribed regions of the ventrolateral medulla. We conclude that the RVLM plays a key role in circulatory control in conscious rabbits. However, we find no evidence for the role of ANG II receptors in the RVLM in the moment-to-moment regulation of AP and RSNA.

The pressor region of the rostral ventrolateral medulla (RVLM) plays a critical role in the generation and maintenance of sympathetic activity as shown by studies in anesthetized animals (10, 11, 20). Neuronal excitation in the RVLM increases blood pressure, whereas lesioning or inhibiting neuronal activity in this area decreases arterial pressure to levels observed after spinal cord transection (11, 20). However, the magnitude of hypotension after lesioning or cooling of the RVLM is anesthetic dependent (7, 17), and sympathetic vasomotor tone has virtually recovered in conscious rats within 1 day after lesioning (8). These data indicate that in the conscious animal, the RVLM is not the only area of the central nervous system contributing to the maintenance of vasomotor tone.

Functional studies in anesthetized animals have also demonstrated that the RVLM is an essential part of the central baroreflex pathways because lesioning the RVLM or blockade of the GABAergic synapses in this area abolishes the vasomotor component of the baroreflex (9, 20). In conscious animals, the RVLM also is likely to be an important part of the brain stem baroreflex circuitry because most of the bulbospinal RVLM neurons respond to decreases in arterial pressure, as shown by studies using a combination of early gene c-fos expression and neuronal tracing (35, 37). However, because a large number of other brain nuclei also responds to changes in arterial pressure (19, 27), the relative functional contribution of the RVLM in mediating the baroreflex cannot be established using the c-fos technique. Furthermore, optical imaging studies found that an increase in arterial pressure causes neuronal inhibition at the rostral ventral medullary surface in the anesthetized, but not in the conscious goat (21), suggesting that, in the conscious state, brain regions other than the RVLM may be recruited to mediate reflex pressor-induced responses. Thus the role of the RVLM in the circulatory control, and in particular in mediating the baroreflex, in conscious animals needs to be reexamined.

Until recently, the ability to microinject into the RVLM was essentially confined to acute, anesthetized preparations, as this area is close to the flexion point of the cervical spinal cord and is subject to movement in the conscious animal. However, rabbits may prove to be a suitable animal for microinjecting into the RVLM while conscious, because when placed in the standard rabbit box, they keep relatively still for a long time due to their natural behavior. Therefore, we developed a new method for bilaterally microinjecting into the RVLM in conscious rabbits, by modifying the technique we used previously to microinject into the noradrenergic...
MATERIALS AND METHODS

In the present study, we used this new technique to examine the role of the RVLM in vasomotor control in conscious rabbits by determining the effect of modulating neuronal activity in this region on renal sympathetic nerve activity (RSNA) at rest and during baroreflex responses. To excite cell bodies within the RVLM, we used local microinjections of glutamate, which have been shown to produce pressor responses when given locally into this region in both anesthetized and conscious animals (3, 10). To inhibit neuronal activity in the RVLM we used local injections of the mixed $\alpha_2$-imidazoline receptor agonist rilmenidine. Previous studies with anesthetized animals have shown that inhibitory action of rilmenidine on the RVLM vasomotor neurons is critical in its depressor effect (6, 22). We also determined the effects of modulating neuronal activity in the RVLM with microinjections of ANG II and the specific, but nonsubtype-selective ANG II receptor antagonist sarile. Previous studies have demonstrated that ANG II can excite vasomotor neurons in the region (16, 26), and ANG II receptors are tonically activated in anesthetized animals and also modulate the RSNA baroreflex (23, 24, 39–41). To validate the selectivity of the new microinjecting system, we have compared the cardiovascular responses to modulating neural activity in the pressor region of the RVLM and in adjacent regions of the ventrolateral medulla.

**Surgical procedures**

Rabbits were implanted with metal guide cannulas for bilaterally microinjecting into the ventrolateral medulla (3–4 wk before experiments) and with an electrode for measuring RSNA (1 wk before experiments). A 2-wk interval was necessary to ensure full recovery from the cannula implantation surgery (31), whereas a reliable RSNA signal can usually be obtained only within 2 wk after the electrode implantation.

**Guide cannula implantation.** Rabbits were premedicated with 4 mg of dexamethasone (Dexason, Troy Laboratories) to prevent inflammation around the guide cannulas. Anesthesia was induced using propofol (Diprivan, 1 mg/kg iv, Zeneca) after which the rabbits were intubated, and anesthesia was maintained with halothane (Fluothane, Zeneca). The animal was placed in a stereotaxic frame, and holes (1.0 mm in diameter) were drilled 3.0 mm bilaterally from the central fissure and 4.2 mm posterior to lambda. The guide cannula (22 gauge, 22.5-mm long) was then inserted into the brain at the angle of 12° and fixed in place with dental cement and stainless steel screws. The lower end of the guide cannula was situated in the lateral recess of the fourth ventricle (LV4V), FN, facial nuclei; IO, inferior olive.

**Implantation of renal nerve electrodes.** A bipolar renal nerve electrode for recording RSNA was implanted under halothane anesthesia according to the method of Dorward and colleagues (12). With the use of a dissecting microscope, the left kidney was exposed by a retroperitoneal approach, and the renal nerve was identified and placed inside a coiled pair of electrodes. The nerve and recording electrode assembly was insulated from the surrounding tissue by SilGel 604 (Wacker-Chemie). The other end of the electrode was tunneled under the skin for retrieval on the day of the experiment, and the incision was sutured.

**Success rate.** In this study, the guide cannulas were implanted in 20 rabbits. In four rabbits, brain injury occurred during the surgery due to intracranial bleeding, as revealed by postmortem examination. These animals were killed within a few hours of the surgery. One rabbit demonstrated no RSNA signal and was excluded from the study. In two rabbits, a strong salivary outflow and dysphagia developed during glutamate infusion presumably due to stimulating the ventral medullary swallowing area (25), because the injection sites were found to be $\sim 1.5$ mm rostral to the...
pressor region of the RVLM. These animals were not included in the following experiments.

**Blood Pressure and RSNA Measurement**

On the day of the experiment, the animal was placed in a standard rabbit box (dimensions 15 × 40 × 18 cm, width × length × height). Under local anesthesia (lignocaine HCI 1%, Delta West), the central ear artery and marginal ear vein were catheterized, and the plug of the renal nerve electrode was retrieved from under the skin and connected for measurements of RSNA. Pulsatile arterial blood pressure was measured with a Statham 23Dc pressure transducer. Sympathetic nerve activity was amplified, filtered between 50 and 5,000 Hz, full-wave rectified and integrated using a low-pass filter with a 20-ms time constant. The integrated neurogram obtained using this low time constant allowed us to analyze oscillations of synchronized bursts of sympathetic activity and served as the input signal for all subsequent computer analysis. The integrated neurogram and pulsatile arterial pressure were continuously monitored throughout the experiment and were sampled at 500 Hz using an analog-to-digital data acquisition card (PC Plus, National Instruments). The bursts of sympathetic activity, beat-to-beat systolic and diastolic pressures, and R-R interval were detected online using a program written in the LabVIEW graphical programming language. For RSNA, the program used a series of fast and slow filters to detect changes in the voltage of the signal from increasing to decreasing levels. Providing these changes were above an operator-defined threshold, they were classified as synchronized bursts of RSNA. The threshold was set to 10–15% of the average maximum burst height, as discussed previously (34). The program also calculated every 2 s the average values of mean arterial pressure (MAP), heart rate (HR), and RSNA, as well as the amplitude of renal sympathetic bursts and frequency of occurrence (32). Because voltages recorded from RSNA electrodes vary considerably between animals, in each experiment the values were normalized to the upper plateau of the first control baroreflex curve, which was taken to equal 100 normalized units (nu).

**Experimental Protocol**

Two groups of rabbits, instrumented for microinjections into the RVLM (n = 7) or IVLM (n = 6), were subjected to the bilateral treatments into these regions with glutamate, ANG II, rilmenidine, and sarile in two separate experiments 2 days apart. In five of these rabbits, during one of the experiments, ANG II was also infused bilaterally into the lateral recess of the fourth brain ventricle.

**Mapping microinjections into the ventrolateral medulla.** At the beginning of the experiment, glutamate (10 and 20 nmol in 100–200 nl) and vehicle (Ringer solution, 200 nl) or ANG II (10, 20, and 30 pmol in 50–150 nl) were injected bilaterally into the ventrolateral medulla to verify the location of the injection sites. A period of stabilization of least 5 min was allowed after insertion of the injection cannulas, and the cannulas remained in place between consecutive injections of glutamate or ANG II to minimize damage to the brain tissue associated with multiple insertions of the cannulas. Fifteen-minute recovery periods were allowed between the consecutive mapping injections and 30–60 min before the remainder of the experiment.

**Baroreflex estimation.** The RSNA and HR baroreflexes as well as respiration rate were assessed before and 10–15 min after starting the following bilateral treatments into the RVLM or IVLM: 1) glutamate infusion (2 nmol/min for 20 min), 2) rilmenidine injection (4 nmol), 3) ANG II infusion (4 pmol/min for 20 min), 4) sarile injection (500 pmol), 5) Ringer infusion (20 nl/min for 20 min) as well as 6) ANG II infusion into the fourth ventricle (4 pmol/min at 200 nl/min for 20 min). Respiration rate was estimated by determining the time required for 30 breaths. Each rabbit was subjected to two or three different treatments per experiment. Treatments with glutamate or ANG II, relatively short-acting substances, were accomplished in the first part of the experiment, followed, after a 40- to 60-min recovery period, by administrations of sarile or rilmenidine. The order of treatments was randomized between experiments. The same treatment was usually not repeated in the same animal, and thus each animal received a total of eight to ten administrations into the ventrolateral medulla over two experiments.

The treatments into the ventrolateral medulla were made through a 30-gauge stainless steel needle (OD 315 μm) connected via polyethylene SP8 tubing to a 250-μl Hamilton syringe driven by a syringe infusion pump (Harvard Apparatus, model 22) at the rate of 200 nl/min for 30–60 s for rapid injections and 20 nl/min for 20 min for slow infusions. The injection volume was controlled by measuring the displacement of a small air bubble in the polyethylene tubing.

Sarile ([Sar1,Ile8]-ANG II), ANG II (human form) and L-glutamate (all obtained from Sigma) and rilmenidine phosphate (I.R.I. Servier) were dissolved in sterile Ringer solution (Baxter).

Upon completion of the experiment, each animal was deeply anesthetized with pentobarbital sodium, and the injection sites were marked with 100 nl of 2% Pontamine sky blue solution. Brains were removed, fixed in 10% formaldehyde solution, and then frozen and sectioned at 30 μm. Each fifth section from the obex to the rostral pole of the facial nucleus was slide mounted and examined under the microscope for distribution of Pontamine sky blue and the course of the cannula track.

**RSNA and HR Baroreflex Relationship**

The baroreflex was assessed by a ramp rise and fall in MAP produced by intravenous infusions of phenylephrine hydrochloride (0.5 mg/ml, 50–100 μl, Sigma) and sodium nitroprusside (1 mg/ml, 100–200 μl, Fluka), respectively. Injections lasted 30–60 s and the rate of change in MAP was controlled between 0.5 and 1 mmHg/s. MAP, RSNA, and HR from individual rabbits were averaged over 2-s intervals and fitted to a sigmoid five-parameter logistic function to produce the RSNA-MAP and HR-MAP curves using a nonlinear regression program as described in detail elsewhere (38). Parameters included the lower plateau, which was the minimum RSNA or HR, the range between the lower plateau and upper plateau (which was a calculated maximum activation), and the median blood pressure at half the reflex range. Two curvature parameters were used, which allowed for a non-symmetrical fit of the data. The gain, defined as the average slope between the two inflection points of the curve, was calculated as the average of the curvature parameters multiplied by the range and divided by 4.562 (38).

**Statistical Analysis**

Values are expressed as means ± SE. A one-factor repeated-measure ANOVA was used to determine treatment effects for each sympathetic and hemodynamic parameter. The between-animal sum of squares (SS) as well as the treatment SS were removed from the total SS to obtain the residual SS (43). The latter was used to calculate the average within-animal SE, indicating the variability within animals. For
each parameter of the sigmoidal baroreflex curve, comparisons were made by partitioning the treatment SS into orthogonal contrasts for each agent used. Effects of different doses of glutamate and ANG II were analyzed by orthogonal partitioning to determine drug effects and dose within drug effect (43). Similarly, the effect of ANG II given via different routes was assessed by appropriate orthogonal partitioning. Contrasts were considered significant and the null hypothesis rejected when $P < 0.05$.

RESULTS

Effects of Treatments into the RVLM on Resting RSNA and MAP

Histological verification of the injection sites revealed that, in all seven rabbits, the diffusion of 100 nl of Pontamine sky blue (which formed a sphere ~1 mm in diameter) was found in both right and left rostral parts of the ventrolateral medulla, at or just caudal to the level of the rostral tip of the inferior olive (Fig. 2) and thus overlapped the pressor region of the RVLM in the rabbit (10, 33).

Bilateral microinjection of glutamate (10 and 20 nmol) into the RVLM in these rabbits (Fig. 2 and 3) caused a rapid increase in RSNA, reaching a maximum within the first minute of completing the injection (average time to peak 0.5 ± 0.2 min for both doses). At the peak time, the injection of 10 and 20 nmol of glutamate increased RSNA by 81 ± 6 and 88 ± 16%, respectively ($P < 0.001$). The increase in RSNA was mediated by changes in both the amplitude and frequency of sympathetic bursts (Fig. 3). The RSNA response was accompanied by an increase in MAP of 17 ± 3 and 25 ± 5 mmHg for 10 and 20 nmol of glutamate, respectively ($P < 0.01$). The pressor response reached a peak within 0.9 ± 0.2 min of completing the injection. HR decreased by 27 ± 3 and 39 ± 3 beats/min, respectively ($P < 0.01$). After the peak response, RSNA rapidly returned to the pretreatment levels (average time to recovery 1.3 ± 0.6 min for both doses), while MAP remained elevated compared with pretreatment values for 4 ± 1 and 5 ± 1 min, for 10 and 20 nmol of glutamate, respectively. Microinjection of Ringer solu-
tion (200 nl) into the RVLM did not affect MAP, HR, or RSNA (Fig. 3).

In the same seven rabbits, a 20-min bilateral microinfusion of glutamate (2 nmol/min in 20 nl/min) into the RVLM caused a gradual increase in resting MAP (Table 1), reaching a plateau within 10 min of starting the infusion. RSNA was increased in four rabbits, not altered in one rabbit, and decreased in two remaining rabbits, resulting in overall increase of 35 ± 10% (P < 0.05). Microinfusion of Ringer solution (20 nl/min for 20 min) into the RVLM did not affect resting MAP, HR, or RSNA (data not shown). Bilateral microinjection of rilmenidine (4 nmol) into the RVLM decreased MAP and RSNA by 8 ± 2 mmHg (P < 0.01) and 24 ± 8% (P < 0.05), respectively, but did not affect HR (Table 1). In all rabbits, the hypotensive and sympathoinhibitory effects reached a maximum within 12 ± 2 min after injection.

Bilateral microinjection of ANG II (10 and 20 pmol) into the RVLM did not affect resting RSNA, MAP, or HR (Fig. 4). In five rabbits, a higher dose of 30 pmol of ANG II was also injected into the RVLM. In these rabbits, MAP gradually increased by 8 ± 3 mmHg (P < 0.05), whereas RSNA remained unaffected (Fig. 4). The time course of the increase in MAP was slow, with MAP starting to increase within 0.5–1 min and reaching a peak within 4 min of completion of the injection (Fig. 4). The 20-min bilateral microinfusion of ANG II (4 pmol/min in 20 nl/min) into the RVLM did not affect resting MAP, HR, or RSNA (Table 1). Bilateral treatment with the ANG II receptor antagonist sarile (500 pmol) into the RVLM also did not change resting hemodynamic and sympathetic parameters (Table 1).

**Effects of Treatments into the IVLM on Resting RSNA and MAP**

Histological verification of the injection sites revealed that, in all six rabbits, the substances were microinjected into the intermediate part of the ventrolateral medulla 0 to +1.4 mm rostral to the obex level and 0.5–2.5 mm caudal to the pressor region of the RVLM (Fig. 2). Bilateral microinjection of glutamate into the IVLM caused a moderate pressor (the rostral part of the IVLM, Fig. 2) or depressor (the caudal part of the IVLM, Fig. 2) response, and as such, the pooled data did not show any overall effect on MAP (+7 ± 4 and +7 ± 5 mmHg for 10 and 20 nmol of glutamate, respectively). Similarly, the 20-min microinfusion of glutamate into the IVLM caused moderate decreases and increases in MAP and RSNA, producing, overall, little effect on these parameters (Table 2).

Rilmenidine microinjection (4 nmol) into the rostral or caudal parts of the IVLM did not affect MAP (−1 ± 1

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**Table 1. Resting and baroreflex parameters before and after bilateral treatments into the RVLM in conscious rabbits**

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<tr>
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<th>Before</th>
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<tr>
<td><strong>Resting parameters</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>79 ± 3</td>
<td>95 ± 3*</td>
<td>85 ± 5</td>
<td>77 ± 4*</td>
<td>80 ± 3</td>
<td>84 ± 3</td>
<td>79 ± 3</td>
<td>80 ± 2</td>
<td>2.0</td>
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<td>HR, beats/min</td>
<td>187 ± 4</td>
<td>207 ± 11</td>
<td>233 ± 12</td>
<td>224 ± 12</td>
<td>212 ± 12</td>
<td>189 ± 15</td>
<td>220 ± 12</td>
<td>212 ± 14</td>
<td>8.9</td>
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<tr>
<td>RSNA, nu</td>
<td>27 ± 3</td>
<td>36 ± 3*</td>
<td>31 ± 5</td>
<td>23 ± 3*</td>
<td>27 ± 3</td>
<td>27 ± 3</td>
<td>26 ± 5</td>
<td>24 ± 6</td>
<td>2.6</td>
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<tr>
<td>Respiration rate, breaths/min</td>
<td>83 ± 10</td>
<td>83 ± 11</td>
<td>93 ± 15</td>
<td>91 ± 10</td>
<td>86 ± 12</td>
<td>90 ± 22</td>
<td>72 ± 12</td>
<td>77 ± 11</td>
<td>11.8</td>
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<td><strong>RSNA baroreflex parameters</strong></td>
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<td>Lower plateau, nu</td>
<td>6 ± 1</td>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>11 ± 3</td>
<td>8 ± 3</td>
<td>10 ± 2</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
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<td>Upper plateau, nu</td>
<td>100</td>
<td>140 ± 7‡</td>
<td>110 ± 11</td>
<td>74 ± 6‡</td>
<td>100</td>
<td>98 ± 7</td>
<td>86 ± 8</td>
<td>92 ± 6</td>
<td>6.2</td>
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<tr>
<td>Range, nu</td>
<td>94 ± 2</td>
<td>130 ± 7‡</td>
<td>101 ± 9</td>
<td>64 ± 6‡</td>
<td>92 ± 3</td>
<td>88 ± 7</td>
<td>79 ± 8</td>
<td>87 ± 7</td>
<td>6.5</td>
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<td>Curvature, −1/mmHg × 100</td>
<td>27 ± 5</td>
<td>28 ± 4</td>
<td>22 ± 5</td>
<td>24 ± 3</td>
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<td>Gain, −nu/mmHg</td>
<td>5.5 ± 0.7</td>
<td>7.8 ± 0.7‡</td>
<td>5.0 ± 0.7</td>
<td>3.0 ± 0.4‡</td>
<td>4.6 ± 0.2</td>
<td>4.8 ± 0.5</td>
<td>3.9 ± 0.6</td>
<td>4.1 ± 0.4</td>
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</table>

Values are means ± between-animal SE (n = 7). RVLM, rostral ventrolateral medulla; RSNA, renal sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate; nu, normalized units. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. pretreatment value.

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**Fig. 4. The average responses to the bilateral microinjection of ANG II into the RVLM of 7 conscious rabbits. ○, 10 pmol; ●, 20 pmol; ▽, 30 pmol.**

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Table 2. Resting and baroreflex parameters before and after bilateral treatment into the IVLM in conscious rabbits

<table>
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<th></th>
<th>Glutamate Infusion</th>
<th>Rilmenidine</th>
<th>ANG II Infusion</th>
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<th>Within-Animal SE</th>
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<tr>
<td>MAP, mmHg</td>
<td>79 ± 3</td>
<td>76 ± 6</td>
<td>82 ± 6</td>
<td>83 ± 5</td>
<td>76 ± 2</td>
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<td>HR, beats/min</td>
<td>212 ± 18</td>
<td>212 ± 26</td>
<td>238 ± 15</td>
<td>259 ± 19</td>
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<td>RSNA, nu</td>
<td>27 ± 6</td>
<td>31 ± 9</td>
<td>28 ± 4</td>
<td>30 ± 4</td>
<td>29 ± 5</td>
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<tr>
<td>Respiration rate, breaths/min</td>
<td>66 ± 10</td>
<td>72 ± 9</td>
<td>71 ± 9</td>
<td>79 ± 12</td>
<td>59 ± 5</td>
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<td>RSNA baroreflex parameters</td>
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<tr>
<td>Lower plateau, nu</td>
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<td>4 ± 1</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Upper plateau, nu</td>
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<td>99 ± 13</td>
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<td>Range, nu</td>
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<td>Curvature, −1/mmHg *100</td>
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<td>20 ± 3</td>
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<td>24 ± 5</td>
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<td>Gain, −nu/mmHg</td>
<td>5.3 ± 1.0</td>
<td>6.8 ± 2.4</td>
<td>4.3 ± 0.5</td>
<td>3.9 ± 0.3</td>
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</table>

Values are means ± between-animal SE (n = 6). IVLM, intermediate ventrolateral medulla.

and +3 ± 1 mmHg, respectively) or RSNA (+5 ± 2 and +1 ± 3 nu, respectively), resulting in no overall change in these parameters (Table 2). Microinjection of ANG II (10 and 20 pmol) into the rostral or caudal part of the IVLM did not alter MAP or RSNA (data not shown). Bilateral microinfusion of ANG II (4 pmol/min for 20 min) or treatment with sarile (500 pmol) into either the rostral or caudal part of the IVLM did not change resting hemodynamic or sympathetic parameters (see Table 2 for pooled data).

Comparison of Effects of Glutamate Infusion and Injection on MAP

Because the spread of drugs with the 20-min infusion protocol (total volume 400 nl) could be larger than after a single injection (100–200 nl) and thus encompass both the pressor and depressor regions of the ventrolateral medulla, we estimated the correlation between MAP responses to infusion and bolus injection of glutamate into the RVLM and IVLM. The data from two additional rabbits, in which glutamate was administered ~1.5 mm rostral to the RVLM, were also used for the analysis. In these two rabbits, glutamate microinfusion did not affect blood pressure (Fig. 5), but caused salivation and dysphagia, and these rabbits were excluded from the following tests (see MATERIALS AND METHODS). Thus, in the total of 15 rabbits, there was a high degree of correlation (r² = 0.86) between the MAP responses to glutamate infusion (20 nl/min for 20 min) and injection (20 nmol) into the ventrolateral medulla (Fig. 5).

Effects of Treatments into the RVLM on the RSNA and HR Baroreflexes

Bilateral microinfusion of glutamate (2 nmol/min for 20 min) into the RVLM increased the range, upper plateau, and gain of the RSNA baroreflex by 38 ± 7, 40 ± 8, and 41 ± 10%, respectively (P < 0.01; Table 1, Fig. 6). The gain of the HR baroreflex was also increased by 37 ± 10% (P < 0.01) during glutamate infusion, whereas the range and upper and lower plateaus of this reflex were not affected (Fig. 6). By contrast, pretreatment with 4 nmol of rilmenidine into the RVLM decreased the range and gain of the RSNA baroreflex by 37 ± 7% (P < 0.001) and 41 ± 12% (P < 0.05), respectively, without affecting the range and gain of the HR baroreflex (Table 1, Fig. 6). Bilateral microinfusion of ANG II (4 pmol/min for 20 min) into the RVLM (Table 1, Fig. 7) did not alter the RSNA and HR baroreflexes, nor did pretreatment with sarile (500 pmol) into the RVLM (Table 1).

To test the efficacy of sarile in blocking the effects of ANG II, both substances were also given intravenously in two rabbits. Bolus injection of ANG II at a 10-times higher dose (120 pmol) than that used for the RVLM injections increased MAP by 35–40 mmHg, whereas pretreatment with a correspondingly higher dose of sarile (500 pmol) decreased this effect by 37–69%.

To elucidate further the role of ANG II in cardiovascular control in conscious animals, ANG II was also infused (4 pmol/min for 20 min) into the fourth ventricle of five rabbits. This treatment increased MAP by 16 ± 3 mmHg from 75 ± 3 mmHg, RSNA from 29 ± 4 to 39 ± 4 nu (P < 0.05), and the range and upper plateau of the RSNA baroreflex by 19 ± 4 and 17 ± 3%,
respectively ($P < 0.01$), without affecting the gain of this reflex (Fig. 7). Infusion of ANG II into the fourth ventricle did not alter the gain or upper or lower plateau of the HR baroreflex (Fig. 7).

Effects of Treatments into the IVLM on the RSNA and HR Baroreflexes

Bilateral microinfusion of glutamate (2 nmol/min for 20 min) into the IVLM in six rabbits resulted in moderate increases (injections into the rostral part of the IVLM, Fig. 2) or decreases (injections into the caudal part of the IVLM, Fig. 2) in the range and gain of the RSNA baroreflex, resulting, overall, in no significant changes in these parameters (Table 2). Microinfusion of glutamate into the IVLM decreased the upper plateau of the HR baroreflex by $9 \pm 3\%$ ($P < 0.05$). This change was mainly due to a large decrease in HR ($21 \pm 3\%$) in three rabbits receiving microinfusions into the caudal part of the IVLM. Microinjections of rilmenidine into either the rostral or caudal part of the IVLM did not affect the RSNA baroreflex parameters (see Table 2 for pooled data), nor did bilateral microinfusion of ANG II (4 pmol/min for 20 min) or treatment with sarile (500 pmol) into the IVLM (Table 2).

Effects of Treatments into the RVLM and IVLM on Respiration and Behavior

The respiratory rate of 70–90 breaths/min in conscious rabbits was not altered by any treatments into the RVLM or IVLM (Tables 1 and 2). Furthermore, there were no observable alterations in behavior such as grooming or locomotion during or just after treatments.

Treatments into the RVLM and IVLM and Brain Tissue Damage

The repeated insertions of the injection cannulas (OD 315 μm) were associated with a small degree of damage to surrounding brain tissue as revealed by postmortem examination (Fig. 8). The damage area could be observed along the course of the cannula, approaching the dorsal surface of the medulla at ~4 mm from the obex. However, this degree of tissue damage was not associated with neurological disorders or changes in behavior between experiments.

DISCUSSION

The present results demonstrate that the newly developed microinjecting system can be used to modulate neuronal activity selectively in highly circumscribed regions of the ventrolateral medulla in conscious rabbits. The major finding of our study is that neuronal excitation and inhibition in the RVLM, respectively, increases and decreases arterial pressure, RSNA, and the renal sympathetic baroreflex range. To our knowledge, this is the first functional evidence in the conscious animal that the RVLM is of major importance in maintaining resting sympathetic activity and mediating the sympathetic baroreflex. Perhaps surprisingly, this region does not appear to be a major site of sympathoexcitatory and baroreflex action for ANG II in...
conscious, chronically instrumented rabbits under resting conditions, as it has been suggested from studies in anesthetized animals (2, 39, 41).

The increases in RSNA caused by glutamate infusion into the RVLM were remarkably similar to those observed by our laboratory in anesthetized rabbits, in which equipressor infusions of glutamate into the RVLM augmented resting RSNA by 26% and the RSNA baroreflex range by 44% (39). Furthermore, decreases in the resting RSNA and RSNA baroreflex range after injections of rilmenidine into the RVLM in the present study had a comparable magnitude to changes in these parameters after treatment with rilmenidine (0.5–4 nmol) into the RVLM in anesthetized rabbits (22). These similar sympathetic responses to either exciting or inhibiting neuronal activity in the RVLM strongly suggest that this vasomotor region makes an equally important contribution in controlling RSNA in conscious and anesthetized animals both at rest and during baroreflex responses.

Microinfusions of glutamate into the IVLM evoked much smaller responses in blood pressure and RSNA than into the RVLM. One may suggest that the spread of glutamate after infusion was larger then after rapid injection (100–200 nl) and could thus encompass both the pressor and depressor regions of the ventrolateral medulla resulting in little net effect on arterial pressure. However, this is unlikely, as there was a very strong correlation between MAP responses to infusion and injection of glutamate into the same sites of the ventrolateral medulla. This correlation might seem unexpected, considering the total volume of infused glutamate at the time of the baroreflex testing was 200–300 nl, significantly exceeding the volume of rapid injections. However, the actual size of the spread of glutamate may be reduced by active uptake mechanisms. A previous finding that the concentration of glutamate declined 3–6 min postinjection into the brain stem supports this possibility (29). The small effect of glutamate injections and infusions ~1.5 mm rostral to the RVLM may further indicate that both treatments had a similar spread. Thus it is most likely that similar functional areas within the ventrolateral medulla were engaged with the injection and infusion protocols.

The changes in the RSNA baroreflex parameters evoked by rilmenidine were observed only if the injection sites were located within the pressor region of RVLM (Fig. 2), whereas rilmenidine microinjections just caudal to the RVLM (+1.0 to 1.4 mm from the obex, Fig. 2) did not affect MAP, RSNA, or the baroreflex. These results demonstrate the ability of the developed microinjecting technique to affect neuronal activity specifically in this very confined vasomotor region of the RVLM. Additionally, these data suggest that, in conscious animals, the \( \alpha_2 \)-adrenergic and/or imidazoline receptors in the pressor region of the RVLM may play a critical role in regulating sympathetic outflow in a fashion similar to their role in anesthetized animals (14, 18, 22).

Microinfusion of glutamate into the RVLM increased the gain of the HR baroreflex and shifted the HR baroreflex curve to the right, whereas treatment with rilmenidine shifted this curve to the left. These data suggest that the RVLM might be an important integrative site in the baroreflex-mediated cardiac responses in conscious animals, a possibility supported by the finding that the baroreflex-mediated tachycardia to a decrease in arterial pressure was abolished at 1 day after bilateral lesion of the RVLM and attenuated at 5 days after lesion in conscious rats (8). The treatment with glutamate did not affect the upper plateau of the HR baroreflex. However, the upper plateau limit of ~360 beats/min has previously been observed under various physiological conditions (4, 13, 32) and presumably represents the physiological limit to the rate of contraction of the heart in the rabbit.

In our study, treatment with two lower doses of ANG II into the same RVLM sites, which were sensitive to both glutamate and rilmenidine, did not affect arterial pressure or RSNA either at rest or during baroreflex responses. These results are in contrast to previous findings in anesthetized animals that both glutamate and ANG II injections into the same sites of the pressor region of the RVLM substantially increase arterial pressure (2, 36, 41) and facilitate the RSNA baroreflex (39, 40). Only the highest dose of ANG II in our study induced a modest pressor response, which was not accompanied by changes in RSNA. However, the time course of this response was slower than that observed after ANG II microinjections into the RVLM in anesthetized cats and rabbits (latency to peak ~1 and 2 min, respectively) (2, 41), indicating that the effect of the highest dose of ANG II might be due to its leakage...
to other brain regions. Furthermore, in these experiments, treatment with sarile into the RVLM also did not affect resting or baroreflex parameters, indicating that endogenous ANG II does not support arterial pressure and RSNA via a sarile-sensitive receptor in the RVLM in conscious rabbits. By contrast, most previous studies in anesthetized animals, using nonselective ANG II receptor peptide antagonists, found that the ANG II receptor in the RVLM is tonically active (24, 40, 41) and also modulates the RSNA baroreflex (40).

The reason for the inability of lower ANG II doses to produce pressor effects in the present study is not clear. Because we used larger injection volumes (100–200 nl) than those used in most previous studies (20–50 nl), there is a possibility that some of the ANG II might have penetrated into the depressor region of the caudal ventrolateral medulla, where ANG II can decrease arterial pressure (36, 41) and inhibit the RSNA baroreflex (40, 42) and thus offset its excitatory effects in the RVLM. However, this is unlikely because ANG II microinjections into both the rostral and caudal parts of the IVLM also did not affect cardiovascular parameters.

It has been reported that, in the anesthetized rabbit, the ANG II-sensitive region of the RVLM is highly restricted, extending only 1 mm caudal from the caudal pole of the facial nucleus (41). Thus another possibility is that the microinjections in our study were not made exactly into this highly circumscribed area sensitive to ANG II. However, in our study, glutamate microinjections into the RVLM always produced marked pressor responses, whereas it has been shown that the magnitudes of the glutamate and ANG II-evoked responses are well correlated in the ventrolateral medulla (2). By contrast, we did not observe any changes in arterial pressure after injections of ANG II (20 pmol), even into the sites of the RVLM, where glutamate produced pressor responses ~40 mmHg. Therefore, it is more likely that the absence of circulatory responses to treatments into the RVLM with lower doses of ANG II or sarile in our study suggests that the role of the renin-angiotensin system in this region in the beat-to-beat regulation of arterial pressure may be of lesser physiological importance in conscious, chronically instrumented rabbits than in anesthetized animals. The difference between our study and previous work may thus relate to such factors as surgical stress and anesthesia. Indeed, a high degree of surgical stress is typically associated with microinjecting into the RVLM in the acute anesthetized preparation, and hormonal systems such as the renin-angiotensin system have been reported to become activated during anesthesia and surgery (5).

Alternatively, both excitatory and inhibitory angiotensin-sensitive inputs to the presympathetic neurons may coexist within the RVLM and may be unequally activated under different physiological conditions. The finding that microinjection of selective AT1 and AT2 receptor antagonists into the RVLM produced pressor effects, whereas the nonselective ANG II receptor antagonist sarathran decreased arterial pressure in conscious rats is in accord with this possibility (16). The inhibitory angiotensin-sensitive inputs might be less active in an anesthetized preparation, at least under resting conditions, because microinjections into the RVLM of selective AT1 receptor antagonists produced little effect on arterial pressure in anesthetized rats (16, 23). By contrast, it has been suggested that, during hemorrhage, endogenous ANG II plays a primary inhibitory role in the RVLM in anesthetized rats (28).

In this study, infusion of ANG II into the fourth cerebral ven-tricle at a dose that was ineffective given into the RVLM increased resting arterial pressure and RSNA and facilitated the renal sympathetic baroreflex in concert with results reported previously (13). The difference in actions of ANG II suggests that the RVLM was not the major sympa-thoexcitatory site for ANG II under these experimental conditions and that other regions of the central nervous system might be involved (1, 15). In particular, ANG II injected into the cerebrospinal fluid of the fourth ventricle might activate the efferent projections from the circumventricular organs to hypothalamic cells, which, in turn, influence the inputs to the nucleus of the solitary tract, the RVLM, and intermediolateral cell column of the spinal cord (15). Additionally, some ANG II might penetrate into the spinal cord and directly excite the sympathetic preganglionic neurons (44).

Limitations. Although relatively high injection volumes and consequently lower spatial resolution are technical limitations of our microinjecting approach, another limitation could be the tissue damage produced by multiple insertions of the injection cannula (OD 0.315 mm). Taking into account that the vasomotor RVLM neurons form a narrow column (~1 mm wide and 1.5 mm long) in the rabbit (41), the damaged area could encompass ~20% of the pressor region of the RVLM. However, good circulatory responses to glutamate and rilmenidine treatments into the RVLM, which were typically observed in the second experiment, strongly indicate that this partial impairment did not affect the functional integrity of the region in the conscious rabbit.

We cannot also rule out that a portion of the neurons located in the immediate vicinity of the injection site was depolarized with the glutamate concentration used (100 mM), while more distant neurons were excited (29). However, the dose-related pressor responses to glutamate injection, as well as the strong correlation between the blood pressure responses to infusion and injection of glutamate into the ventrolateral medulla, suggest that the depolarizing block did not play a functionally significant role with either the injection or the infusion protocol.

In summary, we have demonstrated that a chronically implanted guide cannula microinjecting system can be effectively used to examine the neurotransmitter mechanisms operating in the integrative sites within the ventrolateral medulla of the conscious rabbit. We found that the pressor region of the RVLM is of major importance in maintaining resting RSNA and mediating the renal sympathetic baroreflex in con-
scious rabbits and may also modulate baroreflex-mediated cardiac responses. The prominent sympathoinhibitory effect of rilmenidine administration into the RVLM indicates that the α₂-adrenergic and/or imidazoline receptors in this region may also play a critical role in regulating sympathetic tone in conscious rabbits, a finding previously demonstrated only in anesthetized animals. The difference between the RVLM and fourth ventricle actions of ANG II indicates that ANG II receptors in the RVLM are not of major importance in the moment-to-moment regulation of arterial pressure and RSNA in conscious, chronically instrumented rabbits under resting conditions, and that other regions may be involved.

Perspectives

With the use of a novel microinjecting system, this study has established the feasibility of investigating the neurotransmitter mechanisms within the RVLM in a conscious rabbit and has confirmed the importance of the RVLM in the moment-to-moment regulation of blood pressure. More importantly, it opens a new field for studying the role of synaptic mechanisms within the RVLM in mediating the circulatory responses to emotional and behavioral stimuli, studies that are possible only in the absence of anesthesia. For example, in a recent preliminary study, we showed that administration of the ANG II receptor antagonist sarile into the RVLM, at the dose used in the present study, markedly reduced the pressor response to air-jet stress in conscious rabbits (29). These results indicate that ANG II receptors in the RVLM may mediate excitatory inputs from the suprabulbar and cortical structures primarily conveying excitatory stimuli from environmental factors. The recent finding that the sympathetic excitatory response to hypothalamic stimulation was decreased after the blockade of AT₁ receptors in the RVLM is in accord with this possibility (45). Thus, in addition to their important role in the short-term regulation of blood pressure, interactions of afferent inputs to the RVLM level could be essential in shaping the circulatory response to defense or emotional behavior. These interactions presumably permit optimal circulatory adjustment to environmental requirements to occur. Further research is now needed to characterize their mechanisms and physiological significance in conscious animals.

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