Attenuation of homeostatic responses to hypotension and glucoprivation after destruction of catecholaminergic rostral ventrolateral medulla neurons

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Madden, Christopher J., Sean D. Stocker, and Alan F. Sved. Attenuation of homeostatic responses to hypotension and glucoprivation after destruction of catecholaminergic rostral ventrolateral medulla neurons. Am J Physiol Regul Integr Comp Physiol 291: R751–R759, 2006.—This study determined the effect of destruction of rostral ventrolateral medulla (RVLM)-C1 cells on integrated sympathetic and hormonal responses to hypotension or glucoprivation. Injection of anti-dopamine β-hydroxylase-saporin into the RVLM resulted in 29–90% depletion of RVLM-C1 neurons and ~60% reduction in the number of A5 neurons. As in our previous study in unanesthetized rats, resting mean arterial pressure (MAP) was reduced by ~10 mmHg in rats with >80% depletion of RVLM-C1 cells compared with control rats, although resting heart rate (HR) did not differ significantly. In the present study, resting plasma levels of norepinephrine (NE) did not differ significantly between control rats and rats with >80% depletion of RVLM-C1 cells, although there was a tendency for RVLM-C1 lesioned rats to have lower levels. Also consistent with our previous study, hydralazine (HDZ)-evoked hypotension resulted in smaller increases in HR and plasma levels of NE in rats with >80% depletion of RVLM-C1 cells compared with control rats. Furthermore, the elevated plasma levels of posterior pituitary hormones vasopressin and oxytocin evoked by HDZ were blunted in RVLM-C1 lesioned rats compared with control rats, even though MAP fell to lower levels in the lesioned rats. Plasma renin activity, plasma osmolality, and plasma protein concentrations did not differ between control rats and rats with >80% depletion of RVLM-C1 neurons. In response to systemic administration of 2-deoxyglucose, the circulating level of epinephrine and the resulting hyperglycemia were attenuated in rats with >80% depletion of RVLM-C1 cells compared with control rats. These results demonstrate that RVLM-C1 cells, in addition to playing a role in acute cardiovascular reflexes, play an important role in integrated sympathetic and hormonal responses to homeostatic challenges such as hypotension and glucoprivation.

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these observations is that neurons of the C1 cell population play a role in the glucoprivation-evoked increase in Epi secretion from the adrenal medulla. Ritter et al. (22) demonstrated that destruction of spinally projecting catecholaminergic cell groups eliminated the hyperglycemic response to systemic administration of 2-DG. However, depletions of catecholamine neurons produced by spinal injections of anti-DβH-saporin are not selective for the C1 cell population but also destroy other spinally projecting DβH-containing neurons (e.g., neurons in the C3, A5, and A7 cell groups) (22, 30), and therefore the elimination of the hyperglycemic response in the study by Ritter et al. (22) cannot be attributed to the specific destruction of the C1 cell population. Thus a second general goal of the present study was to determine whether the C1 cell population plays a role in a sympathoadrenal reflex unrelated to CV function, glucoprivation-induced Epi secretion, and the resulting hyperglycemia.

**METHODS**

**General Methods**

Male Sprague-Dawley rats (Zivic Laboratories or Charles River Laboratories; the supplier was switched because of changes in local animal facility regulations) weighing 250–350 g at the start of the study (i.e., time of toxin injection) were used throughout these studies. All rats were singly housed and given ad libitum access to standard rat Chow (Purina 5001) and water. The colony room was maintained at a temperature of 22–23°C and kept on a 12:12-h light-dark cycle. Animal care and experimental procedures were performed with the approval of the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experiments were performed using conscious unrestrained rats. Before experimentation, rats were anesthetized with halothane (2–3% in oxygen) and implanted with femoral arterial (polyethylene-50 tubing filled with heparinized saline, 400 U/ml) and venous catheters (polyvinyl-3 tubing filled with heparinized saline, 40 U/ml) that were tunneled subcutaneously, exteriorized between the scapulae of the rat, and threaded through a tethering harness. Halothane anesthesia was terminated, and rats were allowed at least 1 day to recover before experimentation. To record arterial pressure and heart rate (HR), the arterial catheter was attached to a pressure transducer (Statham P23-ID) and a polygraph recording system (Grass model 7 Physiograph).

**Brain Stem Toxin Injections**

RLVM microinjections were performed as described previously (16). Briefly, all toxin injections were made into rats anesthetized with halothane (2–5% in oxygen). For microinjections of toxin into the RLVM, a glass micropipette (outer tip diameter 40–75 μm) was positioned as follows: with the pipette angled 20° rostrally, the pipette tip was placed on the caudal tip of the area postrema and then moved 1.8 mm lateral and 1.8 mm rostral to this landmark. The tip was then advanced 2.8 mm through the medulla into the RLVM, with coordinates based on our group’s previous publications (11, 15, 16). All injections were given over a 1- to 2-min period using a PicoPump (WPI, New Haven, CT). To bilaterally destroy the C1 cell population, rats (n = 18) received bilateral injections of anti-DβH-saporin [21 ng in 200 nl of artificial cerebrospinal fluid (aCSF) or sterile saline per side] into the RLVM. This dose produces large reductions in the number of C1 cells while still maintaining selectivity for the C1 cell population (15). To control for any nonspecific effects of a saporin-conjugated toxin, other rats (n = 5) received bilateral injections into the RLVM of saporin conjugated to an antibody (Mab-ZAP; Advanced Targeting Systems) raised against mouse IgG (21 ng in 200 nl of aCSF or sterile saline). To control for nonspecific damage created by microinjections into the RLVM, yet other rats (n = 15) received bilateral injections of aCSF or sterile saline into the RLVM or no injection. To control for the depletion of the A5 cell population in anti-DβH-saporin-treated rats, other rats (n = 3) received infusions of 6-hydroxydopamine (6-OHDA) into the A5 area, as described previously (16). Briefly, animals were placed in a stereotaxic instrument with the incisor bar positioned 2.5 mm below the interaural line. Two small holes were drilled in the skull at points 1.0 mm caudal and ±2.6 mm lateral to interaural zero, and pargyline (75 mg/kg ip), a selective monoamine oxidase inhibitor, was administered. A micropipette was lowered into the A5 area (0.2 mm below interaural zero), and 5 μg of 6-OHDA in 2 μl of vehicle (0.1% ascorbic acid) were infused over 20 min in each side. After intraparenchymal injections or infusions, the incision was closed and the animal received an injection of Bicilllin (30,000 units im) and was returned to its home cage. A 2-wk recovery period was allowed before any further experimentation was performed on these rats. This recovery time has been shown to be sufficient for the development of the maximal cell loss in toxin-injected rats and 6-OHDA-infused rats (15, 16). All of the rats used in the glucoprivic experiments were also used in previously reported studies investigating reflex cardiovascular regulation and received injections of phenylephrine, sodium nitroprusside, phenylbiguanide, and potassium cyanide prior to the glucoprivic testing (16).

**Hypotensive Challenge**

Food and water were removed from the cage just before the beginning of the test. A baseline blood sample (1.8 ml) was collected into ~80 units of heparin. Blood was then centrifuged (10,000 g, 1 min), and 300 μl of plasma were collected into 6 μl of 5 N perchloric acid and stored at −80°C until assayed for norepinephrine (NE) and Epi, an additional 500 μl of plasma were collected for determination of protein concentration, osmolality, vasopressin (VP), oxytocin (OT), and renin activity. Plasma protein concentrations were determined by refractometry. Plasma osmolality was measured using the freezing point depression method with a microosmometer (Advanced Instruments, Norwood, MA). Remaining plasma was stored at −80°C until assayed for VP, OT, and plasma renin activity. After the baseline blood sample was collected, hydralazine (HDZ, 10 mg/kg iv; Sigma Chemicals) was administered to decrease pressure and 30 min later, a second blood sample was taken using the same procedure as described for the baseline sample. Ninety minutes after the HDZ injection, rats were deeply anesthetized with urethane (2 g/kg iv) and perfused transcardially. Plasma VP and OT levels were determined by radioimmunoassay after extraction from plasma with the use of C18 cartridges (27, 34). Plasma renin activity was measured using radioimmunoassay (34). Plasma catecholamines were extracted with alumina and measured using HPLC with coulometric electrochemical detection (ESA, Chelmsford, MA) (16).

**Glucoprivic Challenge**

The glucoprivic challenge test was performed in the middle of the light cycle at least 3 h after other reflex testing (reported previously in Ref. 16). Food was removed from the cage at least 1-h before the baseline plasma sample was taken. For plasma samples, 0.9 ml of blood was collected from the arterial line into chilled microcentrifuge tubes containing ~40 units of heparin. Blood was then centrifuged at 10,000 g for 1 min. 100 μl of plasma were collected and stored at −20°C until assayed for glucose, and 300 μl of plasma were collected into 6 μl of 5 N perchloric acid and stored at −80°C until assayed for Epi. 2-DG (200 mg·kg−1·ml−1 iv; Sigma Chemicals) was administered 15 min after the baseline blood sample was collected. Sixty minutes after 2-DG administration, a second blood sample was taken, as described for the baseline condition. Previous work has demonstrated that the peak 2-DG-evoked increase in plasma glucose occurs at approximately this time point (22). Plasma glucose was assayed using the glucose oxidation method with a glucometer (Beckman).
Plasma catecholamines were extracted with alumina, eluted with 0.1 N perchloric acid, and measured using HPLC with coulometric electrochemical detection (ESA).

**Histological Assessment of Lesions**

All histological assessments were performed using standard techniques as previously described (16). Briefly, at the conclusion of experiments, all rats were deeply anesthetized (urethane, 2 g/kg iv) and perfused transcardially. Brains were removed, post-fixed, and cut into 30-µm coronal sections. For immunohistochemical staining, brain stem sections from each rat were incubated in rabbit anti-PNMT or rabbit anti-TH (Protos; 1:1,000 to 1,200 dilutions) and immunohistochemically processed using the avidin-biotin immunoperoxidase method as previously described (15, 16). Cell counts of PNMT-positive and tyrosine hydroxylase (TH)-positive neurons were conducted in every sixth 30-µm brain stem section through the rostrocaudal extent of the RVLM, the A5 area, and the A1 area, as defined previously (16).

**Statistical Analyses**

All statistics were performed using SYSTAT (version 10; SPSS). Control, 6-OHDA-infused, and Mah-ZAP-injected rats were assigned to groups according to the treatment received; however, based on physiological assessments of cardiovascular reflexes in a previous study (16), the anti-DβH-saporin group was subdivided into rats with small depletions of the C1 cell population (<80%) and rats with large depletions of this population (>80%). Experiments examining one variable across several groups at multiple time points were analyzed using a repeated-measures analysis of variance (ANOVA). When a significant interaction between the grouping variable and time was found, an ANOVA with Bonferroni correction was performed at each time point. For the hypotension experiment, data from the group of rats with <80% depletion of the RVLM-C1 cells are shown, although because of the small sample size (n = 3), statistical analyses exclude this group. Based on previous work in our laboratory (16) demonstrating a small decrease in resting mean arterial pressure (MAP) in rats with >80% depletion of the RVLM-C1 cells compared with control rats, a one-tailed t-test was used to compare resting MAP between these groups in the current study. Also, one of the rats in the large C1 cell depletion group and one of the control rats responded to HDZ injection with sustained bradycardia instead of tachycardia; therefore, all reported values and statistical analyses exclude these two rats. Values are expressed as means ± SE. Differences were considered significant when P < 0.05.

**RESULTS**

**Hypotension Experiment**

**Lesion assessment.** Histological assessments in the present study were found to be similar to data previously reported by our group (15, 16). The majority of PNMT-positive cells were located ~1–1.5 mm rostral to the obex, which corresponds to the area just caudal to the caudal pole of the facial nucleus and is ~11.6–12.3 mm posterior to bregma based on the atlas of Paxinos et al. (20). Two weeks after injection of 21 ng of anti-DβH-saporin into the RVLM, the number of PNMT-positive neurons in this area was markedly reduced (Table 1). The reduction in the number of C1 neurons appeared to extend rostrocaudally from the site of injection in a graded fashion (Fig. 1). The most complete depletion was seen ~900–1,800 µm rostral to obex, whereas a smaller reduction was observed in the area extending from the obex to 720 µm rostral to the obex, as previously documented (15). In the RVLM, the region extending caudally for 720 µm from the caudal pole of the facial nucleus, the number of PNMT-positive neurons was reduced by 83 ± 3% (range: 63–96%) in the toxin-injected rats compared with the average number of neurons counted in vehicle-injected animals. Two separate groups of anti-DβH-saporin-injected rats were established, using an 80% reduction in the number of C1 neurons of the RVLM (compared with control rats) as the division point (see Ref. 16). Cell counts of the C1 cell population were significantly reduced in the anti-DβH-saporin-injected groups compared with control rats (P < 0.001) (Fig. 1 and Table 1). In addition, cell counts of the A5 cell population in rats with large (>80%) depletions of the RVLM-C1 cell population were reduced by 64 ± 5% (P < 0.001 compared with control), and counts of this population in rats with small (<80%) depletions of the RVLM-C1 cell population were reduced by 42 ± 14% (P < 0.01 compared with control) (Fig. 1 and Table 1). Injections of toxin into the RVLM did not reduce the number of neurons in the A1 cell group (Fig. 1 and Table 1).

Similar to previous reports (15, 16), in a small subset (n = 2 of 11) of anti-DβH-saporin-injected rats, a small area of necrosis was noted at the injection site. However, this necrotic damage did not correlate with the noted depletion of the C1 cell population; indeed, one of the rats with necrosis belonged to the small (<80%) C1 cell depletion group.

**Physiological evaluation.** Resting MAP was reduced by 10 mmHg in the rats with large (>80%) depletions of the RVLM-C1 population compared with control rats (Fig. 2; P = 0.0275, t-test), as in the previous study (16). Injection of HDZ resulted in a transient increase in MAP (<1 min) followed by a long-lasting hypotension, with MAP falling to lower levels in the rats with large (>80%) depletions of the RVLM-C1 cell population compared with control rats (Fig. 2). Resting HR did not differ significantly between the groups, and the increase in HR evoked by the HDZ hypotension was significantly attenuated in the rats with large depletions of the RVLM-C1 cell population compared with control rats, as in the previous study (16).

Baseline plasma levels of NE and Epi did not differ between groups (Table 2). The plasma level of NE attained following HDZ-evoked hypotension was significantly attenuated in the

**Table 1. Cell counts of catecholaminergic cell populations in rats undergoing the hypotension experiment**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>C1</th>
<th>A1</th>
<th>A5</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>198±5</td>
<td>184±6</td>
<td>205±3</td>
</tr>
<tr>
<td>Anti-DβH-saporin (&lt;80%)</td>
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<td>181±12</td>
<td>119±29*</td>
</tr>
<tr>
<td>Anti-DβH-saporin (&gt;80%)</td>
<td>7</td>
<td>23±4*</td>
<td>188±11</td>
<td>75±10*</td>
</tr>
</tbody>
</table>

Values are bilateral phenylethanolamine-N-methyl transferase (PNMT)-positive (C1 group) or tyrosine hydroxylase (TH)-positive (A1 and A5 groups) cell counts shown in control rats and rats receiving bilateral injections of anti-dopamine β-hydroxylase (DβH)-saporin into the rostral ventrolateral medulla (RVLM). All cell counts are based on the sum of counts in every 6th brain stem section (one 30-µm section of each 180 µm) through each respective area. The C1 cell count is based on the sum of the counts from 4 sections extending caudally for 720 µm from the caudal pole of the facial nucleus. A1 cell counts are taken from 7 sections extending caudally for 1,080 µm from the obex. A5 cell counts are taken from ~16 sections extending rostrally from the facial nucleus through the rostral extent of this cell population (~4,500 µm rostral to the obex). The toxin-injected rats were divided into 2 groups based on the magnitude of C1 cell depletion, with an 80% depletion used as the division point. *P < 0.05, significantly different from all other groups.
Fig. 1. Cell counts of tyrosine hydroxylase (TH)-positive neurons across the rostrocaudal extent of the A1, C1, and A5 cell populations. Bilateral counts are shown in the ventrolateral area of 30-μm coronal sections in control rats (n = 4), anti-dopamine β-hydroxylase (DβH)-saporin-injected rats with large (>80%) depletions of the C1 cell population within the rostral ventrolateral medulla (RVLM; n = 7), and anti-DβH-saporin-injected rats with small (<80%) depletions of the C1 cell population within the RVLM (n = 3). Arrow indicates the level targeted by our coordinates for the toxin injection. Values are means ± SE.

Fig. 2. Time course of hydralazine (HDZ)-evoked changes in mean arterial pressure (MAP) in control rats (n = 10), anti-DβH-saporin-injected rats with <80% depletions of the RVLM-C1 cells (n = 5), and anti-DβH-saporin-injected rats with >80% depletions of the RVLM-C1 cells (n = 7). Values are means ± SE. *P < 0.05, main effect of grouping variable (repeated-measures ANOVA.)

rats with large (>80%) depletions of the RVLM-C1 cell population compared with control rats (Table 2), similar to the previous study (16). HDZ-evoked plasma Epi values did not differ between groups (Table 2). Baseline plasma levels of VP and OT did not differ between groups (Fig. 3). HDZ increased the concentration of VP in plasma in all groups; however, the concentration of VP in plasma resulting from HDZ administration was significantly attenuated in the large (>80%) C1 depletion group compared with the control group (Fig. 3A). Likewise, the plasma concentration of OT was increased by HDZ in all groups, and the plasma level of OT resulting from HDZ administration was significantly attenuated in RVLM-C1 lesioned rats compared with the control group (Fig. 3B). Plasma osmolality did not differ among the groups either in the baseline condition or after HDZ administration, although HDZ did cause a significant increase in plasma osmolality (Table 3). Likewise, plasma protein was not found to differ between groups either in the baseline condition or after injection of HDZ, although HDZ caused a small decrease in plasma protein (Table 3). Plasma renin activity did not differ among the groups either in the baseline condition or after HDZ administration, although HDZ increased plasma renin activity (Table 3).

Glucoprivation Experiment

Lesion assessment. All histological assessments in rats undergoing the glucoprivation experiment were found to be similar to data previously reported by our laboratory (15, 16) as well as the present study hypotension experiment. Two weeks after injection of 21 ng of anti-DβH-saporin into the RVLM, the number of PNMT-positive neurons in this area was markedly reduced (Table 4). The reduction in the number of C1 neurons appeared to extend rostrocaudally from the site of injection in a graded fashion (Fig. 4). In the RVLM, the region extending caudally for 720 μm from the caudal pole of the facial nucleus, the number of PNMT-positive neurons was reduced by 87 ± 8% (range: 29–99%) in the toxin-injected rats compared with the average number of neurons counted in vehicle-injected animals. Because there was only one rat with a small (<80%) depletion of the C1 cell population, data from this animal were not included in the group analyses, however, it is noteworthy that all of the responses of this rat closely resembled those of control rats. Cell counts of the C1 cell population were significantly reduced in the anti-DβH-saporin-injected group compared with all other groups (P < 0.001) (Fig. 4 and Table 4). In addition, cell counts of the A5 cell population in anti-DβH-saporin-injected rats were reduced by 69 ± 4% (P < 0.001 compared with control) (Fig. 4 and Table 4). Injections of toxin into the RVLM did not reduce the number of neurons in the A1 cell population (Fig. 4 and Table 4). A variety of control injections were performed to evaluate the nonselective effects of the immunotoxin injection. Compared with control counts, no reduction in the number of C1 neurons was observed after injection of Mab-ZAP, a saporin toxin conjugate of a mouse IgG antibody, into the RVLM or after infusion of 6-OHDA into the A5 area (Fig. 4 and Table 4).

Similar to previous reports (15, 16), in a small subset of anti-DβH-saporin-injected rats (n = 3 of 11), a small area of...
necrosis was noted at the injection site. A similar area of necrosis at the site of injection was found in a subset of Mab-ZAP-injected rats (n = 2 of 5).

**Physiological evaluation.** Baseline plasma concentrations of Epi did not differ among any of the four groups (Fig. 5A). Systemic administration of 2-DG increased the plasma concentration of Epi in all rats. However, the plasma Epi concentration after administration of 2-DG was significantly attenuated in the >80% C1 depletion group compared with all other groups (P < 0.01, Fig. 5A). Similarly, the resting plasma glucose concentration did not differ among the four groups (Fig. 5B), but the plasma concentrations of glucose resulting from 2-DG administration were significantly attenuated in the >80% C1 depletion group compared with all other groups (P < 0.01, Fig. 5B).

**DISCUSSION**

Previous studies have demonstrated that neurons of the C1 cell population play a role in sympathoexcitatory cardiovascular reflexes (16, 29–31). The present data are consistent with these findings and extend them to suggest a role of the C1 cell population not only in sympathoexcitatory cardiovascular reflexes but also in hormonal responses related to cardiovascular homeostasis (VP and OT secretion in response to hypotension) and in a response unrelated to cardiovascular function (glucose homeostasis (VP and OT secretion in response to hypotension).

Immunohistological evaluations of the anti-DβH-saporin-induced depletions of catecholaminergic cell populations in the current study are quite similar to data previously reported by our group (15, 16). We believe that these depletions reflect the destruction of these neurons, as we and others have previously discussed (15, 30). In addition, because all aspects of the histological assessment in the present study are consistent with those seen in our previous experiments, we assume that there is minimal nonspecific damage within the RVLM, as documented in a previous report (15).

Several lines of evidence suggest that resting sympathetic vasomotor outflow is maintained, although perhaps at slightly reduced levels, in rats with extensive depletions of the C1 cell population. In the present study, resting MAP was reduced by ~10 mmHg in rats with >80% depletions of the RVLM-C1 cells compared with control rats, as noted in a previous study (16). In separate RVLM-C1 lesion experiments utilizing telemetry to measure arterial pressure, toxin treatment reduced arterial pressure by ~10 mmHg from pretreatment control levels (Stedenfeld K and Sved AF, unpublished observation). In the present study, resting plasma NE levels, an index of sympathetic outflow, may have been slightly reduced by destruction of RVLM-C1 neurons; we observed a 30% decrease in basal plasma NE levels, which did not attain statistical significance. Still, given the variability in measurement of resting plasma NE levels, a difference of 30% would be difficult to show; indeed, a power analysis indicated that our sample size was inadequate (power = 0.2) to detect significance given the variability and the magnitude of the effect. Conclusive evidence for or against a small decrease in basal sympathetic vasomotor tone by RVLM-C1 lesions will be difficult to produce, although the existing data (i.e., the decrease in resting MAP) suggest that decreased sympathetic nervous system activity under baseline conditions may occur.

Previous studies have demonstrated an attenuation of baroreceptor-mediated increases in HR (16) and splanchnic symp-
lesions, suggesting that a response that is dependent in part on plasma renin activity were not altered by large RVLM-C1 of the C1 cell population. Still, HDZ-evoked increases in whether it is due to destruction of rostrally projecting neurons resulting attenuation of sympathoexcitatory responses or related to destruction of spinally projecting C1 neurons and the attenuation of plasma OT levels seen in C1-depleted rats is not assessed in studies employing injections of anti-DβH-saporin into the RVLM. No significant differences in pOsm, pProt, or PRA were noted among the groups, *P < 0.05 compared with resting level, overall effect.

The hypertensive nerve activity (30) in RVLM-C1-depleted rats compared with control rats. Similarly, in the present study, the hypertensive-evoked increase in HR and sympathetic vasomotor outflow (as assessed by plasma NE concentration) was attenuated in rats with large (>80%) depletions of the C1 cell population compared with control rats. Given the similarity of these results to those observed in rats with spinal injections of anti-DβH-saporin, in which rostrally projecting neurons of the C1 cell population are not destroyed, the most likely explanation for these results is that the C1 cell population plays a direct role in hypertensive-evoked increases in sympathetic vasomotor outflow by supplying a portion of the supraspinal drive to preganglionic neurons of the spinal cord. However, in the current study, depletion of the C1 cell population also was associated with an attenuation of the hypertensive-evoked increase in plasma levels of OT. It has been shown previously (10) that after HDZ administration, the high circulating level of OT plays a role in the hypertensive-evoked tachycardia and increase in renin secretion (presumably through an action involving augmentation of the sympathetic response). Therefore, the attenuated OT response seen in C1-depleted rats compared with control rats could account for the attenuated responses in HR and sympathetic activation observed in C1-depleted rats in the current study. Unfortunately, hypertensive-evoked plasma OT levels were not assessed in studies employing injections of the immunotoxin into the spinal cord. Thus it is not clear whether the attenuation of plasma OT levels seen in C1-depleted rats is related to destruction of spinally projecting C1 neurons and the resulting attenuation of sympathoexcitatory responses or whether it is due to destruction of rostrally projecting neurons of the C1 cell population. Still, HDZ-evoked increases in plasma renin activity were not altered by large RVLM-C1 lesions, suggesting that a response that is dependent in part on the increase in circulating OT levels was not attenuated under the present conditions.

Another notable observation of the present study is that hypertensive-evoked increases in plasma levels of Epi were not reduced in rats with large (>80%) depletions of the C1 cell population compared with control rats, in contrast to the large reduction in plasma NE levels under these conditions. Thus the hypertensive-evoked increase in sympathetic outflow to Epi-secreting adrenal chromaffin cells appears to be uncompromised in rats with large depletions of the C1 cell population, suggesting a clear distinction between the role of the C1 cell group in the control of sympathetic vasomotor outflow and Epi secretion. The lack of an effect of RVLM-C1 lesions on HDZ-evoked Epi secretion is even more surprising in conjunction with the observation that these lesions produced a substantial attenuation of the Epi secretion in response to 2-DG. Thus C1 cells may be differentially involved in Epi secretion evoked by different stimuli. A functional division in the neurons mediating hypoglycemic and hypertensive responses has been previously demonstrated (1) at the level of the preganglionic neurons (i.e., preganglionic neurons that respond to

### Table 3. Resting and hypotension-evoked levels of plasma osmolality, protein, and renin activity

<table>
<thead>
<tr>
<th></th>
<th>pOsm, mosM</th>
<th>pProt, g/dl</th>
<th>PRA, ng/mL/min⁻¹</th>
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<tr>
<td></td>
<td>Resting</td>
<td>HDZ</td>
<td>Resting</td>
</tr>
<tr>
<td>Control</td>
<td>290±2</td>
<td>294±2*</td>
<td>6.0±0.1</td>
</tr>
<tr>
<td>Anti-DβH-saporin (&lt;80%)</td>
<td>289±0</td>
<td>294±5*</td>
<td>6.2±0.2</td>
</tr>
<tr>
<td>Anti-DβH-saporin (&gt;80%)</td>
<td>287±2</td>
<td>296±3*</td>
<td>6.2±0.1</td>
</tr>
</tbody>
</table>

Values are resting and HDZ-evoked levels of plasma osmolality (pOsm), plasma protein (pProt), and plasma renin activity (PRA) in control rats and rats receiving bilateral injections of anti-DβH-saporin into the RVLM. No significant differences in pOsm, pProt, or PRA were noted among the groups, *P < 0.05 compared with resting level, overall effect.

### Table 4. Cell counts of catecholaminergic cell populations in rats undergoing the glucoprivation experiment

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>C1</th>
<th>A1</th>
<th>A5</th>
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<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>179±4</td>
<td>206±9</td>
<td>191±22</td>
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<tr>
<td>Anti-DβH-saporin (&lt;80%)</td>
<td>3</td>
<td>9±3*</td>
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<td>60±9†</td>
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<td>6-OHDA</td>
<td>7</td>
<td>199±14</td>
<td>211±9</td>
<td>13±0†</td>
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<tr>
<td>Mab-ZAP</td>
<td>5</td>
<td>164±13</td>
<td>201±16</td>
<td>201±18</td>
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</tbody>
</table>

Values are bilateral PNMT-positive (C1 group) or TH-positive (A1 and A5 groups) cell counts in control rats, rats receiving bilateral injections of anti-DβH-saporin into the RVLM with >80% depletions of the C1 cell population, rats receiving infusions of 6-hydroxydopamine (6-OHDA) into the A5 area (A5X; n = 3), and rats receiving injections of Mab-ZAP into the RVLM (MabZap; n = 5). Arrows indicate the level targeted by our coordinates for the anti-DβH-saporin or Mab-ZAP injection (filled arrow) or for the 6-OHDA infusion (open arrow). Values are means ± SE.
administration of 2-DG are not barosensitive). A possible interpretation of the current data is that this functional division extends to the sympathetic premotor neurons supplying the drive to Epi-secreting adrenal chromaffin cells, with the supraspinal neurons supplying the sympathetic premotor drive to Epi-secreting chromaffin cells during hypotension differing from those that supply this drive during glucoprivation.

An interesting finding of the current study is that the increase in the plasma level of VP elicited by HDZ-evoked hypotension is attenuated in rats with large (>80%) depletions of the C1 cell population within the RVLM, compared with all other groups (including a control group with HDZ thalamotomies (43), the vast majority of the neurons destroyed in the present study are likely those with projections to the spinal cord. However, we acknowledge that a small number of C1 neurons with projections to the hypothalamus are found in the area of large C1 cell depletion and that destruction of these cells may contribute to the physiological consequences seen in the current study. Consequently, a potential explanation of the current results is that rostrally projecting neurons of the C1 cell population, like those of the A1 cell population, provide an excitatory input to VP secreting cells of the hypothalamus. Indeed, PNMT-containing neurons project to the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus (2, 14, 26, 41), and electrical stimulation of the PVN area increases plasma VP levels under certain conditions (25).

Alternatively, C1 neurons projecting to the PVN could provide an excitatory input to OT-secreting neurons. A previous study has demonstrated that systemic administration of an OT antagonist blunts the increase in plasma VP in rats made hypotensive with HDZ (10). Another possibility that cannot be excluded is that VP is cleared more rapidly in rats with large depletions of the C1 cell population compared with control rats. A final hypothesis to explain the blunted hypotension-evoked increase in plasma VP in C1-lesioned rats compared with control rats is to propose that increased levels of circulating catecholamines act to augment hypotension-evoked VP secretion. Rats with large depletions of the C1 cell population would therefore secrete smaller amounts of VP in response to hypotension because of a blunted sympathetic response. Notably, sympathetic outflow produced by intravenous injection of 6-OHDA in rats blunts hypotension-evoked VP (and OT) secretion (33), and electrical stimulation of the superior cervical ganglion, which provides the sympathetic input to the posterior pituitary, stimulates VP (and OT) secretion (13).

Glucoprivation

A previous study by Ritter et al. (22) clearly demonstrated a role of spinally projecting catecholaminergic neurons in glucoprivation-induced hyperglycemia (presumably due to impaired sympathoadrenal activation, although this was not tested directly); however, because of the depletion of several different catecholaminergic cell populations in that study (A5, A7, C1, and C3 cell populations), it was not clear which neurons are crucial to these responses. The present study, utilizing a different methodology [brain stem injections of immunotoxin as opposed to the spinal injections used in the study of Ritter et al. (22)] and controls for damage to other catecholaminergic cell groups, has clarified this issue and indicates that the C1 cell population is a critical component of this response. More specifically, in the present study, 2-DG-evoked plasma levels of Epi were attenuated in anti-DβH-saporin-injected rats (having large depletions of the C1 and A5 cell population) compared with all other groups (including a control group with depletions of the A5 cell population alone). Because sympathoadrenal outflow is a major mediator of glucoprivation-induced hyperglycemia (17), it is not surprising that an atten-
ulation of the sympathoadrenal response to 2-DG was associated with an attenuation of the hyperglycemic response. A likely explanation for these effects is that C1 neurons provide a large portion of the sympathetic premotor drive to the preganglionic neurons providing sympathoadrenal outflow in response to glucoprivation. In fact, on the basis of electrophysiological properties of preganglionic neurons and their responses to stimulation of the RVLM, Morrison and Cao (18) previously proposed a role of C1 neurons in driving glucoprivation-induced sympathetic outflow to Epi-secreting chromaffin cells of the adrenal. Alternatively, destruction of the rostrally projecting C1 neurons in the current study could account for the attenuated increase in plasma catecholamines evoked by 2-DG in anti-DJH-saporin-injected rats compared with control rats. Consistent with this explanation, it was previously reported (39) that hypothalamic deafferentation attenuates the increase in plasma catecholamines evoked by 2-DG. However, given that spinal injections of anti-DJH-saporin (which do not destroy rostrally projecting catecholaminergic cells) eliminate the 2-DG-induced hyperglycemia (22) and the observation that the forebrain is not necessary for the 2-DG-induced hyperglycemic response (5), a more likely explanation for the current results is that spinally projecting C1 neurons provide a large portion of the supraspinal drive of sympathoadrenal outflow in response to glucoprivation.

In conclusion, the present data are consistent with the notion that RVLM-C1 neurons are essential for normal cardiovascular reflexes, although they are not essential for maintaining baseline sympathetic vasomotor tone at near normal levels. The role of the RVLM-C1 neurons in cardiovascular regulation is not unique to sympathetic vasomotor outflow, because the loss of these neurons also interferes with secretion of posterior pituitary hormones in response to HDZ-evoked hypotension. Furthermore, RVLM-C1 neurons appear to be involved in sympathoadrenal responses to 2-DG-induced glucoprivation.

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