Inactivation of the PVN during hypoglycemia partially simulates hypoglycemia-associated autonomic failure

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Departments of 1Psychology and of 4Medicine, University of Washington, Seattle 98195; 2Department of Veterans Affairs, Puget Sound Health Care System, Seattle 98108; and 4Geriatric Research, Education and Clinical Center, Department of Veterans Affairs, Puget Sound Health Care System and Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98195

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Evans, Scott B., Charles W. Wilkinson, Pam Gronbeck, Jennifer L. Bennett, Gerald J. Taborsky, Jr., and Dianne P. Figlewicz. Inactivation of the PVN during hypoglycemia partially simulates hypoglycemia-associated autonomic failure. Am J Physiol Regul Integr Comp Physiol 284: R57–R65, 2003. First published October 10, 2002; 10.1152/ajpregu.00439.2002.—The anatomic connections of the paraventricular nucleus of the hypothalamus (PVN) are such that it is ideally situated to modulate and/or control autonomic responses to a variety of stressors, including hypoglycemia. In our experimental model of hypoglycemia-associated autonomic failure (HAAF), a syndrome in which the counterregulatory response to hypoglycemia is partially compromised via unknown mechanisms, activation of the PVN is blunted (15). We hypothesized that this blunted PVN activation during HAAF may be sufficient to cause the impaired counterregulatory response. To test this hypothesis, we anesthetized the PVN with lidocaine during insulin-induced hypoglycemia in rats and measured counterregulatory hormone levels. PVN inactivation decreased indexes of the sympathoadrenal response (plasma epinephrine and norepinephrine) and the hypothalamic-pituitary axis response (ACTH). Inactivation decreased the peak epinephrine response to hypoglycemia by almost half (−42 ± 6% from control; P = 0.04) and the peak norepinephrine response by 34 ± 5% (P = 0.01). The peak plasma ACTH levels attained were suppressed by 35 ± 6% (P = 0.02). Adrenal corticosterone and pancreatic glucagon responses were not impaired. This pattern of neuroendocrine response is unlike that previously seen with our HAAF model. Control infusions of lidocaine ≥1 mm anterior or posterior to the PVN did not simulate this neuroendocrine pattern. Thus it appears that decreased PVN activation, as occurs with HAAF, may be involved in specific components of HAAF (i.e., blunting the sympathoadrenal and hypothalamic-pituitary-adrenocortical axis response), but not in others (i.e., blunting the glucagon response).

The paraventricular nucleus (PVN) of the hypothalamus responds to physiological and psychological stressors (21) by increasing the synthesis and release of vasopressin (VP) and corticotropin-releasing factor (CRF), which stimulate the release of ACTH from the pituitary (57) and, consequently, release of glucocorticoids (e.g., corticosterone) from the adrenal cortex. The PVN also sends direct projections to autonomic preganglionic cells in the spinal cord and parasympathetic nuclei in the brain stem (23, 36, 46, 50) and may activate the sympathetic and parasympathetic nervous systems through these autonomic centers (4, 22, 58). Adrenomedullary epinephrine release secondary to sympathetic activation, in concert with plasma corticosterone, represents the essential neuroendocrine response to stressors. This response may be reduced on repeated challenge with that same stressor (7–10, 17). A clinically important example of a blunted neuroendocrine response to a repeated physiological stressor is the defective counterregulatory response to repeated hypoglycemia in insulin-requiring diabetic patients, known as hypoglycemia-associated autonomic failure (HAAF) (9). Hypoglycemia stimulates the neuroendocrine response described above, as well as glucagon release by the pancreas, and pituitary growth hormone release. HAAF is the blunting of these responses after repeated hypoglycemic episodes, as can happen with intensive insulin therapy.

HAAF has been modeled experimentally in both human subjects (11, 12) and animals (45). We previously reported (15) that repeated bouts of insulin-induced hypoglycemia in rats result in HAAF (reduced epi- nephrine, glucagon, and corticosterone responses to hypoglycemia). Concomitantly, activation of several hypothalamic nuclei, including the PVN, was blunted. Hypoglycemia after prior intracerebroventricular corticosterone did not produce HAAF. In those rats, activation of hypothalamic nuclei was blunted except for the PVN. Therefore, we hypothesized that decreased PVN activation might be a determining factor for the impaired neuroendocrine response seen during repeated hypoglycemia. To test this hypothesis in the...
current study, we induced hypoglycemia in rats to a level comparable to our prior study while inactivating the PVN by infusion of lidocaine. We predicted that anesthetizing the PVN would blunt the neuroendocrine response to hypoglycemia in a manner similar to the blunted neuroendocrine response caused by antecedent hypoglycemia.

METHODS

Subjects. Male Wistar rats (Simonsen Laboratories, Gilroy, CA; 350–400 g) were maintained on a 12:12-h light-dark schedule (lights on at 7 AM; lights off at 7 PM) with ad libitum access to food and water and were studied during the lights-on portion of the light cycle. All procedures were approved by the Animal Studies Subcommittee of the Department of Veterans Affairs Puget Sound Health Care System Research and Development Committee and adhere to the "Guiding Principles for Research Involving Animals and Human Beings" set forth by the American Physiological Society (3).

Surgery. All animals underwent bilateral implantation of intraventricular Silastic catheters according to the method of Scheurink et al. (42) under ketamine-xylazine anesthesia [60 mg/kg ketamine (KetaFlo, Abbott Laboratories, Chicago, IL), 7.8 mg/kg xylazine (Xyla-Ject, Phoenix Pharmaceutical, St. Joseph, MO)] with supplemental doses (25 mg/kg) of ketamine when necessary. One catheter was placed in the lingualveal facial vein and the other in the submaxillary vein and advanced to the right atrium. Catheters were tunneled subcutaneously and exteriorized through a midline incision in the scalp. Rats were then placed in a stereotaxic frame and bilaterally overdosed with pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, IL), 0.3 U·100 g body wt·h−1·iv) over 120 min along with PVN infusions of lidocaine (2%; Sigma; n = 10) or vehicle (n = 11). Infusions were carried out by programmable syringe pumps (SP101i, World Precision Instruments, Sarasota, FL). The time course and number of intracerebral infusions are given in Fig. 2. This timing was based on the functional pharmacokinetics of lidocaine in brain tissue (33, 47) and the goal of establishing a lidocaine block of PVN neuronal activity for the duration of the insulin infusion. Data from a number of studies indicate that a single infusion of lidocaine at this dose anesthetizes various regions of the central nervous system, including the PVN, for approximately 15–20 min (1, 2, 16, 33, 35, 39, 44, 47, 56). Therefore, a 10-min infusion was made every 25 min. Lidocaine was infused at a rate of 0.1 μl/min, a rate that causes no discernible tissue damage on histological evaluation (unpublished observations). The volume of functional spread in brain tissue is ≤1.0 mm³ for this rate and volume of infusion [based on studies using similar and higher rates/volumes (1, 6, 29, 34, 44, 47)]. Blood samples (1.5 ml) were drawn every 30 min and immediately replaced with donor blood drawn from unstressed rats immediately before the day 2 procedure. The same procedure was carried out for the two anatomic control groups, CA and CP.

Histology. After the day 2 infusions, each animal was overdosed with pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, IL), 0.3 μl of fast green dye (VWR, West Chester, PA) was infused into the PVN to mark the site of injection, and then animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and placed in 4% paraformaldehyde at 4 °C for 3 days. Brains were submersed in 30% sucrose in 0.1 M PBS vehicle (pH = 7.4; Oxoid, Basingstoke, UK) to habituate the animals to the infusion procedure. On day 2, the animals were placed in the test chambers for at least 2 h before experimental procedures began. Injection and withdrawal cannulas were connected, and the experiment began once the animals were observed to be “calm” (~10–15 min after connecting cannulas). The preexperiment habituation sessions in the test chambers, preexperiment patency checks, and the day 1 injection procedure ensured well-adapted animals and relatively stress-free baseline conditions on day 2.

Plasma assays. Blood samples were obtained for the measurement of neuroendocrine responses and stored at −80°C until assayed. Assays were as described previously (15). Blood for the catecholamine assays was collected on EGTA: glutathione (2.3 mg/ml/1.5 mg/ml; Sigma). Tubes for glucagon assays contained 10 μl of 1 M benzamidine (Sigma) and 1 U heparin. Blood for glucose, corticosterone, and ACTH assays was collected on EDTA and aprotinin (0.7 trypsin inhibitor unit; Sigma). A radioenzymatic method as described in Evans et al. (14) was used for determination of plasma epinephrine and norepinephrine. An RIA procedure was used for plasma corticosterone measurement as described in van Dijk et al. (53). Plasma glucose was measured spectrophotometrically, after a glucose oxidase reaction, with a Dynatech MR5000 microplate reader connected to a personal computer running Dynatech Biolinx software (Dynatech Laboratories, Chantilly, VA). Glucagon was assayed by the Linco glucagon RIA kit (Linco Research, St. Charles,
MO). Measurements of ACTH were made using the Nichols Institute Diagnostics immunoradiometric assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Statistical analysis. Data from the plasma assays were analyzed using repeated-measures ANOVA with time as the repeated measure and treatment (vehicle or lidocaine) as the between-groups factor. In the event of significant main effects or interactions, Fisher’s protected least significant difference post hoc tests were done to determine significant differences, and t-tests were done where appropriate. Data expressed as percentage of control were analyzed using ANOVA, with treatment (lidocaine, vehicle, or repeated hypoglycemia) as the between-groups factor. Significance for all tests was taken as $P \leq 0.05$.

RESULTS

PVN inactivation and the counterregulatory response. Both vehicle and lidocaine PVN rats demonstrated significant decreases in plasma glucose during intravenous insulin infusion (Table 1; main effect of time: $F_{4,17} = 353, P < 0.0001$). The declines in plasma glucose levels did not differ between the two groups (Table 1; no interaction between time and treatment: $F_{4,68} = 1.02, P = 0.40$).

Tables 1 and 2 present all the raw data for all time points, while Figs. 3 and 4 summarize peak plasma levels of hormones for the lidocaine and vehicle groups.
as percentages of vehicle control. Figure 3 and Table 1 show that intra-PVN lidocaine infusion blunted the ACTH response to hypoglycemia (Fig. 3; treatment effect: $F_{1,17} = 5.1$, $P = 0.03$), as expected with decreased parvocellular PVN activity (31). Lidocaine rats demonstrated a $35 \pm 6\%$ decrease in the peak ACTH response to hypoglycemia ($P = 0.028$ vs. vehicle; Fig. 3). Time course data indicate that PVN lidocaine caused a small ($-11\, \text{pg/ml}$) but significant decrease in ACTH at time 0 ($P = 0.05$; Table 1). This difference disappeared by 30 min into the hypoglycemia ($P = 0.44$), and the plasma ACTH levels in the lidocaine and vehicle groups remained similar to each other until 90 min, at which point the lidocaine group demonstrated lower plasma ACTH at both 90 and 120 min (Table 1; interaction between time and treatment: $F_{4,68} = 2.6$, $P = 0.02$). Despite the effect on plasma ACTH levels, the peripheral release of corticosterone was not significantly affected by intra-PVN lidocaine (Table 1; no effect of treatment: $F_{1,17} = 1.17$, $P = 0.29$; and no interaction between time and treatment: $F_{4,68} = 0.77$, $P = 0.55$).

The plasma catecholamine response to hypoglycemia was also suppressed by PVN anesthetization. Peak plasma epinephrine levels in the lidocaine group were decreased $42 \pm 6\%$ compared with the vehicle group ($P = 0.04$; Fig 3; treatment effect: $F_{1,17} = 4.8$, $P = 0.04$). Plasma epinephrine levels were marginally decreased at the first time point (time 0) by $-82\, \text{pg/ml}$ ($P = 0.009$) and again at 60 min ($P = 0.046$) and maximally blunted at 120 min ($-3,084\, \text{pg/ml}$; $P = 0.044$) in this group (Table 1; interaction between treatment and time: $F_{4,68} = 3.15$, $P = 0.01$). The increase of plasma norepinephrine was also blunted by PVN lidocaine. The peak plasma norepinephrine concentration decreased by $34 \pm 5\%$ ($P = 0.013$ vs. control; Fig 3; effect of treatment: $F_{1,17} = 7.7$, $P = 0.01$). Plasma norepinephrine levels in the lidocaine group were slightly lower at time 0 ($-85\, \text{pg/ml}$; $P = 0.002$) but returned to control levels until 120 min when they were decreased $-453\, \text{pg/ml}$ ($P = 0.01$) relative to vehicle (Table 1; interaction between treatment and time: $F_{4,68} = 2.72$, $P = 0.037$). PVN lidocaine did not alter pancreatic glucagon release in response to hypoglycemia (Fig. 3; Table 1; no interaction between time and treatment: $F_{4,68} = 1.04$, $P = 0.39$).

**Inactivation of regions surrounding the PVN.** Cannulas were placed 1 mm anterior to the PVN (CA) and 1 mm posterior to the PVN (CP) at the same dorsoventral coordinate to test if diffusion of the lidocaine to adjacent structures may have produced the changes in the counterregulatory response. Lidocaine infusion in the CA group did not alter the decline of plasma glucagon (Table 2; no effect of treatment: $F_{1,9} = 0.17$, $P = 0.69$; no treatment-time interaction: $F_{4,36} = 0.65$, $P = 0.63$). Unlike intra-PVN lidocaine, CA lidocaine did not blunt the hypoglycemia-induced rise in plasma ACTH, corticosterone, epinephrine, norepinephrine, or glucagon.

Table 1. Plasma glucose and hormone levels during hypoglycemia after lidocaine or vehicle infusion into the PVN

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Glucose, mg/dl</th>
<th>ACTH, pg/ml</th>
<th>Corticosterone, ng/ml</th>
<th>Epinephrine, pg/ml</th>
<th>Norepinephrine, pg/ml</th>
<th>Glucagon, pmol/ml</th>
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</thead>
<tbody>
<tr>
<td>PVN vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>98 ± 4</td>
<td>21 ± 4</td>
<td>87 ± 15</td>
<td>130 ± 21</td>
<td>288 ± 16</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>30</td>
<td>45 ± 2</td>
<td>58 ± 11</td>
<td>140 ± 12</td>
<td>757 ± 128</td>
<td>497 ± 66</td>
<td>308 ± 27</td>
</tr>
<tr>
<td>60</td>
<td>32 ± 2</td>
<td>161 ± 24</td>
<td>179 ± 9</td>
<td>2,933 ± 550</td>
<td>747 ± 94</td>
<td>366 ± 46</td>
</tr>
<tr>
<td>90</td>
<td>29 ± 1</td>
<td>230 ± 24</td>
<td>207 ± 10</td>
<td>3,440 ± 350</td>
<td>859 ± 71</td>
<td>660 ± 74</td>
</tr>
<tr>
<td>120</td>
<td>24 ± 1</td>
<td>200 ± 22</td>
<td>220 ± 9</td>
<td>6,654 ± 1087</td>
<td>1,323 ± 121</td>
<td>510 ± 47</td>
</tr>
<tr>
<td>PVN lidocaine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>91 ± 4</td>
<td>10 ± 5a</td>
<td>74 ± 15</td>
<td>49 ± 9a</td>
<td>203 ± 16a</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>30</td>
<td>45 ± 2</td>
<td>56 ± 12</td>
<td>123 ± 13</td>
<td>390 ± 106</td>
<td>347 ± 33</td>
<td>250 ± 29</td>
</tr>
<tr>
<td>60</td>
<td>32 ± 2</td>
<td>121 ± 11</td>
<td>185 ± 7</td>
<td>1,358 ± 245a</td>
<td>566 ± 42</td>
<td>460 ± 156</td>
</tr>
<tr>
<td>90</td>
<td>28 ± 2</td>
<td>150 ± 14a</td>
<td>202 ± 6</td>
<td>3,100 ± 640</td>
<td>801 ± 73</td>
<td>560 ± 117</td>
</tr>
<tr>
<td>120</td>
<td>25 ± 3</td>
<td>136 ± 13a</td>
<td>195 ± 6</td>
<td>3,570 ± 450a</td>
<td>870 ± 70a</td>
<td>586 ± 96</td>
</tr>
</tbody>
</table>

Values are means ± SE. $^aP < 0.05$ vs. paraventricular nucleus (PVN) vehicle.
Table 2. Plasma glucose and hormone levels during hypoglycemia after lidocaine or vehicle infusion into areas surrounding the PVN

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Glucose, mg/dl</th>
<th>ACTH, pg/ml</th>
<th>Cort, ng/ml</th>
<th>Epi, pg/ml</th>
<th>NE, pmol/ml</th>
<th>Glucagon, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>105 ± 2</td>
<td>15 ± 4</td>
<td>93 ± 25</td>
<td>102 ± 18</td>
<td>420 ± 59</td>
<td>112 ± 15</td>
</tr>
<tr>
<td>30</td>
<td>49 ± 5</td>
<td>49 ± 20</td>
<td>126 ± 27</td>
<td>402 ± 102</td>
<td>474 ± 64</td>
<td>412 ± 60</td>
</tr>
<tr>
<td>60</td>
<td>37 ± 3</td>
<td>112 ± 40</td>
<td>190 ± 35</td>
<td>1,362 ± 401</td>
<td>666 ± 141</td>
<td>634 ± 205</td>
</tr>
<tr>
<td>90</td>
<td>29 ± 3</td>
<td>222 ± 57</td>
<td>228 ± 29</td>
<td>4,588 ± 970</td>
<td>1,300 ± 242</td>
<td>978 ± 243</td>
</tr>
<tr>
<td>120</td>
<td>27 ± 4</td>
<td>200 ± 35</td>
<td>252 ± 13</td>
<td>4,534 ± 883</td>
<td>1,228 ± 300</td>
<td>683 ± 109</td>
</tr>
<tr>
<td><strong>Lidocaine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>105 ± 6</td>
<td>7 ± 4</td>
<td>53 ± 20</td>
<td>117 ± 54</td>
<td>325 ± 18</td>
<td>107 ± 9</td>
</tr>
<tr>
<td>30</td>
<td>51 ± 4</td>
<td>40 ± 16</td>
<td>117 ± 23</td>
<td>943 ± 374</td>
<td>583 ± 96</td>
<td>330 ± 28</td>
</tr>
<tr>
<td>60</td>
<td>35 ± 3</td>
<td>70 ± 27</td>
<td>212 ± 27</td>
<td>2,083 ± 327</td>
<td>735 ± 78</td>
<td>266 ± 78</td>
</tr>
<tr>
<td>90</td>
<td>34 ± 2</td>
<td>175 ± 25</td>
<td>261 ± 24</td>
<td>3,608 ± 900</td>
<td>950 ± 97</td>
<td>559 ± 147</td>
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<td>120</td>
<td>26 ± 3</td>
<td>187 ± 11</td>
<td>280 ± 27</td>
<td>6,053 ± 400</td>
<td>1,386 ± 129</td>
<td>468 ± 113</td>
</tr>
</tbody>
</table>

Values are means ± SE. Cort, corticosterone; Epi, epinephrine; NE, norepinephrine. *P < 0.05 vs. vehicle.

DISCUSSION

Figure 5 summarizes the primary findings of this study and compares the results of PVN inactivation with those we observed in HAAF (15). Although plasma glucose levels decreased equivalently for vehicle and lidocaine treatments within the PVN group (Table 1), the counterregulatory response was not equivalent for those treatments. Lidocaine block of PVN activity resulted in two types of changes in the levels of plasma ACTH and the catecholamines measured during the 2-h insulin infusion. One change was a small decrease (compared with later changes) in the baseline levels of these hormones. This change disappeared during the session (see RESULTS and Table 1). The other change, larger in magnitude, was a blunting of the rise in the levels of these hormones. This change disappeared during the session (see RESULTS and Table 1).

Both of these changes may reflect a role of the PVN in increasing the level of activation or the “tone” of the hypothalamic-pituitary-adrenocortical axis and autonomic nervous system. This is consistent with other findings of modest baseline effects of PVN inactivation/stimulation on indexes of autonomic function (see below) (16, 27, 32, 39, 55).

PVN lidocaine resulted in decreased release of ACTH. Because CRF and AVP, two factors that regulate ACTH release from the pituitary, are released by parvocellular PVN neurons (31), this is an expected outcome. In fact, this degree of blunting of the ACTH response appears to be the same as that achieved with prior hypoglycemia in the HAAF model (15; Fig. 5). A complete block of ACTH release was not observed, perhaps because 1) CRF and AVP are not the sole factors that regulate ACTH release (25, 37, 52), nor is the PVN the sole regulator of ACTH release (5); 2) even with permanent physical lesion of the PVN, stimulated ACTH release is not always completely abolished; and 3) the lidocaine infusions may not have inactivated the...
60 min was not different from the level at our animals, because the level of corticosterone at ticosterone response. This might in fact be occurring in lidocaine rats might decrease the duration of the cor-

cles that increases in plasma ACTH can increase the amplitude of that response (26, 28, 54). Thus in the current study, decreased plasma ACTH in the PVN lidocaine group translates into a shortened duration of corticosterone response. It has been shown across species that increases in plasma ACTH can increase the duration of the glucocorticoid response without affecting the amplitude of that response (26, 28, 54). Thus in the current study, decreased plasma ACTH in the PVN lidocaine rats might decrease the duration of the corticosterone response. This might in fact be occurring in our animals, because the level of corticosterone at $t = 60$ min was not different from the level at $t = 120$ min in the PVN lidocaine rats ($P = 0.33$ by 2-tailed $t$-test; $185 \pm 7$ vs. $195 \pm 6 \mu g/dl$), while in the PVN vehicle rats, corticosterone was still rising from $t = 60$ min to $t = 120$ min ($P = 0.005; 179 \pm 8$ vs. $219 \pm 8 \mu g/dl$). However, this remains speculative because we observed no difference between the treatment groups (lidocaine vs. vehicle) over time (no time-treatment interaction: $P = 0.55$). Another possibility, but also speculative, is based on evidence that autonomic innervation of the adrenal gland may decrease adrenal sensitivity to ACTH, an effect that is removed (i.e., adrenals are disinhibited) by denervating the adrenals (24). If, as reflected in plasma norepinephrine and epinephrine, PVN lidocaine results in decreased sympathetic activity, the adrenals may be more sensitive to ACTH (i.e., disinhibited) and thus produce increased plasma corticosterone at lower levels of ACTH.

PVN lidocaine also resulted in blunted adrenomedullary epinephrine release as reflected in decreased plasma epinephrine levels and in decreased plasma norepinephrine relative to vehicle controls (Table 1; Fig. 3). This indicates that PVN inactivation reduces the adrenomedullary response to hypoglycemia as well as the overall sympathetic nervous system response. Thus the PVN plays a role in maintaining the sympathoadrenal response to hypoglycemia. This is consistent with the anatomic data showing that a number of neurons in the PVN project directly to autonomic nuclei in the brain stem and spinal cord (23, 30, 36, 46, 50). These neurons are found throughout the PVN (especially in the dorsal and lateral areas) and include a small number originating in the magnocellular PVN (see Figs. 2 and 4 in Ref. 30). The functionality of these neurons has been demonstrated in numerous physiological experiments (e.g., Refs. 16, 27, 32, 39, 55) demonstrating changes in autonomic indexes such as blood pressure, sympathetic nerve activity, gastric motility, and plasma epinephrine and norepinephrine levels with PVN stimulation. To our knowledge the current study is the first demonstrating that alteration of activity of PVN neurons changes the sympathoadrenal response to hypoglycemia, although the probable anatomic basis for this has been established (23, 36, 38, 46, 50). The somewhat modest effect seen here is consistent with findings by others either measuring plasma catecholamines (32, 41) or other physiological endpoints of sympathetic activity (39, 56) after PVN manipulations. Interestingly, the plasma norepinephrine response was not altered or blunted with HAAF in our

Fig. 4. Changes in counterregulatory response to hypoglycemia after Lido or Veh infusions into sites $\sim$1 mm anterior to the PVN (A) or $\sim$1 mm posterior to the PVN (B). Values are peak plasma levels of hormones for the Lido and Veh groups as percentages of Veh control. Bars represent means $\pm$ SE. NS, not significant. * $P \leq 0.05$.

Concomitant with the decrease in ACTH, one might have predicted a decrease in plasma corticosterone as well. In the HAAF model, corticosterone was indeed modestly but significantly blunted at 120 min. In the current study, the rise in corticosterone was not significantly blunted by PVN lidocaine (Table 1). It is possible that the adrenal cortex response may be maximal even at the blunted level of ACTH seen in the lidocaine rats: ACTH-induced corticosterone secretion plateaus even with increasing levels of plasma ACTH (26, 51). It is also possible that the reduction in ACTH in the lidocaine group translates into a shortened duration of corticosterone response. It has been shown across species that increases in plasma ACTH can increase the duration of the glucocorticoid response without affecting the amplitude of that response (26, 28, 54). Thus in the current study, decreased plasma ACTH in the PVN lidocaine rats might decrease the duration of the corticosterone response. This might in fact be occurring in our animals, because the level of corticosterone at $t = 60$ min was not different from the level at $t = 120$ min in the PVN lidocaine rats ($P = 0.33$ by 2-tailed $t$-test; $185 \pm 7$ vs. $195 \pm 6 \mu g/dl$), while in the PVN vehicle rats, corticosterone was still rising from $t = 60$ min to $t = 120$ min ($P = 0.005; 179 \pm 8$ vs. $219 \pm 8 \mu g/dl$). However, this remains speculative because we observed no difference between the treatment groups (lidocaine vs. vehicle) over time (no time-treatment interaction: $P = 0.55$). Another possibility, but also speculative, is based on evidence that autonomic innervation of the adrenal gland may decrease adrenal sensitivity to ACTH, an effect that is removed (i.e., adrenals are disinhibited) by denervating the adrenals (24). If, as reflected in plasma norepinephrine and epinephrine, PVN lidocaine results in decreased sympathetic activity, the adrenals may be more sensitive to ACTH (i.e., disinhibited) and thus produce increased plasma corticosterone at lower levels of ACTH.

Fig. 5. Comparison of changes in counterregulatory response with either hypoglycemia (Hypo)-associated autonomic failure (HAAF) or PVN inactivation with lidocaine. Bars represent means $\pm$ SE. * $P \leq 0.05$. 

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previous study (15), even though c-FOS staining indicated that PVN activation was markedly blunted with HAAF. This may indicate that the activity of other brain structures involved in the control of the sympathetic response resulted in a stimulation of norepinephrine output masking the inhibitory component of PVN blunting.

The pancreatic glucagon response is blunted in our HAAF model, but was not after PVN inactivation (Fig. 5). This response has been shown to be maintained by redundant sympathetic and parasympathetic autonomic inputs. All of these inputs need to be compromised to significantly decrease the glucagon response (19, 20). This suggests that HAAF disrupts these inputs more completely than PVN inactivation alone, again indicating the involvement of other brain regions and/or peripheral structures in addition to the PVN in HAAF. Recently, Schöfl et al. (43) have demonstrated that in a population of patients with damage to the hypothalamus as a result of craniopharyngioma, the sympathoadrenal response to hypoglycemia was abolished while the sympathetic response to orthostatic challenge was unchanged. They found no change in the glucagon response in these patients, indicating that central nervous system sites that may modulate the glucagon response may not be in the hypothalamus (see below) and are not the same sites modulating sympathetic responses. Although the extent of damage in these patients was not reported, and basal medial structures such as the ventromedial nucleus probably regulate sympathetic activity as well (41), the finding does suggest that activation of hypothalamic structures is important and specific for the sympathoadrenal component of the response to hypoglycemia.

We are confident of the anatomic specificity of the effects of our lidocaine infusions into the PVN for a number of reasons. The literature indicates that the volume of functional spread of lidocaine in brain tissue is ≈ 1.0 mm³ for this rate and volume of infusion [based on studies using similar and higher rates/volumes (1, 6, 29, 34, 39, 44, 47)]. Additionally, Patel and Schmid (39) showed that lidocaine injected just dorsal or lateral to the PVN did not produce alterations of autonomic function, while intra-PVN injections did (see Fig. 4 in Ref. 39). In the CP group, lidocaine infusion 1 mm posterior to the PVN produced an effect that was distinct from that in the PVN group. CP lidocaine did not alter baseline (time 0) levels of hormones, and the catecholamine response to hypoglycemia was curtailed at the final time point. There was no change in the ACTH response, suggesting no diffusion into the PVN. Examination of the brain sections from the CP group revealed that the infusions occurred just above the dorsomedial nucleus of the hypothalamus (DMH). The DMH is known to have anatomic projections to the PVN, as well as autonomic cell groups in the brain stem (48, 49), and studies have demonstrated that stimulation or inhibition of the DMH results in autonomic changes (13, 18, 59). We are currently evaluating the potential contribution of the DMH to the normal response to hypoglycemia and to HAAF. In the CA group, injections 1 mm anterior to the PVN had no effect on the rise in catecholamines or ACTH in response to hypoglycemia, indicating no diffusion beyond 1 mm into the PVN.

Perspectives

We previously reported that there is a dramatic decrease in hypoglycemia-induced activation of the PVN after prior hypoglycemia (15). Anatomically, the blunting was relatively homogeneous across the PVN, not favoring what has been considered an “autonomic region.” In attempting to replicate this homogeneous inactivation, we infused lidocaine into the PVN during hypoglycemia. Anesthetizing the PVN resulted in a blunting of the autonomic and HPA components of the counterregulatory response, similar to that produced by repeated hypoglycemia. The complete counterregulatory response, however, may require activation of both PVN and areas such as the arcuate and dorsomedial hypothalamic nuclei and posterior hypothalamic regions. Additionally, endocrine tissues (e.g., pancreatic β-cells, adrenomedullary cells, etc.) and/or the peripheral innervation of these tissues may have a role in these phenomena, changing release parameters and/or sensitivity to neurotransmitters and humoral factors. Thus while PVN activation must play a critical role in the sympathoadrenal response to hypoglycemia, it is not responsible for the complete counterregulatory response to a single bout of hypoglycemia, and its inactivation cannot be solely responsible for the impaired counterregulatory response that occurs with multiple bouts of hypoglycemia.

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REFERENCES


