Inactivation of the DMH selectively inhibits the ACTH and corticosterone responses to hypoglycemia

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Inactivation of the DMH selectively inhibits the ACTH and corticosterone responses to hypoglycemia. Am J Physiol Regul Integr Comp Physiol 286: R123–R128, 2004; 10.1152/ajpregu.00328.2003.—We have previously reported that repeated bouts of insulin-induced hypoglycemia (IIH) in the rat result in blunted activation of the paraventricular, arcuate, and dorsomedial hypothalamic (DMH) nuclei. Because DMH activation has been implicated in the sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) responses to stressors, we hypothesized that its blunted activation may play a role in the impaired counterregulatory response that is also observed with repeated bouts of IIH. In the present study, we evaluated the role of normal DMH activation in the counterregulatory response to a single bout of IIH. Local infusion of lidocaine (n = 8) to inactivate the DMH during a 2-h bout of IIH resulted in a significant overall decrease of the ACTH response and a delay of onset of the corticosterone response compared with vehicle-infused controls (n = 9). We observed suppression of the ACTH response at time (t) = 90 and 120 min (50 ± 12 and 63 ± 6%, respectively, of control levels) and early suppression of the corticosterone response at t = 30 min (59 ± 13% of the control level). The epinephrine, norepinephrine, and glucagon responses were not altered by DMH inactivation. Our finding suggests that DMH inactivation may play a specific role in decreasing the HPA axis response after repeated bouts of IIH.

METHODS

Subjects. Male Wistar rats (350–400 g; Animal Technologies Limited, Kent, WA) were maintained on a 12:12-h light-dark schedule (lights on at 6:00 A.M., off at 6:00 P.M.), 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 Veterans Affairs Puget Sound Health Care System Research and Development Committee and adhere to the guiding principles for research set forth by the American Physiological Society (3).

Surgery. All animals underwent bilateral implantation of intravenous Silastic catheters under ketamine-xylazine anesthesia [60 mg/kg ketamine (KetoFlo; Abbott Laboratories, Chicago, IL), 7.8 mg/kg xylazine (Xyla-Ject; Phoenix Pharmaceutical, St. Joseph, MO)] as described by Evans et al. (16). Catheters were tunneled subcutaneously and exteriorized through a midline incision in the scalp. Each rat also received bilateral 26-gauge stainless steel guide cannulas (Plastics One, Roanoke, VA) aimed at the DMH using the stereotaxic coordinates [−2.2 anterior-posterior, ± 0.5 medial-lateral, −7.0 dorso-ventral] from bregma according to the atlas of Paxinos and Watson (28)]. The intracranial cannula and intravenous catheters were held in place with acrylic cement (Lang Dental, Wheeling, IL) and four skull screws (Small Parts, Miami Lakes, FL). Animals received subcutaneous 3 ml lactated Ringer solution (Baxter Pharmaceutical Products, New Providence, NJ) and intramuscular 0.2 ml Gentamicin antibiotic (Bayer, Leverkusen, Germany) and were maintained on a circulating water heating pad until recovery from anesthesia. Catheter lines were filled with 25–60% polyvinylpyrrolidone (PVP10; Sigma, St. Louis, MO)/heparin (1.000 U/ml; Elkins-Sinn, Cherry Hill, NJ) and kept patent by a heparin (100 U/ml) flush every 3 days. All animals regained weight to at least the presurgical level and were on a positive weight gain trajectory before study. Figure 1A shows examples of DMH placements to illustrate the range of typical DMH cannula placements and a plotting of the actual placements. Animals that were

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found to have placements outside of the DMH were included in an anatomic control group. Additionally, this anatomic control group consisted of animals receiving cannulas aimed 1 mm dorsal to the DMH coordinates listed above (final n = 10; 5 vehicle infused, 5 lidocaine infused). Figure 1B shows the placements for the anatomic control group.

**Experimental procedures.** Before performing experiments, animals were familiarized with square acrylic test chambers (~30 cm × 30 cm × 30 cm) and the microinjection procedure, as described by Evans et al. (16). Subsequent to this, the animals were placed in the test chambers with ad libitum access to food and water for at least 2 h before experimental procedures began. Food was removed 1 h before the experiment, injection and withdrawal cannulas were connected, and the experiment began once the animals were observed to be calm (~10–15 min after connecting cannulas; 10:00 AM). Animals received insulin (Novolin R, regular human insulin, recombinant DNA origin; Novo Nordisk, Princeton, NJ; 0.5 U·100 g body wt-1·h-1) intravenously over 120 min along with DMH infusions of lidocaine (2%; n = 8; Sigma) or vehicle (0.1 M PBS, pH = 7.4; n = 9; Oxoid, Basingstoke, UK). Lidocaine is a local anesthetic that blocks sodium channels and some calcium channels in neurons, thus blocking conduction (7, 29, 41). It has been widely used in a variety of studies to inactivate specific CNS targets (for example, Refs. 2, 17, 32, 35). Microinjections and intravenous infusions were carried out by programable syringe pumps (SP101i; World Precision Instruments, Sarasota, FL), as described in Evans et al. (16). A 10-min microinjection was made every 25 min. Lidocaine or vehicle was infused at a rate of 0.1 μl/min, a rate that causes no discernible tissue damage upon histological evaluation (unpublished observations). The dose, infusion rate, and timing were chosen based on studies showing inactivation for ~15–20 min and diffusion of ≤1.0 mm2 for similar rates and volumes (1, 2, 18, 26, 35). Blood samples (1.5 ml) were drawn every 30 min and immediately replaced with donor blood drawn from unstressed rats immediately before the procedure.

**Histology.** After the termination of infusions, each animal was overdosed with pentobarbital sodium (Nembutal; Abbott Laboratories). Fast green dye (0.3 μl; VWR, West Chester, PA) was infused in the DMH 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 followed by freezing at ~80°C in embedding media (Fisher, Pittsburgh, PA), until sectioning at 40 μm. Tissue sections were mounted on slides to verify cannula placement.

**Plasma assays.** Blood samples were obtained for the measurement of neuroendocrine counterregulatory responses to IIH and stored at −80°C until assayed. Blood for the catecholamine assays was collected on EGTA-glutathione (2.3:1.5 mg/ml; Sigma). Tubes for glucagon assays contained 10 μl of 1 M benzamidine (Sigma) and 1 unit heparin. Blood for glucose, corticosterone, and ACTH assays was collected on EDTA and aprotinin (0.7 tissue inhibitor unit; Sigma). The assays have been described previously (15). Briefly, a radioenzymatic method as described by Evans et al. (14) was used for determination of plasma epinephrine and norepinephrine. An RIA procedure was used for plasma corticosterone measurement, as described by van Dijk et al. (39). Plasma glucose was measured spectrophotometrically, after a glucose oxidase reaction, with a Dyanatech MR5000 microplate reader connected to a PC computer running Dynatech Biolinx software (Dyanatech 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 (lidocaine or vehicle) 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. Significance for all tests was taken as P ≤ 0.05.

**RESULTS**

**DMH lidocaine blunts the HPA response.** Table 1 presents the neuroendocrine data for the experimental DMH groups.

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**Table 1.** The DMH lidocaine group had significantly lower plasma glucagon compared with the DMH control group. However, there were no significant differences between the groups for plasma glucose, corticosterone, or ACTH. The data for the plasma assays were analyzed using repeated-measures ANOVA with time as the repeated measure and treatment (lidocaine or vehicle) 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. Significance for all tests was taken as P ≤ 0.05.
ROLE OF THE DMH IN THE COUNTERREGULATORY RESPONSE TO HYPOGLYCEMIA

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

<table>
<thead>
<tr>
<th>Time Point, min</th>
<th>Glucose, mg/dl</th>
<th>ACTH, pg/ml</th>
<th>Corticosterone, μg/dl</th>
<th>Epinephrine, pg/ml</th>
<th>Norepinephrine, pg/ml</th>
<th>Glucagon, pmol/ml</th>
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<tr>
<td>0</td>
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<td>7.8±1.5</td>
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**DMH Vehicle (n = 9)**

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<th>Time Point, min</th>
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<th>Corticosterone, μg/dl</th>
<th>Epinephrine, pg/ml</th>
<th>Norepinephrine, pg/ml</th>
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<td>61±17</td>
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**DMH Lidocaine (n = 8)**

Values are means ± SE; n, no. of rats. DMH, dorsomedial hypothalamus. *P < 0.05 vs. DMH vehicle.

DMH lidocaine did not affect baseline (t₀) values of any of the neuroendocrine parameters (Table 1). Both DMH vehicle (n = 9) and DMH lidocaine (n = 8) rats demonstrated significant decreases in plasma glucose during intravenous insulin infusion [Table 1; main effect of time: F(4,15) = 406, P < 0.0001]. The declines in plasma glucose levels did not differ between the two groups [no interaction between time and treatment: F(4,60) = 0.88, P = 0.48].

Table 1 and Fig. 2B show that intra-DMH lidocaine infusion blunted the ACTH response to IIH [treatment effect: F(1,15) = 8.1, P = 0.01; time × treatment interaction: F(4,60) = 3.65, P = 0.0099]. Lidocaine rats demonstrated a significantly decreased ACTH response to IIH at both 90 and 120 min into the insulin infusion (Fig. 2B; P = 0.02 and P = 0.007, respectively). The release of corticosterone was also decreased by intra-DMH lidocaine [Table 1 and Fig. 2A; time × treatment interaction: F(4,60) = 2.48, P = 0.05]. Corticosterone remained at baseline at 30 min in the lidocaine group (P = 0.89, t₀ vs. t₃₀) and then proceeded to steadily increase over 120 min (Table 1 and Fig. 2A). In contrast, the corticosterone in the vehicle group had increased already at 30 min (P < 0.0001, t₀ vs. t₃₀) and was significantly higher than the lidocaine group (P = 0.03, vehicle vs. lidocaine at t₃₀).

The plasma catecholamine response to IIH was not suppressed by DMH inactivation. Plasma epinephrine levels increased in both groups across the 120-min session (Table 1), with no effect of intra-DMH lidocaine [no treatment effect: F(1,15) = 0.255, P = 0.62; no time × treatment interaction: F(4,60) = 0.224, P = 0.92]. The increase of plasma norepinephrine was also not affected by DMH lidocaine [Table 1; no treatment effect: F(1,15) = 0.406, P = 0.53; no time × treatment interaction: F(4,60) = 0.228, P = 0.92]. DMH

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Fig. 2. A: plasma corticosterone levels (μg/dl) during a 2-h insulin-induced hypoglycemia (IIH) in which the DMH was infused with lidocaine or vehicle. Error bars indicate ± SE. *P < 0.05, vehicle vs. lidocaine. Solid symbols indicate data points significantly different (P ≤ 0.05) from time 0 (t₀). B: plasma ACTH levels (pg/ml) during a 2-h insulin-induced IIH in which the DMH was infused with lidocaine or vehicle. Error bars indicate ± SE. *P < 0.05, vehicle vs. lidocaine. Solid symbols indicate data points significantly different (P ≤ 0.05) from t₀. C: plasma corticosterone levels (μg/dl) during a 2-h IIH in which a region ~1 mm dorsal to the DMH was infused with lidocaine or vehicle. Error bars indicate ± SE. Solid symbols indicate data points significantly different (P ≤ 0.05) from t₀. D: plasma ACTH levels (pg/ml) during a 2-h IIH in which a region ~1 mm dorsal to the DMH was infused with lidocaine or vehicle. Error bars indicate ± SE. Solid symbols indicate data points significantly different (P ≤ 0.05) from t₀.
lidocaine had no effect on pancreatic glucagon release in response to IIH [Table 1; no treatment effect: F(1,15) = 0.218, P = 0.65; no interaction between time and treatment: F(4,60) = 0.068, P = 0.99].

Control injections do not blunt the response. Data for the anatomic control group are presented in Table 2. Figure 2, C and D, shows the corticosterone and ACTH data graphically for comparison with the DMH group data in Fig. 2, A and B. As is evident, lidocaine injected 1 mm dorsal to the DMH did not blunt the indexes of the counterregulatory response measured in the current study [time × dose: glucagon, F(4,32) = 0.27, P = 0.89; epinephrine, F(4,32) = 0.46, P = 0.77; norepinephrine, F(4,32) = 0.45, P = 0.77; ACTH, F(4,32) = 0.53, P = 0.72; corticosterone, F(4,32) = 0.49, P = 0.74].

DISCUSSION

Inactivation of the DMH (and not regions dorsal to it; see Fig. 1B and Table 2) delayed and reduced the increase of ACTH resulting from IIH (Fig. 2B). Despite having a drop in blood glucose that was closely matched to the vehicle group (Table 1), the lidocaine group did not experience a rise in blood glucose that was closely matched to the vehicle group in the current study [time × dose: glucagon, F(4,32) = 0.27, P = 0.89; epinephrine, F(4,32) = 0.46, P = 0.77; norepinephrine, F(4,32) = 0.45, P = 0.77; ACTH, F(4,32) = 0.53, P = 0.72; corticosterone, F(4,32) = 0.49, P = 0.74].

PVN are principally responsible for ACTH release by the anterior pituitary (see Ref. 4 for a summary). Evidence also indicates that the projection from the DMH to the parvocellular PVN is excitatory (5, 20). Infusion of muscimol, a GABA_A receptor agonist, in the DMH blunts the ACTH response to air stress and the associated rise in the number of activated neurons in the PVN, as assessed by c-Fos expression in the PVN (13). The parsimonious explanation for this would be that the DMH projection neurons to the PVN were inhibited by muscimol, removing an excitatory input to CRH-producing neurons in the PVN.

Because ACTH stimulates corticosterone release from the adrenal cortex, one would predict a decrease in the corticosterone response to IIH with the blunted ACTH response. Although the corticosterone response was initially delayed, it was progressively less reduced thereafter. The terminal t_{120} corticosterone levels were identical for both groups (vehicle vs. lidocaine for t_{120}: P = 0.40). We have observed in past studies that ACTH must be blunted substantially before it is associated with a decreased corticosterone response at this level of IIH (Refs. 15 and 16 and unpublished observations). Additionally, a blunted ACTH response in the lidocaine group might primarily decrease the duration of the corticosterone response (23, 25, 40). It would only be possible to confirm this if we extended our observations beyond the plateau, i.e., beyond 120 min. A blunted corticosterone response after DMH inhibition is consistent with the findings of Keim and Shekhar (22), in which chemical stimulation of the DMH increased corticosterone levels.

The effect of DMH inactivation was very specific to the HPA response to IIH. The plasma catecholamine profile, reflecting the sympathoadrenal component of the counterregulatory response, was not altered by DMH lidocaine. The present finding coupled with our previous observations (15, 16) suggests a fundamental difference in the roles of different regions of the hypothalamus in the counterregulatory response to IIH. Our previous study, in which we inactivated the PVN during IIH, demonstrated that the PVN, unlike the DMH, is important for the sympathoadrenal response to IIH (16), particularly the last 30 to 60 min of the response. Inactivation of the PVN did not delay the ACTH response (or for that matter the corticosterone or catecholamine responses); it decreased the magni-

Table 2. Plasma glucose and hormone levels during hypoglycemia after lidocaine or vehicle infusion

<table>
<thead>
<tr>
<th>Time Point, min</th>
<th>Glucose, mg/dl</th>
<th>ACTH, pg/ml</th>
<th>Corticosterone, μg/dl</th>
<th>Epinephrine, pg/ml</th>
<th>Norepinephrine, pg/ml</th>
<th>Glucagon, pmol/ml</th>
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Values are means ± SE; n = 5 rats in each group.
The role of the DMH is stressor specific; importantly, as DiMicco et al. (13) point out in a recent review, the effects of the DMH on the sympathetic nervous system. This literature nevertheless argues for the potential stimulatory (42). Although there are some discrepancies, taken together, the decreased firing rate of sympathetic nerves innervating iBAT (42). Although there are some discrepancies, taken together, this literature nevertheless argues for the potential stimulatory effects of the DMH on the sympathetic nervous system. Importantly, as DiMicco et al. (13) point out in a recent review, the role of the DMH is stressor specific; i.e., although muscimol injection in the DMH inhibits the increases of blood pressure, heart rate, and Fos-positive neurons in the PVN accompanying air stress (27, 33, 34), the same treatment does not do this in the context of hemorrhagic stress. It appears that, in the context of IIH, the DMH may be more important for the HPA components of the response than the sympathoadrenal components.

Our previous studies have shown that PVN activation plays a critical role in the sympathoadrenal response to IIH but that it is not responsible for the complete counterregulatory response to a single bout of IIH, and its inactivation cannot be solely responsible for the impaired counterregulatory response, which occurs with multiple bouts of IIH. Another hypothalamic region that is both activated by IIH and inhibited by repeated IIH in our HAAF model is the DMH. In the current study, we found the DMH to be important for the HPA component of the counterregulatory response to IIH, particularly during the initial phase of this response. The DMH was not important for the sympathoadrenal component of the response, or for the glucagon component. Thus DMH inhibition in the context of HAAF would likely contribute to the inhibition of the HPA response. The complete blunted neuroendocrine profile of HAAF is the result of varying levels of PVN, DMH, and Arc inhibition (with the lack of inhibition of other structures, such as the thalamic PVN), and perhaps inhibition of other CNS sites in the brain stem (31) and peripheral end organs, which we have not yet investigated.

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REFERENCES