Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint

D. Zelena,1 Z. Mergl,1 A. Földe,2 K. J. Kovács,2 Z. Tóth,3 and G. B. Makara1

Laboratories of 1Stress Research and 2Molecular Neuroendocrinology, Institute of Experimental Medicine, Hungarian Academy of Sciences, and 3Laboratory of Neuromorphology, Semmelweis University Medical School, 1450 Budapest, Hungary

Submitted 15 May 2003; accepted in final form 22 July 2003

Zelena, D., Z. Mergl, A. Földe, K. J. Kovács, Z. Tóth, and G. B. Makara. Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint. Am J Physiol Endocrinol Metab 285: E1110–E1117, 2003; 10.1152/ajpendo.00219.2003.—The role of hypothalamic structures in the regulation of chronic stress responses was studied by lesioning the mediobasal hypothalamus or the paraventricular nucleus of hypothalamus (PVH). Rats were acutely (60 min) and/or repeatedly (for 7 days) restrained. In controls, a single restraint elevated the plasma adrenocorticotropin (ACTH), corticosterone, and prolactin levels. Repeated restraint produced all signs of chronic stress, including decreased body and thymus weights, increased adrenal weight, basal corticosterone levels, and proopiomelanocortin (POMC) mRNA expression in the anterior pituitary. Some adaptation to repeated restraint of the ACTH response, but not of other hormonal responses, was seen. Lesioning of the mediobasal hypothalamus abolished the hormonal response and POMC mRNA activation to acute and/or repeated restraint, suggesting that the hypothalamo-pituitary-adrenal axis activation during repeated restraint is centrally driven. PVH lesion inhibited the ACTH and corticosterone rise to the first restraint by ~50%. In repeatedly restrained rats with PVH lesion, the ACTH response to the last restraint was reduced almost to basal control levels, and the elevation of POMC mRNA level was prevented. PVH seems to be important for the repeated restraint-induced ACTH and POMC mRNA stimulation, but it appears to partially mediate other restraint-induced hormonal changes.

IN ACUTE STRESS, the hypothalamic paraventricular nucleus of hypothalamus (PVH) is known to be a key site in the activation of the hypothalamo-pituitary-adrenal (HPA) axis by providing the hypophysiotropic neuropeptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) to stimulate ACTH secretion from the anterior pituitary (see Ref. 2). Various stress paradigms act through different pathways, because in some cases the elimination of PVH abolished acute stress-induced ACTH elevation [hemorrhage (10), early response after bacterial lipopolysaccharide (13), elevated platform stress (28)], but with some other stressors, considerable ACTH and/or corticoste-
MATERIALS AND METHODS

Male rats (220–400 g) of Wistar strain were obtained from Charles River (Budapest, Hungary). Before surgery, the animals were housed five per cage at 23–24°C and 50–60% humidity with a 12:12-h light-dark cycle (light on 0600, off 1800). Animals were given rat chow and tap water ad libitum. Lesions of the MBH and the PVH were performed in separate experimental series. The experiments were performed in accord with regulations set by the Hungarian Council for Animal Care and were supervised by the Institutional Animal Care and Use Committee, Institute of Experimental Medicine, Hungarian Academy of Sciences.

Hypothalamic Surgeries

Hypothalamic lesions were placed 5 days before starting the chronic stress, and the lesioned animals were housed singly post-surgery. Anesthesia was induced by intraperitoneal injection of ketamine (50 mg/kg, SelBruHa Allatgyo- yaszati Kft, Hungary)-xylazine (20 mg/kg, Spofa, Czech Republic)-promethazine chloratum (0.2 ml/kg, EGIS, Budapest, Hungary). The lesions were placed as described previously, using wire knives fashioned from the stainless steel mandrel of a 20-gauge spinal needle and manipulated with the help of a stereotaxic frame (D. Kopf, Tujunga, CA). MBH lesion transected all hypothalamic axons, terminating in the median eminence region by a cone-shaped lesion (20). PVH lesion destroyed all CRH-containing cell bodies in the PVH by use of an inverted cone-shaped lesion (3.0–3.8 mm in diameter at its base) (28, 30, 31). Control rats were subjected by use of an inverted cone-shaped lesion (3.0 mm below the brain surface, i.e., just above the hypothalamus). This restraint procedure minimized the space around the animal, prevented turning, and provided a rather strong stressful stimulus without being harmful in the long run. Restraint sessions lasted for 1 h in the morning (0900–1200) and were repeated daily for 7 or 8 days.

Controls were non-stressed rats. An acute-restraint group was killed at the end of the first restraint; a repeated-restraint group at rest was killed on day 8, 24 h after the 7th restraint; an acute-restraint-after-repeated-restraint group was killed at the end of the 8th restraint.

Experimental Groups

**MBH lesion series.** The MBH lesion series consisted of four sham-operated groups: control (n = 11), acute restraint (n = 12), repeated restraint (n = 10), and acute restraint after repeated restraint (n = 10). MBH-lesioned groups were control (n = 18), acute restraint (n = 18), repeated restraint at rest (n = 15), and acute restraint after repeated restraint (n = 21).

**PVH lesion series.** In the PVH lesion series were four sham-operated groups: control (n = 18), acute restraint (n = 13), repeated restraint (n = 20), and acute restraint after repeated restraint (n = 12). PVH-lesioned groups were control (n = 17), acute restraint (n = 10), repeated restraint at rest (n = 11), and acute restraint after repeated restraint (n = 9).

Hormone Measurements

Trunk blood was collected into ice-cold tubes containing 50 µl of 20% K-EuTDA. Hormones were measured by radioimmunoassay. Plasma ACTH was measured as described earlier (46). The intra- and interassay coefficients of variation (CVs) were 4.7 and 7%, respectively. Plasma corticosterone was measured from 10 µl of unextracted plasma with an RIA by use of a specific antiserum developed in our institute in rabbits against the corticosterone-3-carboxymethyloxime-BSA. An 125I-labeled carboxy methyl-tyrosine methyl ester derivative was used as tracer (catalog no. I-RBO-36, Institute for Isotopes, Budapest, Hungary). The corticosterone antibody cross-reactivity with other naturally occurring...

Table 1. Weight changes induced by repeated restraint

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight Gain, g</th>
<th>Relative Adrenal Weight, mg/kg</th>
<th>Relative Thymus Weight, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBH</td>
<td>PVH</td>
<td>MBH</td>
</tr>
<tr>
<td>Sham-Control</td>
<td>43.8 ± 2.9</td>
<td>33.6 ± 1.0</td>
<td>162.0 ± 4.9</td>
</tr>
<tr>
<td>Sham-RR</td>
<td>13.2 ± 6.2</td>
<td>4.4 ± 2.5*</td>
<td>174.8 ± 6.9</td>
</tr>
<tr>
<td>Lesion-Control</td>
<td>36.4 ± 3.0</td>
<td>22.3 ± 7.7†</td>
<td>79.1 ± 3.9</td>
</tr>
<tr>
<td>Lesion-RR</td>
<td>21.2 ± 6.1</td>
<td>7.5 ± 2.9*</td>
<td>81.9 ± 4.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. RR, repeated restraint. MBH and PVH, mediodessal hypothalamus and paraventricular nucleus of hypothalamus lesion series, respectively. Body weight gain was reduced by repeated restraint [1 h daily for 7 days (n = 20–32), P < 0.001], and a significant interaction was seen between PVH lesion and repeated restraint (P < 0.001). When all sham-operated animals were compared, the relative weight of adrenal gland (mg/kg) was significantly increased by chronic stress [C: 152.8 ± 3.0 (n = 61); RR: 175.9 ± 3.8 (n = 59); P < 0.001]. Both lesions had significant reducing effect (P < 0.001). When all sham-operated animals were compared, the relative weight of the thymus was significantly decreased by chronic stress [C: 1,786 ± 54 mg/kg (n = 61); RR: 1,371 ± 45 (n = 59); P < 0.001]. MBH lesion had a significant effect (P < 0.001). PVH lesion and RR had a significant interaction (P < 0.001). *P < 0.01, significant difference from control group above it in same column; †P < 0.01, significant difference from Sham-Control in same column. [AJP-Endocrinol Metab • VOL 285 • NOVEMBER 2003 • www.ajpendo.org]
adrenal steroids was <0.05%, except desoxycorticosterone (1.5%) and progesterone (2.3%). Final dilution of the antibody was 1:40,000. Incubation time was 24 h at 4°C, and a second antibody (anti-rabbit from goat) and 6% polyethylene glycol solution was used for separation. A calibration curve was prepared from corticosterone (Calbiochem) and ranged from 0.27 to 40 pmol/tube. The intra- and interassay CVs were 12.3% and 15.33%, respectively. For prolactin RIA, we used materials donated by the Pituitary Program (National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD). The intra-assay and interassay CVs were 15.8 and 24.5%, respectively.

**Northern Blot**

To assess proopiomelanocortin (POMC) mRNA levels, pituitaries were rapidly removed, and anterior lobes were carefully dissected under microscope to avoid contamination of intermediate lobe cells. Samples were stored at −80°C until RNA isolation. The tissue samples were homogenized and lysed in RNA-Clean solution (AGS, Heidelberg, Germany), and total RNA was extracted with chloroform according to the manufacturer’s instructions.

For preparation of Northern blots, 20 μg of total RNA samples were size-fractionated on a 1% agarose-8% formaldehyde denaturing gel and transferred onto Hybond N membranes (Amersham, UK) with the capillary transfer method. The POMC 48-mer synthetic oligonucleotide probe (kindly provided by G. Aguiler, National Institute of Child Health and Human Development, Bethesda, MD) was labeled by terminal transferase (Boehringer Mannheim), and the β-actin riboprobe was labeled by the random primer method (HexaLabel DNA Labeling Kit, MBI Fermentas) using 32P-labeled dCTP (Izinta, Hungary). Prehybridizations (42°C, 4 h) and hybridizations (42°C, overnight) were carried out in a solution containing 50% (vol/vol) formamide, 6× SSC, 5× Denhardt’s solution, 0.5% SDS, 50 mM Na-phosphate buffer, 100 μg/ml tRNA, 10 μg/ml polyU-homopolymer, and 7.5 μg/ml denatured salmon sperm DNA. The labeled probes were added to the hybridization solution at 1 × 10^6 counts·min⁻¹·ml⁻¹. Filters were washed at high-stringency conditions (RT in 2× SSC-0.1% SDS for 5 min, 68°C, in 2× SSC-0.1% SDS for 30 min, 68°C, and in 0.2× SSC-0.1% SDS for 30 min). Blots were exposed to X-ray films (Kodak XAR, Sigma) for 1 or 2 days at −70°C with intensifying screens. Between hybridizations, filters were washed in a solution containing 5 mM Na-phosphate-0.1% SDS at 100°C for 30 min to remove the labeled probe. Quantification of the hybridization signals was achieved by use of an image analysis system (ScionImage, Scion) to obtain plot profiles of autoradiograms. All comparisons were made from RNA samples hybridized on the same filter and normalized to the content of β-actin RNA detected in each individual sample.

**In Situ Hybridization Histochemistry**

To determine CRH and AVP mRNA expression in the hypothalamus, frozen sections were hybridized with 35S-UTP-labeled riboprobes. Plasmids containing CRH and AVP cDNA were generously provided by Dr. W. S. Young (National Institutes of Health). Hybridization and autoradiographic techniques were performed according to a protocol described by Simmons (42) and as reported previously (35). After decapitation, brains were quickly frozen in isopentane, and frontal sections were cut on a cryostat, mounted on poly-l-lysine-coated slides, and stored at −70°C until hybridization. Hybridized sections were dipped into NTB-3 emulsion and exposed for 10–14 days.

---

**Fig. 1.** Plasma ACTH (pM) levels after repeated (7R) and acute restraint (1R) in Wistar rats: influence of hypothalamic lesions. A: restraint for 1 h increased ACTH levels, and 7 × repeated stress slightly reduced the response to the last restraint (P = 0.0559, compared with 1R). B: mediobasal hypothalamus lesion minimized all kinds of restraint stress-induced ACTH elevations, although a small but significant elevation after acute stress was still present. C: paraventricular lesion reduced the response to the first restraint stress by ~50% and almost abolished the response to the 8th daily restraint session. *P < 0.05; **P < 0.01 vs. control (unstressed) animals; #P < 0.01 vs. respective sham-operated group in A.
Sterone was increased by acute restraint. Statistical Analysis

A statistical analysis was performed using two- or three-way ANOVA or MANOVA models in the STATISTICA software package (Tulsa, OK). The models included lesion, lesion × acute stress, and lesion × chronic stress factors as main effects. The interaction of lesion × stress was tested for significance. In the case of MBH or PVH lesion, the effects and all interactions were tested for significance. To achieve homogeneity of the variances, the hormonal data were transformed using logarithms in the analysis. Analysis factors were identified as follows: acute stress, chronic stress, and lesion. Multiple pairwise comparisons where appropriate were made by the Newman-Keuls method. In the absence of statistical interaction, only the main treatment effects were evaluated, and they are described in legends to Figs. 1–4 and Tables 1 and 2. Values are presented as means ± SE.

RESULTS

The timing of the lesions (5 days before beginning of chronic stress) was based on the evidence that histological and endocrine effects are stabilized 5–7 days after surgery. At this time, long-term tropic changes in the HPA system are not yet expected (28–30, 44).

Lesioned rats recovered from the adverse effects of surgery before the beginning of repeated restraint. All of the MBH and PVH lesions were verified by histological examination. In the case of MBH lesion, verification included histology as well as measurement of well-known physiological and biochemical consequences. Enhanced water consumption (ml/day; sham: 30.6 ± 6.2 (n = 42), MBH lesion: 139 ± 8 (n = 70)); elevated plasma prolactin levels (see Table 2) and reduced AVP [ng/gland; sham: 645 ± 33 (n = 43), MBH lesion: 22 ± 4.7 (n = 71)] and oxytocin [ng/gland; sham: 1,456 ± 82 (n = 43), MBH lesion: 29 ± 4.7 (n = 71)] content of the neurointermediate lobe were also measured (31).

Weight Changes

One-week restraint caused consistent weight changes typical for chronic stress (body and thymus weight loss, adrenal mass increase, Table 1). Lesions stimulated weight gain during the first 5 postoperative days (g, MBH lesion series: initial body wt 263 ± 4 (n = 93), increment in sham: +31.1 ± 3.3 (n = 37); increment in MBH lesion: +45.6 ± 3.3 (n = 56); and PVH lesion series: initial body wt 362 ± 9 (n = 156), increment in sham: +13.5 ± 2.0 (n = 81); increment in PVH lesion: +24.3 ± 3.2 (n = 75)). Lesions did not block the effects of repeated restraint on body weight.

Table 2. Plasma prolactin levels after acute and/or repeated restraint in MBH- or PVH-lesioned rats

<table>
<thead>
<tr>
<th></th>
<th>MBH Lesion</th>
<th>PVH Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Acute stress</td>
</tr>
<tr>
<td>Sham-Control</td>
<td>8.5 ± 4.0</td>
<td>47.5 ± 6.0</td>
</tr>
<tr>
<td>Sham-RR</td>
<td>3.0 ± 0.4</td>
<td>51.6 ± 11.4</td>
</tr>
<tr>
<td>Lesion-Control</td>
<td>40.2 ± 5.7</td>
<td>51.6 ± 5.0</td>
</tr>
<tr>
<td>Lesion-RR</td>
<td>33.0 ± 4.1</td>
<td>40.9 ± 3.9</td>
</tr>
</tbody>
</table>

Plasma prolactin values are means ± SE expressed in ng/ml. Acute restraint, 1 h; RR, restraint repeated for 1 h daily for 7 days. MBH and PVH lesions refer to mediobasal-hypothalamus- and paraventricular-lesioned rats, respectively. In sham-operated rats, only the acute stress had significant effect (P < 0.001). The MBH lesion and MBH lesion-acute stress interaction were also significant (P < 0.001) by 2-way ANOVA.

Fig. 2. Effect of acute (1R) and repeated (7R) restraint and hypothalamic lesions on plasma corticosterone (nM) levels. A: basal corticosterone was increased by acute (P < 0.00001) and chronic stress (P = 0.0008), but previous chronic stress did not influence the effect of an acute restraint. B: mediobasal hypothalamus lesion prevented corticosterone elevations except the minimal rise after the 1st restraint. C: paraventricular lesion abolished the chronic stress-induced baseline elevation and lowered the acute rise elicited by restraint. **P < 0.05, ***P < 0.01 vs. control (unstressed) animals. #P < 0.05; ##P < 0.01 vs. respective sham-operated group in A.
loss but did reduce repeated restraint-induced adrenal hypertrophy. The MBH lesion resulted in an enlargement of the thymus, but the reduction after repeated restraint could still be seen. Repeated restraint resulted in a significant reduction of thymus weight. PVH lesion prevented this effect; however, PVH lesion itself reduced the thymus weight.

Hormone Levels

Plasma ACTH. Plasma ACTH levels were elevated only in acutely stressed animals, but not at 24 h after the last stress session in repeated-stress paradigms (Fig. 1A). Previous repeated restraint slightly reduced ACTH elevation observed after the 8th restraint [significant chronic × acute interaction in 3-way ANOVA (P = 0.02), pairwise comparison to the single restraint (P = 0.056)]. As expected, MBH lesion abolished the stress-induced ACTH elevations except the small rise to the acute restraint (Fig. 1B). PVH lesion significantly reduced the acute stress-induced elevation by ~50%, but after repeated restraint the inhibitory effect of the PVH lesion on restraint-induced ACTH elevation was stronger (Fig. 1C).

Plasma corticosterone. Plasma corticosterone levels were elevated by repeated restraint at baseline conditions (P < 0.01), but the 8th restraint session caused further elevation (Fig. 2A). Corticosterone response to acute restraint was similar in restraint-naive and repeatedly restrained rats (Fig. 2A). MBH lesion markedly reduced the corticosterone response (P < 0.01) to the first restraint and abolished the acute response to the 8th bout of restraint (Fig. 2B). PVH lesions prevented the repeated restraint-induced increase in basal plasma corticosterone and reduced the acute response to the 1st and 8th bouts of restraint by 50% (Fig. 2C).

Plasma prolactin. Plasma prolactin levels were increased by 1 h of restraint (Table 2). The chronic stress had no effect on basal or restraint-induced elevated prolactin values. The extensive lesioning (MBH lesion) elevated the prolactin levels in all groups to acute stress level because of the loss of hypothalamic dopaminergic inhibition. The PVH lesion did not change the stress responses of prolactin.

POMC mRNA

Anterior pituitary POMC mRNA levels (Fig. 3) were increased in chronically and in chronically + acutely stressed sham-operated rats compared with their appropriate controls, whereas acute restraint alone did not cause any changes (POMC mRNA is known to rise with a latency of several hours after a single exposure to strong stress). MBH or PVH lesion had no effect on POMC mRNA in the anterior pituitary in the absence of repeated restraint, but both lesions prevented the repeated restraint-induced elevation of POMC mRNA.

Hypothalamic CRH and AVP mRNA Expression

In accord with previous studies, on sections hybridized for CRH mRNA, repeated restraint elevated the number of autoradiographic silver grains, as well as the number of CRH-expressing cells in the medial dorsal parvocellular subdivision of the PVH (Fig. 4, A and B). In addition, cells in this subdivision also express AVP mRNA in chronically stressed rats. The
AVP mRNA signal in the supraoptic nucleus (SON) was not affected by the stress or by the PVH lesion. Neurons expressing CRH mRNA were revealed in the region just below the fornix in PVH-lesioned rats (Fig. 4C). These cells, however, do not express a detectable AVP mRNA signal in chronically restrained rats.

DISCUSSION

The present findings show that, in acute and repeated restraint, the HPA axis stimulation is centrally driven and the PVH has an important role in some, but not all, of the HPA responses to repeated-restraint stress.

In sham-operated rats, the repeated-restraint stress resulted in long-term, sustained elevation of the HPA axis, as it decreased body and lymphoid organ weights and increased the baseline corticosterone level in the plasma and POMC mRNA levels in the anterior pituitary in a way typical of chronic stress (6, 12, 24).

Lesioning of the MBH abolishes all connections between hypothalamus and hypophysis, resulting in a hypophysis without direct central regulation. MBH-lesioned animals show only minimal response to acute, and no response to chronic, restraint, showing that the hypothalamo-hypophysial connection is necessary to maintain HPA axis activation in repeated-restraint stress.

Not only the MBH lesion but also the PVH lesioning prevented the elevation of basal corticosterone levels, the increased POMC mRNA expression in the anterior pituitary, the increase in adrenal weight, and the decrease in thymus weight that are normally seen after repeated restraint. All of these changes suggest that PVH lesioning, like MBH lesion, is likely to block the sustained release of CRH, which might be needed for the POMC mRNA increase and chronic adrenal hyperactivity. It is of interest that PVH lesion also blocked adrenalectomy-induced pituitary POMC mRNA and plasma ACTH increase (23, 30, 39). In contrast, the PVH lesion and also the MBH lesion failed to block the POMC rise accompanying an immune-related chronic situation, adjuvant-induced arthritis (28). The difference between the MBH and PVH lesion effects on repeated restraint and adjuvant-induced arthritis suggests that some, but not all, chronic stressful stimuli activate the HPA axis centrally through stimulating the hypophysiotropic CRH and/or AVP neurons in the PVH.

In different models of chronic stress, basal corticosterone levels are often elevated without any significant increase in baseline ACTH concentrations, perhaps because of the different timing characteristic of the two hormones (1, 3, 33). Moreover, basal ACTH levels were normal after 14 days of intraperitoneal hypertonic saline, when pituitary POMC mRNA levels were increased (21). Because we killed the animals at the end of 1-h restraint (as this is optimal for single hormone measurements), we did not find POMC mRNA changes in the groups subjected to a single stress only (15, 45), where changes usually appear only at 4 h or later.

The moderate adaptation of the HPA system to repeated restraint is shown by the inhibition of the plasma ACTH rise after the last restraint in the sham-operated rats, when the plasma prolactin and corticosterone responses to the last restraint were unchanged. These findings suggest that only some of the hormonal responses habituate to the stressful stimulation and that the stimulus remained effective after seven repetitions. According to Aguiler (1), repeated painful stimuli (footshock or intraperitoneal hypertonic saline) or metabolic disturbances do not result in adaptation to the primary repeated stress, but physical/psychological stress paradigms, such as immobilization or cold exposure, are characterized by adaptation of the ACTH response to the persistent primary stress. Restraint and immobilization are similar stimuli, differing in stimulus strength and technical details. Previous reports on repeated restraint used different strain, conditions, and timing, which might explain the variable adaptations in ACTH and corticosterone previously reported (9, 11, 19, 26, 43). Similar to our observations, Popovic (38) and Pitman et al. (37) reported no adaptation to restraint during 18–21 days. Hauger et al. (16) also described a difference between ACTH and corticosterone inhibition with 2.5 h of daily immobilization. In considering adaptation to repeated stress, it is also possible that various measures may show different results. Peak levels of plasma ACTH and corticosterone might be almost normal, but time course and area under the curve might be influenced more by adaptation. We suggest that adaptation of the hormonal responses may be stimulus and intensity dependent and that responses adapt mainly to stimuli weaker and/or psychological in nature. It should be noted that, in our study, the method...
of restraint left no room for the movement of the animals and probably provided a stronger stimulus than that in other reports. Whether altered feedback plays a role in adaptation to restraint is not known.

PVH lesions are known to inhibit acute stress-induced ACTH and corticosterone responses to many stimuli by variable degrees. After PVH lesion, the plasma ACTH response to short and relatively weak neurogenic stressors may be completely blocked, but most, if not all, medium-to-strong, acute, stressful stimuli will elicit an almost normal ACTH and corticosterone response (7, 8, 10, 27, 29, 40). The present findings are in agreement with these data.

After repeated restraint in the PVH-lesioned rats, a further homologous stimulus was almost ineffective on ACTH release, but the plasma corticosterone response from the same rats showed only partial inhibition. Plasma corticosterone might be less impaired than ACTH, because either maximal adrenocortical stimulation occurs with plasma ACTH levels in the low-stress range in the PVN-lesioned group or the sensitivity of the adrenal cortex may rise and maintain corticosterone secretion even in the presence of plasma ACTH levels well below their acute stress peak. It is also possible that a direct neural influence on the adrenal cortex may play a role in elevating plasma corticosterone in restraint. Because the effects of adaptation to repeated restraint (~50%) and PVH lesion (~50%) seemed to be additive, the marked inhibition of ACTH secretion in chronically stressed PVH-lesioned rats might reflect a stronger inhibition by corticosterone feedback in those few hypophysiotropic CRH neurons that are outside the PVH (e.g., in the periforcal region) and might have escaped lesioning. In addition, the hypothalamic mechanisms, including CRH neurons outside the PVH and AVP/CRH from the SON (18), may not receive all of the stimulatory inputs to the SON from the hypothalamic mechanisms, including CRH neurons in the hypothalamic areas outside the PVH (for example in the periforcal region). However, the CRH-releasing neurons in and outside the PVH seem to behave differently, because PVH lesion strongly inhibited the restraint-related ACTH elevation only in repeatedly stressed animals.

**DISCLOSURES**

These studies were supported by Hungarian Scientific Research Fund Grants T0-25845 to G. B. Makara and T0-43056 to K. J. Kovács.

**REFERENCES**


AJP-Endocrinol Metab • VOL 285 • NOVEMBER 2003 • www.ajpendo.org


