Chronic blockade of hindbrain glucocorticoid receptors reduces blood pressure responses to novel stress and attenuates adaptation to repeated stress

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Bechtold AG, Patel G, Hochhaus G, Scheuer DA. Chronic blockade of hindbrain glucocorticoid receptors reduces blood pressure responses to novel stress and attenuates adaptation to repeated stress. Am J Physiol Regul Integr Comp Physiol 296: R1445–R1454, 2009. First published May 11, 2009; doi:10.1152/ajpregu.00095.2008.—Exogenous glucocorticoids act within the hindbrain to enhance the arterial pressure response to acute novel stress. Here we tested the hypothesis that endogenous glucocorticoids act at hindbrain glucocorticoid receptors (GR) to augment cardiovascular responses to restraint stress in a model of stress hyperreactivity, the borderline hypertensive rat (BHR). A 3- to 4-mg pellet of the GR antagonist mifepristone (Mif) was implanted over the dorsal hindbrain (DHB) in Wistar-Kyoto (WKY) and BHRs. Control pellets consisted of either sham DHB or subcutaneous Mif pellets. Rats were either subjected to repeated restraint stress (chronic stress) or only handled (acute stress) for 3–4 wk, then all rats were stressed on the final day of the experiment. BHR showed limited adaptation of the arterial pressure response to restraint, and DHB Mif significantly (P ≤ 0.05) attenuated the arterial pressure response to restraint in both acutely and chronically stressed BHR. In contrast, WKY exhibited a substantial adaptation of the pressure response to repeated restraint that was significantly reversed by DHB Mif. DHB Mif and chronic stress each significantly increased baseline plasma corticosterone concentration and adrenal weight and reduced the corticosterone response to stress in all rats. We conclude that endogenous corticosterone acts via hindbrain GR to enhance the arterial pressure response to stress in BHR, but to promote the adaptation of the arterial pressure response to stress in normotensive rats. Endogenous corticosterone also acts in the hindbrain to restrain corticosterone at rest but to maintain the corticosterone response to stress in both BHR and WKY rats.

corticosterone; brain; nucleus of the solitary tract; hypothalamic-pituitary-adrenal axis; chronic stress

EXAGGERATED CARDIOVASCULAR responses to acute stress and chronic stress increase the risk for hypertension and cardiovascular disease (1, 3, 13, 15, 31, 38, 42, 58, 68). Acute stress rapidly increases blood pressure, heart rate, plasma glucocorticoid concentration, and blood glucose levels (8), while chronic or repeated stress is associated with increases in baseline blood pressure and glucocorticoids (17). Chronically elevated glucocorticoids increase morbidity and mortality from cardiovascular disease and alterations in the glucocorticoid receptor (GR), and in glucocorticoid metabolizing enzymes are associated with hypertension and cardiovascular disease in humans (28, 30, 35, 36, 39, 48, 49, 61, 65, 67, 70, 71). Thus, chronic stress-induced elevations in glucocorticoids likely contribute to the adverse effects of stress on cardiovascular health.

The mechanisms by which glucocorticoids modulate cardiovascular stress responses are not fully understood. A review by Sapolsky et al. (52) concluded that glucocorticoids act permissively to enhance the arterial pressure response to many physiological stressors, in part by supporting the peripheral effects of catecholamines. Other studies indicate that chronic, systemic elevations in glucocorticoids enhance cardiovascular and catecholamine responses to acute novel psychological stress (33, 64). We demonstrated that chronic administration of the glucocorticoid corticosterone to the dorsal hindbrain (DHB) enhances the blood pressure and glucose responses to acute novel stress, without altering the systemic corticosterone or heart rate responses (56). Thus, exogenously administered glucocorticoids can act within the brain to modulate the cardiovascular response to psychological stress. The goal of the present study was to test the hypothesis that endogenous corticosterone also acts within the DHB to enhance the cardiovascular and glucose responses to both acute and repeated (chronic) stress. Borderline hypertensive rats (BHR) and normotensive outbred Wistar-Kyoto (WKY) rats were subjected to either a single or repeated episodes of restraint stress. BHR are the offspring of female spontaneously hypertensive rats (SHR) and normotensive male WKY rats (51). SHR exhibit exaggerated cardiovascular responses to acute stress and develop glucocorticoid-dependent hypertension (20, 41, 50). BHR also have enhanced stress reactivity and are susceptible to stress-induced hypertension (21, 51). Thus, we hypothesized that endogenous glucocorticoids would exert a greater influence on stress responsiveness in BHR relative to WKY rats.

Both rats and humans secrete glucocorticoids that bind to two receptor subtypes, the mineralocorticoid receptor (MR) and the GR (46). MR are sometimes colocalized with an enzyme, 11β-hydroxysteroid dehydrogenase type II, that degrades corticosterone; in the absence of this enzyme, MR are occupied by glucocorticoids even at baseline hormone concentrations (7, 46). Glucocorticoids have a lower affinity for GR compared with MR, and GR are only fully occupied at very high concentrations of glucocorticoids (7). This study focused on the role of GR in mediating the effects of elevated endogenous glucocorticoids on stress reactivity, and tested the hypothesis that endogenous corticosterone acts in the hindbrain via GR to enhance the blood pressure response to stress selectively in BHR. Actions of corticosterone within the DHB were chronically blocked by implantation of a 3- to 4-mg pellet of the GR antagonist mifepristone (Mif), as previously described (55). Control animals received either DHB sham pellets or subcutaneous Mif.
Surgical Procedures

Physiological data were obtained from 57 male BHR (385 ± 4 g body wt) and 46 outbred male WKY rats (414 ± 6 g body wt) purchased from Taconic Farms. Twenty additional male WKY rats (440 ± 9 g body wt; Taconic Farms) and 6 male Sprague-Dawley rats (487 ± 25 g body wt; Charles River) were used to estimate brain Mif levels. All animals were allowed at least 1 wk to recover from transport prior to being subjected to any procedures and were then randomly assigned to treatment groups. All animals were housed in American Association for Accreditation of Laboratory Animal Care International (AAALAC) accredited animal care facilities on a 12:12-h light-dark cycle and maintained on a normal sodium diet. Food and water were provided ad libitum. Experimental protocols were approved by the Institutional Animal Care and Use Committees at the University of Missouri Kansas City, Kansas City, MO and the University of Florida, and were performed with strict adherence to all AAALAC and National Institutes of Health and National Research Council guidelines.

Methods

General

Physiological data were obtained from 57 male BHR (385 ± 4 g body wt) and 46 outbred male WKY rats (414 ± 6 g body wt) purchased from Taconic Farms. Twenty additional male WKY rats (440 ± 9 g body wt; Taconic Farms) and 6 male Sprague-Dawley rats (487 ± 25 g body wt; Charles River) were used to estimate brain Mif levels. All animals were allowed at least 1 wk to recover from transport prior to being subjected to any procedures and were then randomly assigned to treatment groups. All animals were housed in American Association for Accreditation of Laboratory Animal Care International (AAALAC) accredited animal care facilities on a 12:12-h light-dark cycle and maintained on a normal sodium diet. Food and water were provided ad libitum. Experimental protocols were approved by the Institutional Animal Care and Use Committees at the University of Missouri Kansas City, Kansas City, MO and the University of Florida, and were performed with strict adherence to all AAALAC and National Institutes of Health and National Research Council guidelines.

Surgical Procedures

Animals used for the physiological experiments underwent two surgical procedures: implantation of pellets either on the DHB or subcutaneously, and implantation of arterial catheters. The additional animals underwent pellet implantation only (n = 20), pellet implantation followed by adrenalectomy (n = 4), or no surgery (n = 2). All surgical procedures were performed using aseptic technique with the depth of anesthesia maintained such that there was no reflex response to pinching the hind paw. Rats were placed in warm, padded cages following surgery and monitored until they could move about and groom normally. Buprenorphine (0.05 to 0.1 mg/kg sc), nalbuphine (4 mg/kg sc), or carprofen (5 mg/kg sc) was given as needed to alleviate postsurgical pain. Rats were singly housed following implantation of arterial catheters.

Pellet Implantation. Selective chronic blockade of DHB GR was achieved by implantation of small pellets of the GR receptor antagonist Mif (Sigma-Aldrich) on the surface of the DHB as previously described and validated (55). Briefly, powdered Mif was melted and pipetted into a mold to form 3- to 4-mg pellets with the approximate dimensions of 1.5 mm (l) × 1.75 mm (w) × 1.0 mm (h). Sham pellets were made of hardened Silastic (Kwik-Sil; World Precision Instruments) and carved to the same dimensions as the Mif pellets. Previous results indicate that Silastic pellets serve as an appropriate control in this model (55). To control for systemic effects of the DHB Mif, 3- to 4-mg pellets were implanted subcutaneously. Pellets were implanted using Domitor (metametidine hydrochloride, 0.5 mg/kg ip; Pfizer Animal Health, Exton, PA) and ketamine (75 mg/kg ip; Fort Dodge Animal Health, Fort Dodge, IA) or inhaled isoflurane (2–3% in 100% oxygen at a flow rate of 1 l/min) anesthesia. Propylactic penicillin (600,000 U/kg sc Pen-Pro-G; Henry Schein) was given to each rat. Animals were placed in a stereotaxic headframe with the head slightly ventroflexed, and a midline incision was made between the caudal aspect of the occipital bone and the first vertebra. Subcutaneous Mif pellets were implanted at this location. To implant Mif or sham DHB pellets, a small hole was made in the dura, and the pia was removed from the dorsal surface of the hindbrain. The bottom surface of the DHB pellet was coated with mineral oil to assist diffusion of the Mif into the brain. The pellet was placed on the surface of the hindbrain with approximately one-third of the pellet caudal to calamus scriptorius. The pellet was secured in place with a drop of Vetbond surgical glue and covered with a thin layer of Silastic gel (Kwik-Sil; World Precision Instruments). Rats anesthetized with Dormitor-ketamine received 6 to 8 ml of saline subcutaneously to replace lost fluid and Antisedan (1 mg/kg ip atipamezole hydrochloride) was administered to reverse the anesthesia.

Catheter Implantation. Rats were anesthetized with inhaled isoflurane (2–4% isoflurane in oxygen at a 1-L/min flow rate), and a small skin incision was made to expose the femoral artery as previously described (54). A Teflon-tipped catheter was introduced into the artery and advanced to the descending aorta until the tip was estimated to be 1–2 cm below the left renal artery. The catheter was tunneled subcutaneously to exit between the scapulae, filled with sterile heparin (1,000 U/ml), and closed with a sterile plug.

Adrenalectomy. The adrenals were removed via a retroperitoneal approach under isoflurane anesthesia through small bilateral dorsal flank incisions. Adrenalectomized rats were offered both 0.45% saline and 7% sucrose to drink ad libitum.

Experimental Protocol

Physiological experiments were performed in six experimental groups in each rat strain: rats with DHB sham, DHB Mif, or systemic Mif pellets were subjected to either chronic (i.e., repeated) or acute (i.e., once only) restraint stress. Restraint stress was achieved by placing rats in clear Plexiglas restrainers. Repeatedly, stressed rats were weighed and subjected to restraint stress 5 to 7 days per week for 1–2 h per restraint session for 21 to 25 days, beginning 4–7 days following pellet implantation. The time of day of restraint was also varied so that the stress was not entirely predictable. Acutely stressed rats were weighed each day that the corresponding chronically stressed rats were restrained. Adrenal cortical were then implanted in all rats, and the rats were brought to the laboratory daily in their home cages for the next 4 days for the measurement of arterial pressure. Chronically stressed rats continued to be stressed daily while acutely stressed rats were stressed only on the final day. Arterial pressure was measured by connecting each catheter via extension tubing to a pressure transducer (Maxxim Medical) so the rat was free to move normally within the cage until it was placed in the restrainer. The arterial pressure signal was processed using a MacLab system (ADInstruments) connected to a Macintosh computer. Mean arterial pressure and heart rate were calculated online. Arterial pressure was recorded continuously for 3 h each day with the last hour of the final day used to determine baseline arterial pressure and heart rate. Following the baseline arterial pressure measurement on the final day, all rats were subjected to 1 h of restraint stress. In addition, blood samples (300 μl) were obtained from the arterial catheter following the baseline period prior to restraint and at 10 and 60 min during restraint for the measurement of blood glucose (One Touch Ultra glucose meter; LifeScan, Johnson & Johnson) and plasma corticosterone. The blood used for measurement of corticosterone was placed in heparinized tubes and kept in ice. The blood samples were then centrifuged, and the plasma removed and stored at −20°C or less until assayed for plasma corticosterone using the MP Biomedicals rat 125 I-RIA kit. Rats were euthanized with an overdose of inhaled isoflurane and both adrenals, and the abdominal fat pad were removed and weighed.

Assessment of Brain Mif Levels

Immunohistochemical Assay. Mif and corticosterone both bind to the GR and cause the subsequent translocation of the ligand-bound receptor from the cytoplasm to the nucleus (22, 45). We previously used immunohistochemical identification of GR to demonstrate that 4 days of treatment with DHB Mif or corticosterone caused nuclear translocation of the GR within the DHB, but not in the ventral hindbrain or the forebrain (55). The present experiment utilized the same technique to determine the effect of prolonged DHB Mif treatment on nuclear translocation of the GR. Rats were treated with either DHB sham or DHB Mif pellets as described above. Six or 8 wk later they were deeply anesthetized and perfused transcardially with phosphate-buffered saline followed by 4% paraformaldehyde, and the brains were harvested. Three to 5 days prior to perfusion they were adrenalectomized to eliminate endogenous corticosterone. Untreated...
adrenal-intact rats were restraint stressed for 60 min to stimulate endogenous corticosterone release and were then anesthetized and perfused as described above. These rats were used as a positive control for GR nuclear translocation. Immunohistochemistry was performed as previously described using a rabbit anti-GR antibody (1:1,000 PA1–511, Affinity Bioreagents) that has a higher affinity for the occupied GR compared with the unoccupied receptor (55). Representative photomicrographs from adrenalectomized rats treated for 6 wk with either a DHB sham or a DHB Mif pellet are provided in Fig. 1 (rows A and B, respectively). Figure 1, row C shows photomicrographs from an adrenal-intact rat stressed just prior to perfusion. The DHB sham-treated rats exhibited light cytoplasmic staining for the GR in the DHB, including the nucleus of the solitary tract (NTS), a prominent nucleus in the DHB), the ventral hindbrain including the rostral ventrolateral medulla, and the hippocampus. DHB Mif-treated rats showed dark nuclear GR staining in the NTS, but only lighter cytoplasmic staining in the rostral ventrolateral medulla, and the hippocampus. DHB Mif-treated rats showed dark nuclear GR staining in the NTS, but only lighter cytoplasmic staining in the rostral ventrolateral medulla, and the hippocampus. Adrenal-intact stressed rats showed dark nuclear staining in all three regions of the brain. These results demonstrate that the DHB pellet can produce prolonged delivery of Mif to the DHB, but that Mif was not present in the ventral medulla or hippocampus in sufficient quantities to cause GR translocation to the nucleus that was detectable using immunohistochemistry. Therefore, any effects of Mif from the DHB Mif pellets were likely due to blockade of DHB GR.

HPLC. We also attempted to measure tissue concentrations of Mif by HPLC following extraction of the Mif from brain tissue. Rats were treated with either DHB sham (n = 7), DHB Mif (n = 6) or subcutaneous Mif (n = 7) pellets as described above, and 4 wk later they were then deeply anesthetized with isoflurane and the brains rapidly removed and frozen. Samples of brain tissue were obtained from the DHB, the ventral hindbrain, and the forebrain with average weights of 28 ± 2 mg, 27 ± 2 mg, and 36 ± 2 mg, respectively. The tissue samples were homogenized in ethanol/saline (1:1), extracted twice with methylene chloride, and the samples were then dried under vacuum. The samples were reconstituted in acetonitrile/water (60:40) and analyzed by reversed-phase HPLC (Phenomenex Ultragreg 5 ODS; 150 × 4.60 mm, 1.2 ml/min, 100 μl injection volume; UV detection at 254 nm) using acetonitrile-water (60% vol/vol) and calibration samples ranging from 0.5 to 10 μg/ml (r² = 0.997). Initial tests ensured that Mif and corticosterone did not coelute (retention times of 10.3 and 2.5 min, respectively). The limit of quantification (LOQ) was defined as the lowest concentration included in the calibration curve (0.5 μg/ml; equivalent to 50 ng per injection), and peak height was approximately fourfold higher than background noise. Based on the average tissue weight and lower limit of the assay, the minimum detectable concentration of the DHB samples was ~1,800 ng Mif per gram of brain tissue. Each DHB tissue sample from the DHB Mif-treated rats was run separately. Some samples had apparent peaks at 10.3 min, but only one was above LOQ. When all ventral hindbrain samples from DHB Mif-treated rats were combined, a small Mif peak was detected; however, this was below the LOQ. Forebrain samples from all of the DHB Mif-treated rats were likewise combined, and there was no detection of a Mif peak. Samples from the subcutaneous Mif-treated rats were run together as described above, and no indication of a Mif peak was observed. No further attempts were made to obtain precise measurements of brain Mif concentrations due to the relative insensitivity of the assay.

**Statistical Analysis**

Group data are expressed as means ± SE. The data from the DHB sham and subcutaneous Mif animals were not significantly different, so the results from these animals were collapsed into a single control group. Data were initially analyzed by three- or four-way ANOVA. The between-subject factors were strain (BHR compared with WKY), stress (acute compared with chronic) and the effect of Mif (control compared with DHB Mif). When applicable, the time was also included as a repeated measure. When the overall ANOVA detected significant interactions, additional ANOVA was performed on the appropriate subgroups of data. Mean arterial pressure and heart rate values during stress were averaged into 5-min bins, and the changes from baseline were calculated. The data were divided into 2 × 30-min

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**Fig. 1.** Immunohistochemical localization of occupied glucocorticoid receptors (GR). Rats were treated with dorsal hindbrain (DHB) sham (row A) or DHB mifepristone (Mif) (row B) pellets for a total of 6 wk and were adrenalectomized at least 72 h prior to collection of the brains. Each row contains data from a single animal. The rat in row C was adrenal-intact and subjected to restraint stress for 60 min just prior to collection of the brain. Immunohistochemistry was performed using an antibody that has higher affinity for the occupied, compared with the unoccupied, GR. Scale bars are all equal to 100 μm. NTS, nucleus of the solitary tract; RVLM, rostral ventrolateral medulla.
time segments for analysis. Adrenal weights were normalized to body weight on the day of euthanasia for quantification and analysis. Body weights for the first 3 wk of stress or corresponding period for the acutely stressed rats (i.e., the period prior to surgical implantation of the vascular catheters) were pooled into 2-day periods, and changes from day 1 were calculated for analysis. Significance was accepted at $P \leq 0.05$.

**RESULTS**

**Blood Pressure and Heart Rate**

Baseline mean arterial pressure was higher, and baseline heart rate was lower in BHR relative to WKY rats (Table 1). Chronic stress did not significantly alter baseline heart rate or mean arterial pressure. DHB Mif treatment had no significant effect on baseline arterial pressure, but reduced baseline heart rate selectively in chronically stressed WKY rats. Restraint stress produced a rapid, significant increase in mean arterial pressure and heart rate in all groups of rats (Fig. 2). The chronically stressed WKY rats had a significantly smaller increase in arterial pressure compared with the acutely stressed WKY rats during the entire 60 min of restraint, whereas chronically stressed BHR had a significantly smaller arterial pressure response to restraint only during the first 30 min of stress. Thus, chronically stressed BHR rats exhibited less adaptation of the arterial response to restraint stress compared with WKY rats. The effects of DHB Mif treatment on the arterial pressure response to stress also differed between rat strains. DHB Mif attenuated the arterial pressure increase during acute restraint in BHR, but had no effect on this response in WKY rats. DHB Mif also attenuated the arterial pressure increase during restraint in chronically stressed BHR, but enhanced this response in chronically stressed WKY rats. These data indicate that endogenous corticosterone acted in BHR to enhance the arterial pressure response to either novel or repeated restraint stress, but acted in the opposite way in WKY rats to promote the adaptation of the blood pressure response to repeated stress. The heart rate response to stress was significantly attenuated in chronically compared with acutely stressed rats throughout the 60 min of restraint in both strains, and DHB Mif did not significantly alter the heart rate response.

**Corticosterone and Glucose**

Baseline plasma corticosterone was significantly increased by both chronic stress and DHB Mif treatment, with no difference between rat strains (Fig. 3). The increase in plasma corticosterone at both 10 and 60 min of restraint stress was significantly greater in BHR compared with WKY rats, although chronic stress blunted the restraint stress-induced increase in corticosterone in both strains. DHB Mif treatment had an overall effect to reduce the corticosterone response to stress at 10 min of restraint in both acutely and chronically stressed rats, an effect that was independent of rat strain.

Chronic stress significantly ($P = 0.04$) lowered baseline plasma glucose in WKY rats, while it tended to increase plasma glucose in BHR ($P = 0.09$; Fig. 4). As a result, baseline glucose was higher in chronically stressed BHR compared with chronically stressed WKY rats. The restraint stress-induced increase in plasma glucose was significantly attenuated in all chronically stressed rats compared with acutely stressed rats. DHB Mif treatment had no effects on plasma glucose in these experiments.

**Adrenal and Body Weights**

Adrenal gland weight was greater in BHR compared with WKY rats. Chronic stress and DHB Mif treatment each significantly increased adrenal weight in both BHR and WKY rats (Fig. 5). Chronic stress significantly attenuated body weight gain during the entire duration of repeated stress in WKY rats, but only after the first 7 days of stress in BHR. DHB Mif treatment reduced body weight gain in BHR rats during the initial phase of treatment, but had no significant effect on body weight gain in WKY rats.

**DISCUSSION**

**Effects of Rat Strain and Endogenous Corticosterone on Responses to Acute Stress**

These experiments reveal that endogenous corticosterone can act within the hindbrain in rats to modulate cardiovascular and hypothalamic-pituitary-adrenal (HPA) axis function, but the effects are influenced by the stress state and genetic background (strain) of the animal. We previously demonstrated that chronic treatment of the DHB with exogenous corticosterone increased the arterial pressure and glucose responses to acute restraint stress in normotensive rats (56). The present study demonstrates that blockade of DHB GR with Mif attenuates the arterial pressure response to acute restraint stress in BHR, but has no affect on this response in WKY rats. BHR had a larger glucose response to stress compared with WKY rats, but there were no effects of DHB Mif on this response. The data suggest that in BHR, but not WKY rats, endogenous corticosterone acts via GR within the DHB to enhance the arterial pressure response to acute stress even in the absence of a background level of chronic stress. This cannot be due to increased baseline corticosterone in BHR relative to WKY rats, since the baseline values for corticosterone were similar between the two strains of rats. DHB Mif increased baseline corticosterone similarly in both BHR and WKY rats, so this increase in corticosterone also cannot explain the differential effect of Mif on the arterial pressure response to acute stress in the two rat strains. However, the BHR had a larger corticoste-

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**Table 1. Average baseline mean arterial pressure and heart rate**

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mifepristone</th>
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<tr>
<td><strong>Mean Arterial Pressure, mmHg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY acute stress</td>
<td>110±2 (n = 11)</td>
<td>115±4 (n = 7)</td>
</tr>
<tr>
<td>WKY chronic stress</td>
<td>109±1 (n = 18)</td>
<td>104±2 (n = 7)</td>
</tr>
<tr>
<td>BHR acute stress</td>
<td>122±2* (n = 16)</td>
<td>125±3* (n = 9)</td>
</tr>
<tr>
<td>BHR chronic stress</td>
<td>121±3* (n = 17)</td>
<td>123±4* (n = 9)</td>
</tr>
<tr>
<td><strong>Heart Rate, beats/min</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY acute stress</td>
<td>312±6 (n = 11)</td>
<td>322±12 (n = 7)</td>
</tr>
<tr>
<td>WKY chronic stress</td>
<td>320±5 (n = 18)</td>
<td>295±9† (n = 7)</td>
</tr>
<tr>
<td>BHR acute stress</td>
<td>295±7* (n = 16)</td>
<td>305±5* (n = 9)</td>
</tr>
<tr>
<td>BHR chronic stress</td>
<td>293±7* (n = 17)</td>
<td>292±5* (n = 9)</td>
</tr>
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Values are mean ± SE. * $P < 0.05$ for an overall effect of strain; mean arterial pressure was higher and heart rate lower in borderline hypertensive rats (BHR) compared with Wistar-Kyoto (WKY) rats. † $P < 0.05$ for effect of mifepristone to reduce heart rate selectively in chronically stressed WKY rats.
ron response to stress compared with the WKY rats, indicating some enhanced activity of the HPA axis in these animals. The BHR also had larger adrenals compared with the WKY rats, but we did not determine whether this corresponded to a greater glucocorticoid synthesizing capacity of the BHR adrenals. Qualitative comparison of the data in Fig. 2 reveals that blockade of DHB GR in the BHR reduced the arterial pressure response to acute stress to the level observed in control-treated WKY rats, suggesting that endogenous corticosterone may mediate the enhanced stress reactivity observed in the BHR.

Effects of Rat Strain and Endogenous Corticosterone on Responses to Chronic Stress

Repeated restraint increased adrenal weight and baseline plasma corticosterone in both BHR and WKY rats, indicating that this protocol produced chronic stress in both rat strains (7, 43). However, the two strains of rats exhibited differential physiological responses to the chronic stress. WKY rats showed greater adaptation to the repeated stress compared with BHR, as evidenced by a significantly greater reduction in the arterial pressure response and an apparently larger reduction in the corticosterone response to repeated restraint (Figs. 2 and 3). Baseline glucose was significantly reduced by chronic stress in WKY rats, but not in BHR (Fig. 4), and chronic stress had a greater effect to attenuate body weight gain in WKY rats compared with BHR. Furthermore, the role of endogenous corticosterone in chronically stressed rats was different in the two rat strains. Blockade of DHB GR inhibited the adaptation of the arterial pressure response repeated stress in the WKY rats, effectively increasing the arterial pressure response to repeated restraint. These data extend previous findings that systemic corticosterone can promote the adaptation of the heart.
rate, immediate early gene expression, and the initial amyg-
dalar corticotropin releasing hormone responses to repeated
psychological stress (6, 57). Corticosterone is possibly facili-
tating the acquisition of the memory of the stress, since
activation of GR within the NTS enhances memory consolida-
tion (47). In contrast, the blood pressure response to stress in
the BHR exhibited little adaptation with repeated exposure
even in the absence of GR blockade, and Mif inhibited the
arterial pressure response to restraint even in the chronically
stressed rats. Others have reported that BHR fail to show
adaptation of immediate early gene responses to repeated stress
(44). The action of DHB Mif to inhibit body weight gain in the
BHR is consistent with recent reports that corticosterone can
act within the brain to stimulate food intake, especially in
response to stress (9). The reasons for between-strain differ-
ences in stress responsiveness and adaptation are currently
unknown.

Effects of DHB Mif on Plasma Corticosterone

We have previously shown that chronic treatment with DHB
Mif elevated baseline plasma corticosterone concentration in
the evening, but not in the morning (55). Similar effects of Mif
on circadian glucocorticoid secretion have been reported with
intracerebroventricular administration of Mif in rats and sys-
temic administration in humans (23, 66). Data from the present
study demonstrate that endogenous glucocorticoids act at DHB
GR to inhibit baseline corticosterone secretion, but to enhance
the corticosterone response to stress. These results are consist-
ent with other studies demonstrating that the NTS both toni-
cally inhibits baseline HPA axis function (24) and can mediate
stimulation of the HPA axis (4, 40). The finding that cortico-
sterone can act at DHB GR to maintain the corticosterone
response to repeated psychological stress is also in agreement
with a study by Laugero et al. (34) reporting positive feedback

Fig. 3. Baseline plasma corticosterone (left) and change in plasma corticosterone (right) at 10 and 60 min of restraint stress in BHR (top) and WKY rats (bottom).
Control rats were treated with either a DHB sham pellet or subcutaneous Mif pellet. Chronically stressed rats were exposed to repeated restraint, while acutely
stressed rats were restrained only on the final day of the experiment. *P ≤ 0.05 for a difference between BHR and WKY rats (i.e., effect of strain). The number of animals per group
is as follows: acute stress control WKY = 11, BHR = 15; acute stress Mif WKY = 7, BHR = 9; chronic stress control WKY = 16, BHR = 17; chronic stress
Mif WKY = 7, BHR = 7.
of centrally administered corticosterone on the HPA axis activity with repeated restraint stress. The present results identify the DHB as one site of action for this positive feedback.

**Chronic Delivery of Mif to the DHB**

Other investigators have administered glucocorticoid agonists and antagonists into the brain to investigate the central nervous system actions of glucocorticoids on blood pressure regulation, but the results have been varied due in part to differences in dose, stress level of the animals, and route of administration (26, 27, 59, 60, 62, 69). Most of the previous studies have utilized intracerebroventricular administration of these drugs, confounding interpretation of the results since glucocorticoids might have opposing effects on blood pressure at different locations within the central nervous system. The approach used in this study circumvents some of the problems with intracerebroventricular administration by providing chronic local increases in Mif. We previously demonstrated that DHB Mif reduced arterial pressure in rats with systemic corticosterone treatment but not in control rats, while systemic Mif at the same dose had no effect (55). The immunochemical localization of the GR to the nucleus only in the DHB (Fig. 1) strongly suggests that the Mif from the DHB pellets did not diffuse to other brain regions in quantities sufficient to bind GR. The HPLC data suggest that some Mif may have diffused into the ventral hindbrain, and we cannot completely rule out a role for ventral hindbrain GR in mediating the effects reported in this paper. The HPLC results and the immunohistochemistry analysis both indicate that Mif did not reach the forebrain GR. A short diffusion distance within brain tissue has been reported for structurally similar compounds (18). Mif also acts as an antagonist at progesterone receptors. However, we previously demonstrated that the cardiovascular effects of systemic Mif in male rats were abolished after elimination of endogenous corticosterone by adrenalectomy (53). The present results do not rule out a role for MR in mediating the effects of DHB corticosterone on the stress response. In fact an important role for MR is likely since it is known that activation of central MR increases baseline blood pressure and sympathetic activity, and enhances stress reactivity (2, 19, 25, 63).

The region of the DHB includes two areas that are important for cardiovascular regulation: the area postrema and the NTS (37). There are no reports that GRs are expressed in the area postrema, so the NTS is the most likely site of action for Mif in the present study. The catecholaminergic neurons in the NTS express a high density of GR (29). NTS catecholaminergic neurons project to the hypothalamus, where they release norepinephrine, which can modulate the HPA axis activity and the release of corticosterone.
gic neurons are activated with psychological stress and project to forebrain areas that are important for cardiovascular regulation including the paraventricular nucleus of the hypothalamus (10, 11). Further studies are needed to determine the role of these and other NTS neurons in the cardiovascular responses to acute and chronic stress.

**Perspectives**

There is growing evidence that both stress and glucocorticoids contribute to the etiology of cardiovascular disease; however, the role of glucocorticoids in the stress response has long been a matter of debate (1, 3, 5, 13, 15, 28, 30, 31, 35, 36, 38, 39, 42, 48, 49, 52, 61, 65, 67, 68, 70, 71). Recent research has led to the general hypothesis that stress-induced increases in glucocorticoids act in the brain to prepare the organism for future events (12, 52). With chronic stress, that could include attenuation of the cardiovascular response to a familiar, nonthreatening stress, while enhancing the response to a novel stress that might prove to be a threat to survival. If the chronic stress is prolonged, this could also lead to a state of constant vigilance that includes an increase in baseline blood pressure. However, little is known regarding central actions of chronic increases in glucocorticoids on cardiovascular regulation during stress, and the present study provides novel insight into this area of investigation. There is also considerable variability among people in their sensitivity to stress and to glucocorticoids, making some humans susceptible and some resistant to the adverse effects of stress (12, 14, 16, 32). It is intriguing that the BHR showed increased stress reactivity and poor adaptation of the cardiovascular response to repeated...
stress relative to the normotensive rats, and blockade of DHB GR attenuated the blood pressure response to stress selectively in BHR. Understanding the intertwined roles of glucocorticoids, stress, and genetic susceptibility in the development of cardiovascular disease will lead to improved methods for treatment and prevention.

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