Chronic Activation of Dorsal Hindbrain Corticosteroid Receptors Augments the Arterial Pressure Response to Acute Stress

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Abstract—Augmented cardiovascular responses to acute stress can predict cardiovascular disease in humans. Chronic systemic increases in glucocorticoids produce enhanced cardiovascular responses to psychological stress; however, the site of action is unknown. Recent evidence indicates that glucocorticoids can act within the dorsal hindbrain to modulate cardiovascular function. Therefore, we tested the hypothesis that the endogenous glucocorticoid corticosterone can act in the dorsal hindbrain to enhance cardiovascular responses to restraint stress in conscious rats. Adrenal-intact animals with indwelling arterial catheters were treated for 4 or 6 days with 3- to 4-mg pellets of corticosterone or silastic (sham pellets) implanted on the dorsal hindbrain surface. Corticosterone pellets were also implanted either on the surface of the dura or subcutaneously to control for the systemic effects of corticosterone (systemic corticosterone). The integrated increase in arterial pressure during 1 hour of restraint stress was significantly (*P* <0.05) greater in dorsal hindbrain corticosterone (912±98 mm Hg per 60 minutes) relative to dorsal hindbrain sham (589±57 mm Hg per 60 minutes) or systemic corticosterone (592±122 mm Hg per 60 minutes) rats. The plasma glucose response after 10 minutes of stress was also significantly higher in dorsal hindbrain corticosterone-treated rats relative to both other groups. There were no significant between-group differences in the heart rate or corticosterone responses to stress. There were no differences in baseline values for any measured parameters. We conclude that corticosterone can act selectively in the dorsal hindbrain in rats with normal plasma corticosterone levels to augment the arterial pressure response to restraint stress. (Hypertension. 2007;49:127-133.)

Key Words: glucocorticoids ▪ hypertension ▪ brain ▪ autonomic nervous system ▪ sympathetic nervous system ▪ nucleus of the solitary tract

Chronic elevations in glucocorticoids increase cardiovascular disease risk.1–3 Recent evidence suggests that glucocorticoid-mediated changes in neuroendocrine control of cardiovascular function could contribute to these adverse actions of glucocorticoids. For example, glucocorticoids reduce the sensitivity and increase the midpoint of the arterial baroreceptor reflex; similar changes in baroreflex function are positively associated with increased cardiovascular disease risk.4–7 Other studies have demonstrated that chronic systemic elevations in glucocorticoids increase the arterial pressure or norepinephrine response to acute stress.8,9 Similar enhanced stress responses in humans have also been linked to increased cardiovascular disease risk.10 However, the sites of action for stimulatory effects of glucocorticoids on cardiovascular function remain unknown.

We demonstrated recently that, analogous to systemic increases in glucocorticoids, chronic treatment of the dorsal hindbrain (DHB) with the endogenous glucocorticoid corticosterone (Cort) attenuated the gain and increased the midpoint of baroreflex control of heart rate.7 These alterations in baroreflex function could promote the enhanced stress responses observed with elevated systemic Cort.8,9 The DHB includes the nucleus of the solitary tract (NTS), an important cardiovascular regulatory site in the central nervous system.11 The NTS receives direct input from arterial baroreceptor afferents and has reciprocal connections with more rostral sites involved in cardiovascular control.11 Both ascending and descending pathways can activate NTS neurons in response to acute stress, suggesting that neurons within the NTS influence the arterial pressure response to stress.12–14 Therefore, we performed the present experiments to test the hypothesis that elevated Cort within the DHB would enhance the arterial pressure response to restraint stress.

Methods

General

Experiments were performed on male Sprague–Dawley rats purchased from Charles River Laboratories. Rats were housed under...
standard housing conditions in an Association for Assessment and Accreditation of Laboratory Care International–accredited facility. They weighed 367±3 g. At the conclusion of all of the experiments, the rats were humanely euthanized according to American Veterinary Medical Association guidelines. The University of Missouri Kansas City Institutional Animal Care and Use Committee approved all of the procedures, and the experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

**Experimental Protocol 1: Effects of DHB Cort on Responses to Stress**

Data were obtained from 3 groups of rats: DHB sham (to control for surgery and placement of a pellet on the brain, n=17), DHB Cort (to treat the DHB chronically with Cort, n=15), and systemic Cort (to control for systemic effects of the DHB Cort, n=13). The systemic Cort group included both dura Cort (n=8) and subcutaneous Cort (n=5) animals.

### DHB Pellets

To selectively and chronically activate DHB Cort receptors, small pellets of Cort were implanted on the surface of the DHB, as has been described previously and validated. Briefly, powdered Cort was melted and pipetted into a mold to form pellets with the approximate dimensions of 1.5 mm (length)×1.75 mm (width)×1.0 mm (height). The pellets weighed between 3 and 4 mg. Sham pellets were made of hardened silastic (Kwik-Sil, World Precision Instruments) and carved to the same dimensions as the Cort pellets. To control for systemic effects of the DHB Cort pellets, a 3- to 4-mg Cort pellet was implanted outside the brain either on the surface of the dura or subcutaneously.

### Surgical Preparation

Pellets and arterial catheters were implanted on the same day under Domitor (metamitomidine hydrochloride, 0.5 mg/kg, IP, Pfizer Animal Health) and ketamine (75 mg/kg, IP, Ft Dodge Animal Health) anesthesia with prophylactic penicillin (Pen-Pro-G 600 000 U/kg, SC, Henry Schein) as described previously. Briefly, catheters were introduced into the femoral artery and advanced to the descending aorta until the tip was estimated to be 1 to 2 cm below the left renal artery. The catheter was tunneled subcutaneously to exit between the scapulae, filled with sterile heparin (1000 U/mL), and closed with a sterile obturator. Animals were then placed in a stereotactic head frame with the head slightly ventroflexed for implantation of DHB or systemic pellets. A midline skin incision was made between the rostral pole of each kidney, and the adrenal glands were removed, cleaned, and weighed. In 5 animals, the plasma Cort concentration is the sum of endogenous to exogenous Cort could affect the results. Specifically, significant systemic leakage of Cort from the DHB pellet could feedback on the brain to alter corticotropin-releasing hormone and adrenocorticotropic hormone synthesis, potentially influencing interpretation of the results. Thus, it was important to determine whether a measurable amount of Cort diffused from the implanted pellets into the systemic circulation and whether the amount of Cort that entered the systemic circulation depended on the location of pellet implantation. To achieve this, plasma Cort was measured at 5 to 6 days of treatment in 5 groups of adrenalectomized rats (n=4 per group): DHB sham, DHB Cort, dura Cort, SC 4-mg Cort, and, to serve as a positive control, SC 200-mg Cort.

### Experimental Protocol 2: Plasma Cort Measurements in Adrenalectomized Rats

In protocol 1, we measured Cort in adrenal-intact rats. In these animals, the plasma Cort concentration is the sum of endogenous Cort plus any exogenous Cort that may have diffused from the pellets into the systemic circulation. Even with similar total plasma Cort concentrations among the experimental groups, alterations in the ratio of endogenous to exogenous Cort could affect the results.

The rats were brought to the laboratory in the morning in their home cages. The arterial catheter was connected to a pressure transducer that was connected in series to a bridge amplifier and a Maclab computerized data acquisition system. Before this, the rats were brought to the laboratory in their home cages and prepared for the measurement of blood pressure for ≥2 days to accustom them to the experimental procedures. Arterial pressure and heart rate were recorded continuously for 3 hours until baseline arterial pressure and heart rate were stabilized. The final 10 minutes of arterial pressure and heart rate data were used to determine baseline values. Then, in 30 rats, 300 µL of blood were obtained from the arterial catheter for the measurement of baseline plasma Cort and glucose. The animals were observed for another 15 minutes. Each animal was then placed in a clear plexiglas restrainer for 60 minutes. Additional blood samples were obtained at 10 and 60 minutes during the period of restraint. Plasma glucose was measured immediately in each sample using a hand-held glucose meter (One Touch Ultra, LifeScan, Johnson & Johnson). The animals were subsequently euthanized and the adrenal glands removed, cleaned, and weighed. In 5 animals, baseline arterial pressure or heart rate increased after the blood sampling and did not return to control values within 15 minutes. These animals were not included in the study.

### Surgical Preparation

Surgery was first performed as described above to implant an arterial catheter and a DHB (silastic or Cort), dura, or SC 3- to 4-mg Cort pellet. To provide a positive control using an additional group of rats, a much larger dose of Cort pellets (2×100 mg) was implanted subcutaneously in the flank region as has been described previously. Then with a dorsal approach a small incision was made on the flank above the rostral pole of each kidney, and the adrenal glands were excised. Each rat was given 6- to 8-mL of 0.45% sodium chloride with 7% sucrose into the retroperitoneal space, and the incisions were sutured. After adrenalectomy, rats were given both 0.45% saline and 7% sucrose in water to drink ad libitum.

### Experimental Protocol

After surgery for implantation of DHB or systemic pellets, on the evening of day 5 (within 2 hours before lights off) and morning of day 6 (within 2 hours after lights on) 300-µL blood samples were taken from the arterial catheters, except in 1 DHB sham rat from which only the evening blood sample was obtained. The data from 1 dura Cort rat were excluded, because the plasma Cort concentrations of 3.6 and 8.1 µg/dL in the morning and evening, respectively, indicated that the adrenalectomy was incomplete. Because all of the rats were adrenalectomized, morning and evening plasma Cort concentrations, when available, were averaged to provide a single value for each animal.
Measurement of Plasma Cort

Blood samples were immediately placed on ice in tubes containing 2 µL of heparin (1000 U/mL) and then centrifuged at 4°C for 15 minutes. The plasma was stored at -20°C until being assayed for Cort using a commercially available radioimmunoassay kit (rat Cort 125I, MP Biomedicals) as described previously.²⁰

Data Analysis

Preliminary testing using ANOVA was performed on the results from experiment 1 for data from the dura Cort and subcutaneous Cort rats to determine whether there were any significant differences in the effects of these systemic Cort treatments with respect to the variables we measured. No significant differences were found, so the results from the Dura and subcutaneous Cort were combined and analyzed as a single systemic Cort group. Preliminary analysis also demonstrated that the blood sampling protocol did not alter the integrated arterial pressure and heart rate responses to restraint.

Mean arterial pressure and heart rate were calculated online by the Maclab software. Heart rate was quantified as beats per minute. The values during stress were averaged into 5-minute time bins, and the changes from baseline were calculated. The results (including the “0” time point) were analyzed by 2-way ANOVA (between subjects for treatment group and repeated measures for time). Because the analysis revealed that there was no interaction between time and treatment, the total integrated increase from baseline for the hour of stress was calculated from the 5-minute averages, and the results were analyzed by 1-way ANOVA for group. Regression analysis was used to determine whether the arterial pressure response to stress was correlated to baseline arterial pressure in the DHB Cort-treated rats. Changes in plasma Cort and blood glucose concentrations in response to stress were calculated and analyzed by 2-way ANOVA for time (repeated measures) and group. There was a significant interaction between time and treatment group for plasma glucose values, so 1-way ANOVA was then performed on the 10- and 60-minute values separately. Baseline arterial pressure and heart rate, adrenal weight, and plasma Cort in adrenalectomized rats were analyzed by 1-way ANOVA. Duncan’s new multiple range test was used for posthoc analysis. P values for the overall ANOVA are provided in the Results section, with significant differences accepted at P<0.05. All of the values are presented as mean±SE.

Results

Protocol 1: Cardiovascular Responses to Stress in Adrenal-Intact Rats

There were no differences in baseline mean arterial pressure among DHB Cort (105±2 mm Hg), DHB sham (105±2 mm Hg), and systemic Cort (106±3 mm Hg) groups. DHB Cort significantly increased the arterial pressure response to 60 minutes of restraint stress relative to both DHB sham and systemic Cort animals (P=0.02 overall effect of treatment group; Figure 1, left and right). There was also a significant effect of time on the change in arterial pressure (P<0.01; Figure 1, left). However there was no interaction between the effects of time and experimental group (P=0.28), indicating that the effect of Cort to increase the stress response was present throughout the stress period. Calculating the integrated arterial pressure response to 60 minutes of stress revealed that DHB Cort treatment increased the stress response by 54% relative to both the DHB sham and systemic Cort groups (Figure 1, right). The lack of an increase in baseline arterial pressure in the DHB Cort–treated rats did not account for the enhanced stress response in this group, because there was no correlation between baseline arterial pressure and the integrated arterial pressure response to stress (r²=0.03; P=0.55).

Baseline heart rate was not significantly different among DHB sham (328±5 bpm), DHB Cort (335±7 bpm), and systemic Cort (352±10 bpm) groups (P=0.11). There was also no effect of treatment group on the change in heart rate in response to stress (Figure 2; P=0.22), although there was a significant effect of time (P<0.01). There was no interaction between the effects of experimental group and time (P=0.20).
Protocol 1: Glucose Response to Stress in Adrenal-Intact Rats
There were no significant differences in baseline plasma glucose concentration among the DHB Sham (87±4 mg/dL, n=8), DHB Cort (92±3 mg/dL, n=9), and systemic Cort (96±2, n=13) treatment groups. There were significant effects of treatment group (Figure 3; P=0.01) and time (P<0.01) on the glucose response to stress, with a significant interaction between group and stress (P=0.01). Further analysis revealed that after 10 minutes of stress, plasma glucose was elevated (P=0.02) in DHB Cort rats relative to both DHB sham and systemic Cort groups. After 60 minutes of stress, plasma glucose was significantly lower in the systemic Cort compared with the DHB sham rats (P=0.03).

Protocol 1: Cort Response to Stress in Adrenal-Intact Rats
Baseline plasma Cort concentration before the acute stress was not different between treatment groups. The values were 6.8±2.5, 7.0±2.9, and 9.7±2.8 µg/dL in the DHB sham, DHB Cort, and systemic Cort treatment groups, respectively. There were no significant between-group differences (P=0.321) for the increase (P<0.01) on plasma Cort concentration in response to stress (Figure 3). Adrenal weight was measured to provide an index of the effect of treatment with DHB or systemic Cort on baseline hypothalamic-pituitary-adrenal axis activity. Adrenal weights were not significantly different among DHB sham (65±2 mg), DHB Cort (69±3 mg), and systemic Cort (67±2 mg) treatment groups.

Protocol 2: Plasma Cort in Adrenalectomized Rats
In rats with the 200-mg SC Cort treatment, plasma Cort averaged 24±1 µg/dL, ≈10-fold higher than any other group. This group served as a positive control, and the data were not included in the between-group ANOVA for comparison of plasma Cort in the adrenalectomized rats. Average plasma Cort concentrations in the other groups of adrenalectomized rats were: DHB sham, 1.30±0.24 µg/dL; DHB Cort, 2.61±0.32; dura Cort, 2.67±0.29; and subcutaneous Cort, 2.83±0.24 µg/dL. All of the groups with 3- to 4-mg Cort pellets had significantly elevated plasma Cort values relative to the DHB sham rats (P<0.01). However, the plasma values in the Cort-treated rats were low and did not exceed the lowest plasma Cort concentrations normally observed in adrenal-intact rats during the nadir of the diurnal cycle. Importantly, there were no differences in systemic Cort values among the 3 groups of rats with 3- to 4-mg Cort pellets, regardless of the site of implantation. These results, combined with the data demonstrating no differences in baseline plasma Cort among the groups of adrenal-intact rats, indicate that systemic effects of Cort because of diffusion of Cort from the DHB Cort pellet cannot account for the increased arterial pressure and glucose responses to stress observed in the DHB Cort-treated rats.

Discussion
Effects of Cort on Cardiovascular Stress Responses
Arterial pressure and heart rate responses to psychological stress are mediated primarily by the sympathetic nervous system. Previous studies have examined the effects of systemic elevations in or elimination of glucocorticoids on the cardiovascular and/or sympathetic nervous system response to stress. Earlier studies reported that adrenalectomy without corticosteroid replacement increased baseline and stress-stimulated norepinephrine secretion, leading to the conclusion that glucocorticoids restrain the sympathetic nervous system response to acute stress. However, adrenalectomy has variable effects on the heart rate and arterial pressure responses to acute stressors. Furthermore, more recent studies report that increases in systemic Cort elevate arterial pressure, epinephrine, and/or norepinephrine responses to stress, although the role of the central nervous system in this action of glucocorticoids is unclear. Investigations of central effects of glucocorticoids on arterial pressure regulation have used intracerebroventricular administration of agonists and antagonists or acute microinjection of drugs into the NTS. However, none of these studies investigated the effect of chronic elevations of central glucocorticoids on the cardiovascular response to acute stress. The current results provide novel evidence indicating that chronically elevated Cort can act within the central nervous system to enhance the arterial pressor response to acute stress. The effects observed in this study were qualitatively similar to the effects of elevated systemic Cort reported by van den Buuse et al.

Effect of Cort on Baseline Arterial Pressure
Several studies have examined the effects of central Cort on baseline arterial pressure. These studies indicated that higher doses of Cort administered intracerebroventricularly produce small increases in baseline arterial pressure, whereas low doses were without effect. We reported previously that DHB Cort pellets increased baseline arterial pressure by 5 to 6 mm Hg after 4 days of treatment, although in the present study there were no differences in baseline arterial pressure between groups. This was not because of a difference in
duration of DHB Cort treatment, because baseline arterial pressures in rats treated with DHB Cort for 4 days (105 ± 4 mm Hg, n = 8) or 6 days (104 ± 3 mm Hg, n = 7) were not different. We have no explanation for the lack of effect of DHB Cort on baseline arterial pressure other than to point out that even when it has been observed, it was not a robust effect. Importantly, the arterial pressure response to acute stress was not correlated with baseline arterial pressure in the DHB Cort–treated rats, so the lack of an elevation in baseline pressure does not account for the enhanced stress response in these rats.

Effects of Cort on Glucose and Hypothalamic–Pituitary–Adrenal Stress Responses

Endogenous glucocorticoids combined with sympathetic activation promote the increase in blood glucose during stress.16 DHB Cort enhanced the glucose response after 10 minutes of stress compared with both other groups. Because there were no significant effects of treatment or time on the Cort response to stress in these experiments, the effect of DHB Cort to increase the glucose response to stress is likely because of changes in sympathetic activation. However, further experiments are needed to determine the role of the sympathetic nervous system.

Site of Cort Action

We have reported previously that implantation of small Cort pellets on the surface of the DHB selectively increases Cort levels in a circumscribed region of the DHB.15 Three brain stem structures that influence autonomic control of the cardiovascular system lie within this area: the NTS, the dorsal motor nucleus of the vagus, and the area postrema. Immuno-histochemical methods have not been able to demonstrate the presence of mineralocorticoid receptors (MRs) or glucocorticoid receptors (GRs) in the area postrema, whereas the NTS and the dorsal motor nucleus clearly express both MRs and GRs.32–34 However, in rats, the dorsal motor nucleus has minimal influence over cardiovascular autonomic efferent activity.35 Thus, it is likely that primarily cells within the NTS mediate the effects of Cort within the DHB, although a role for the area postrema cannot be ruled out. Ascending and/or descending inputs activate NTS neurons in response to a variety of stressors, including restraint stress.12–14 At least some of these stress-activated neurons are catecholaminergic and express GRs.12–14,36 However, the role of the NTS in mediating or modulating the cardiovascular responses to acute psychological stress, such as restraint, is not known. The present study reports novel findings that strongly suggest that the physiological status of neurons within the DHB, including the NTS, can influence the arterial pressure response to restraint stress.

Mechanism of Cort Action

In the present experiments, we used a previously validated method to selectively and chronically treat the DHB with Cort.15 This method increases Cort levels locally in DHB areas without altering plasma Cort concentration in adrenal-intact rats.7,15 We now have shown in adrenalectomized animals that the systemic spillover of Cort from the DHB pellet is both minimal and independent of the site of pellet implantation. Thus, the systemic pellet implantation serves as an adequate control for any systemic effect of the exogenous Cort. Because there was no effect of DHB Cort on the Cort response to stress, we also conclude that differences in plasma Cort levels during stress did not contribute to the enhanced arterial pressure response in the DHB Cort–treated rats.

Because the arterial pressure and heart rate responses to psychological stress are mediated primarily by the sympathetic nervous system,21 these studies support the hypothesis that chronic increases in glucocorticoids can enhance sympathetically mediated cardiovascular responses to an acute psychological stress. In a recent study, we reported that DHB Cort treatment attenuated the gain and increased the midpoint of the arterial baroreceptor reflex.7 Thus, it is possible that attenuation in baroreflex control of sympathetic nerve activity contributed to the effect of DHB Cort to enhance the arterial pressure response to stress observed in this study. However, if there was enhanced activation of the sympathetic nervous system, it was not universal, because the heart rate response to stress was not elevated in the DHB Cort rats. Measurements of plasma catecholamines and/or sympathetic nerve activity will be required to determine the contribution of the sympathetic nervous system.

Cort can activate both MRs and GRs.37 Cort has a higher affinity for MRs than GRs so that at lower doses Cort primarily activates MRs, whereas at higher doses both the MRs and GRs are activated.37 van den Buuse et al9 reported that in adrenalectomized rats low-dose Cort replacement only enhanced the heart rate response to an acute stress, whereas high-dose replacement selectively enhanced the arterial pressure response to the stress, suggesting an important role for GRs. Chronic central infusion of the endogenous mineralocorticoid aldosterone also enhanced the arterial pressure increase during acute novel stress, suggesting a role for MRs.38 Also, a selective MR antagonist blunted the arterial pressure response to stress but only after the animals had repeated exposure to the same stress.39 A subpopulation of the MR-expressing neurons in the NTS also expresses an enzyme that degrades Cort (11-β-hydroxysteroid dehydrogenase 2).34 Thus, the MRs in these neurons are activated primarily by aldosterone, although it is possible that high levels of Cort could exceed the capacity of the enzyme. These neurons are involved in sodium appetite,40 but their influence on the neurohumoral responses to psychological stress has not been studied. Results from these various studies considered together suggest that chronic elevations in Cort act centrally to increase cardiovascular responses to stress by complex interactions of MR- and GR-mediated effects that remain to be elucidated.16,41

Perspectives

There is substantial evidence that elevations in glucocorticoids increase morbidity and mortality from cardiovascular disease.1–3 These adverse actions of glucocorticoids have important implications for human health, because many people take glucocorticoids by prescription or have elevated endogenous glucocorticoids. Many peripheral mechanisms
for detrimental cardiovascular effects of glucocorticoids have been investigated. In contrast, actions of glucocorticoids on central neural control of cardiovascular function have received little attention, in part because initial studies suggested that elevated glucocorticoids acted centrally to reduce rather than increase arterial pressure or sympathetic nervous system activity. However, these studies were limited to single doses of steroids. More recent work suggests that chronic elevations in glucocorticoids can act centrally to elevate arterial pressure.

The present results extend recent findings to demonstrate that selective chronic increases in Cort in the DHB enhance arterial pressure and glucose responses to acute stress. Enhanced reactivity to stress is associated with elevated risk for cardiovascular disease. Elevated cardiovascular stress reactivity is observed with chronic stress, which also increases endogenous glucocorticoids. For example, the chronic stress of lower socioeconomic status is associated with elevated glucocorticoids. Thus, elucidating common mechanisms of elevated stress reactivity is critical for understanding the etiology of cardiovascular disease.

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