Glucocorticoids modulate baroreflex control of renal sympathetic nerve activity

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Glucocorticoids modulate baroreflex control of renal sympathetic nerve activity. Am J Physiol Regulatory Integrative Comp Physiol 280: R1440–R1449, 2001.—Experiments were performed to determine the effects of glucocorticoids on arterial baroreceptor reflex control of renal sympathetic nerve activity (RSNA). Intravenous infusions of phenylephrine and nitroprusside were used to produce graded changes in arterial pressure (AP) in Inactin-anesthetized male Sprague-Dawley rats. Baroreflex control of RSNA was determined during a baseline period and 2 and 3 h after administration of the glucocorticoid type II receptor antagonist Mifepristone (30 mg/kg sc) or vehicle (oil). Corticosterone (cort) treatment (100 mg cort pellet sc for 2–3 wk) increased baseline AP from 115 ± 2 to 128 ± 1 mmHg. Cort treatment also decreased the gain coefficient and increased the midpoint of the baroreflex curve. Treatment of cort rats with Mifepristone decreased AP within 2 h and increased the gain coefficient and decreased the midpoint of the baroreflex function curve back toward values measured in control rats. Mifepristone altered the baroreflex function curve even when AP was maintained at baseline levels. Therefore, these data demonstrate for the first time that glucocorticoids can modulate baroreflex control of RSNA by a mechanism that is, in part, independent of changes in AP.

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Ganglionic blockade produced larger decreases in arterial pressure in glucocorticoid-treated rats compared with controls (23). Several studies have demonstrated that exogenous administration or endogenous overproduction of glucocorticoids in rats or humans results in exaggerated increases in arterial pressure in response to adrenergic agonists (23, 30, 36, 54). Conversely, it has also been shown that rats treated with a glucocorticoid type II receptor antagonist or adrenalectomy have decreased pressor responses to adrenergic agonists (7, 13). However, other data show that glucocorticoids either do not change or decrease basal plasma epinephrine or norepinephrine concentration (3, 12, 24, 27, 44, 48, 53) or spillover (45). Plasma catecholamines may not accurately reflect sympathetic nerve activity. A few studies have determined the effects of glucocorticoids on directly recorded sympathetic nerve activity. Basal and/or stimulated sympathetic nerve activity has been reported to increase, decrease, or not change with glucocorticoid treatment (8, 9, 12, 22, 27, 37, 42, 49). Some of the disparity in the results of these studies appears to be due to differences in the regional sympathetic outflow being recorded. Generally, glucocorticoid treatment increases renal sympathetic nerve activity (RSNA) (22, 42, 49) and decreases or does not change lumbar or muscle sympathetic nerve activity (8, 9, 12, 27, 37).

Another factor that could contribute to the variability of glucocorticoid effects on sympathetic function is the duration of glucocorticoid administration. It has been recognized for a number of years that feedback regulation of glucocorticoid release is mediated within at least three distinct time domains, including a very rapid (seconds to minutes) time frame (20). Such rapid effects of glucocorticoids, which presumably employ nongenomic mechanisms, have also been observed with behavior, neuronal activity, blood pressure, and sympathetic nerve activity (4, 9, 16, 20, 28, 33, 34, 49). Haller et al. (16) suggested that, in the context of behavior, acute effects of glucocorticoids are stimulatory, whereas chronic effects are inhibitory. In agreement with this hypothesis, rapid excitatory effects of...
glucocorticoids have been observed in neurons in the rostral ventrolateral medulla (RVLM), and intracerebroventricular administration of cortisol rapidly increased arterial pressure and RSNA (33, 49). However, rapid inhibitory effects of cortisol on neuronal activity in the paraventricular nucleus have also been reported (34). Another study showed no acute change in muscle sympathetic nerve activity in humans after administration of cortisol (9). Similarly, both excitatory and inhibitory effects of glucocorticoids acting in a delayed time domain to modulate sympathetic function and neuronal activity have been reported (8, 12, 19, 22, 27, 37, 42, 45). Unfortunately, the data from most of these studies are difficult to interpret, because the quantification of nerve activity requires normalization of the data, and thus baseline values cannot be compared between groups. Therefore measurements of neuronal activity, blood pressure, and sympathetic nerve activity have yielded no consensus regarding the nature of rapid actions of glucocorticoids on arterial pressure regulation.

The baroreceptor reflex is an important component of arterial pressure homeostasis. Arterial baroreceptor reflex control of sympathetic nerve activity is important for short-term, and probably long-term, control of arterial pressure (46). Some data are available suggesting that glucocorticoids may facilitate baroreflex control of heart rate (HR); however, the results are inconsistent (7, 11, 42, 47, 50). One explanation for the inconsistencies is that glucocorticoids can increase the sensitivity of the myocardium to β-adrenergic stimulation (47). Direct measurement of nerve activity eliminates the confounding effects of changes in end-organ sensitivity. Surprisingly, the effects of glucocorticoids on baroreflex control of sympathetic nerve activity have not been studied.

There are many similarities in basic aspects of the control and function of glucocorticoids in humans and rats, and there is little structural difference between cortisol and corticosterone (cort), the major glucocorticoid in humans and rats, respectively (6). In both rats and humans, glucocorticoids bind to two distinct receptor types: high affinity/low capacity (type I receptors), which also bind aldosterone, and low affinity/high capacity (type II receptors), which are selective for glucocorticoids (31). In the periphery, aldosterone is the endogenous ligand for the type I receptor because of the presence of an enzyme (11β-hydroxysteroid dehydrogenase) that metabolizes cort and cortisol to inactive compounds (10, 26). This enzyme appears to be absent in many sites in the CNS, allowing glucocorticoids, which are normally present in much higher concentrations than aldosterone, to be the primary endogenous ligand for both the type I and type II receptors. However, 11β-hydroxysteroid dehydrogenase is present in the several CNS nuclei that are important for cardiovascular regulation, including the hypothalamus, the subcommissural organ, and the nucleus of the solitary tract, which is the site of termination of the baroreceptor afferents (32). Furthermore, the low-capacity type I receptors are fully occupied at relatively low plasma glucocorticoid concentrations (6). Therefore, the type II receptor would be expected to play an important role in mediating the effects of elevated glucocorticoids on baroreflex control of arterial pressure. In both rats and humans, the selective glucocorticoid type II receptor Mifepristone can normalize arterial pressure in glucocorticoid-hypertensive subjects (14, 29). We previously reported that Mifepristone significantly reduces arterial pressure in anesthetized cort-treated rats within 1.5 h of subcutaneous administration (39). Although this effect is not rapid enough to determine if the mechanism is nongenomic, it provides a valuable tool to study the effects of cort on sympathetic nerve activity, because within-subjects measurements can be made.

In the present study, experiments were performed in anesthetized male Sprague-Dawley rats to test the hypothesis that glucocorticoids modify baroreflex reflex control of RSNA. Baroreflex control of RSNA was determined in Inactin-anesthetized rats that were either treated with a subcutaneous cort pellet for 1–3 wk or left untreated (control rats). Baroreflex function was determined before and 2 and 3 h after administration of the glucocorticoid type II receptor antagonist Mifepristone (30 mg/kg ip sc).

METHODS

Surgical Preparation

Survival surgery. Experiments were performed using male Sprague-Dawley rats purchased from Charles River Laboratories. Increases in plasma cort concentration were produced (n = 12) by subcutaneous implantation of ∼100-mg pellets of cort. Pellets were made using an established technique (1, 39). Briefly, cort was liquefied and pipetted into a mold designed specifically for manufacturing the pellets. Surgery for implantation of the pellets was performed under methoxyflurane anesthesia using aseptic techniques. A small skin incision was made in the dorsal lumbar region, and a pellet was inserted subcutaneously. The incision was sutured closed and washed with an antiseptic solution. Control rats either underwent the same surgical procedure, except that no pellet was implanted (n = 7), or did not undergo survival surgery (n = 7).

Nonsurvival surgery. Experiments were performed 1–3 wk after survival surgery or in naive rats that had been in the laboratory animal facility for a minimum of 1 wk. The animals weighed between 325 and 475 g on the day of the experiment. Rats were anesthetized with the long-acting rodent anesthetic thiobutabarbital sodium (Inactin; Research Biomedical) at the initial dose of 110 mg/kg ip. Supplemental anesthetic was given in doses of 10 mg/kg ip as required to maintain a surgical plane of anesthesia (i.e., an absence of withdrawal to pinch of the hindpaw and no evidence of fluctuations in blood pressure in response to surgical manipulation or pinch of the hindpaw). Body temperature was maintained at 36–38°C using a ventral heating pad and, when necessary, an infrared lamp. The animal was intubated through a tracheotomy and ventilated with room air supplemented with oxygen. A catheter of PE-50 tubing was inserted into the abdominal aorta via the femoral artery and was used for the measurement of arterial pressure and the removal of blood samples for the measurement of plasma cort. Three venous catheters of Tygon tubing (ID 0.01 in., OD 0.03 in.)...
were inserted into the abdominal vena cava via the femoral veins for the infusion of drugs. A left thoracotomy was made at the third or fourth intercostal space to reduce respiratory-related artifact (due to movement of the diaphragm) in the RSNA recording. In one rat, a vascular occluder was placed around the descending aorta. The occluder was used to maintain arterial pressure constant throughout the experiment. Pancuronium bromide (0.5 mg/kg−1·h−1) was infused intravenously to paralyze the respiratory muscles. After administration of the pancuronium, the depth of anesthesia was maintained by assuring that there were no fluctuations in arterial pressure in response to surgical manipulation or pinch of the hindpaw. Unfortunately, pancuronium dampens the reflex control of HR, with the result that baroreflex control of HR could not be determined in these experiments.

For recording RSNA, a left flank incision was made to expose the renal nerves within the retroperitoneal space. Bipolar electrodes made of Teflon-coated stainless steel wires were placed around a bundle of the renal nerves. The electrodes were secured in place using President light body dental impression material (Coltene).

**Experimental Protocol**

The experimental protocol was initiated at least 30 min after completion of the surgical procedures. At this time, 200 μl of blood for the measurement of plasma cort was removed from the arterial catheter, placed in an Eppendorf tube containing 20 μl of 0.3 M EDTA, and placed on ice. Fifteen minutes later, the baseline baroreflex function curve was determined by the infusion of phenylephrine HCl (50 μg/ml) to increase arterial pressure and sodium nitroprusside (100 ng/ml) to decrease arterial pressure. The rate of infusion was adjusted to produce continuous changes in arterial pressure in the range of 1–2 mmHg/s. The rate for phenylephrine infusion generally ranged approximately from <0.4 to 12 ml/h, which equals doses ranging from <0.4 to 42 μg/min. Occasionally, higher infusion rates were used for 1–2 s to be sure a minimum in sympathetic nerve activity had been achieved. The rate for nitroprusside also ranged approximately from <0.4 to 12 ml/h, which equals doses ranging from <0.8 to 84 ng/min. Fifteen minutes after completion of the reflex function curve or after blood pressure had returned to baseline, whichever took longer, the guccorticoid type II receptor antagonist Mifepristone (30 mg/kg) or vehicle (mineral oil, 0.4–0.5 ml) was administered subcutaneously. Blood samples 2 and 3 were taken at 1.5 and 2.5 h after administration of Mifepristone or vehicle. Baroreflex function curves and were obtained at 2 and 3 h after administration of Mifepristone or vehicle. Mifepristone was a gift from Re-search Biomedicals as part of the National Institute of Mental Health synthesis program.

Experiments were performed in four groups of rats. The first group consisted of control rats (no cort) that received the glucocorticoid receptor antagonist (control + Mifepristone; n = 7). The second group consisted of rats that received both cort treatment and Mifepristone (cort + Mifepristone). The third group was a time control, and these rats received neither cort treatment nor Mifepristone (control + vehicle, n = 7). Mifepristone reduced arterial pressure in both control and cort rats. To determine whether changes in baroreflex function were dependent on this reduction in arterial pressure, a fourth group of rats was treated with cort and given Mifepristone. Additionally, in these rats either phenylephrine (250 μg/ml iv, n = 4) or an abdominal aortic occluder (n = 1) was used to prevent pressure from falling subsequent to Mifepristone administration (cort + phenylephrine; n = 5).

The phenylephrine was infused at rates ranging from 0.4 to 1.2 ml/h. Infusion rates at the upper end of the range were infrequently required. The maximum dose was <5 μg/min.

**Methods of Measurement and Analysis**

**Cardiovascular data.** Arterial pressure was measured from the arterial catheter using a pressure transducer (Max- xim Medical, Athens, TX) connected to a bridge amplifier (Coulbourn Instruments, Allentown, PA). The output from the bridge amplifier was fed into a MacLab (ADInstruments) analog-to-digital processor connected to a Macintosh computer. Mean arterial pressure (MAP) and HR were determined online from pulsatile pressure using the MacLab software. Average values for MAP and HR were determined just before each baroreflex function curve, and the values were analyzed by one- and two-way ANOVA as appropriate. Repeated-measures ANOVA was used for analysis of time as a factor, and between-subjects ANOVA was used to compare groups. For post hoc analysis, Tukey compromise for between-subjects variables and least square means for within-subjects variables were used as needed to determine significance. Significance was accepted at P < 0.05.

**RSNA.** Nerve activity was recorded in its raw form and amplified (×10,000–50,000) with the band-pass filters set between 10 and 3,000 Hz. The raw signal was monitored on the oscilloscope. For quantification purposes, the signal was rectified and integrated using 20- and 600-ms time constants. The 20-ms time constant was used to determine the (electrical) noise level during maximal reductions in nerve activity observed when arterial pressure was elevated with phenyl-

The 600-ms time constant was used to generate the data that were used to construct the baroreflex function curves. The noise level was used to determine “0%” RSNA. The maximum RSNA observed during the decrease in arterial pressure produced by the infusion of nitroprusside in the baseline (first) baroreflex function curve was used to determine “100%” RSNA. After calibrating the RSNA signal using 0 and 100%, the values for RSNA during the reflex function curves were averaged into 1-mmHg bins of arterial pressure. The data were then analyzed using a sigmoid logistic function curve according to the equation, RSNA = P4 + P1/[1 + exp(P2(MAP – P3))], where P1 is range of RSNA, P2 is the coefficient to calculate the gain as a function of pressure, P3 is the pressure at the midrange of the curve, and P4 is the minimum value for RSNA. Therefore, P1 + P4 = the maximum value for RSNA that is set to 100% in the baseline curve. The best-fit curve was calculated using SigmaPlot software (SPSS). The fit of the curve to the data was estimated by calculating an r² value for each curve. Values for r² ranged from 0.77 to 0.99, with 67 of 78 curves having r² values of 0.9 or higher. Once the values for each parameter were calculated for each curve, average values for each group were calculated and compared by ANOVA as described above. The simulated curves illustrated in results were then produced from the average parameter values.

**Plasma corticosterone.** During most experiments, plasma samples (200 μl) for the measurement of plasma cort were obtained from the arterial catheter ~15 min before each baroreflex curve. Samples were taken in only one of five rats in the cort + phenylephrine group because of the technical difficulty of the experiments. A few other samples were lost because of miscellaneous causes. The blood samples taken for the measurement of plasma cort were centrifuged at 4°C, and the plasma was stored at ~70°C until being assayed. Plasma cort was determined as previously described (39) using a commercially available kit (ImmuChem double antibody cor-
Exogenous cort decreases thymus and adrenal weights (1). Therefore, at the conclusion of each experiment, the thymus and adrenal glands were removed, patted dry, and weighed. Thymus and adrenal weights were calculated as fractions of body weight. Values for plasma cort and thymus and adrenal weights were analyzed by ANOVA as described above. Thymus and adrenal weights were not obtained from one control rat.

RESULTS

Under normal conditions, there is a diurnal rhythm in glucocorticoid secretion, with a peak occurring just before onset of the active period (night in rats) and a nadir occurring during the onset of the inactive period (day in rats) (6). The 100-mg cort pellets produced an average plasma cort concentration of 11.8 ± 1.3 μg/dl (n = 21) in the morning and 12.1 ± 1.9 μg/dl (n = 20) in the evening in conscious rats. Morning plasma cort in control rats was 1.4 ± 0.9 μg/dl (n = 28). This is a normal basal plasma concentration for cort, indicating that the method of blood sampling did not stimulate cort release within the time required to obtain the sample (6). Evening plasma cort was not measured in the control rats. Other investigators have established that normal evening plasma cort concentration in rats averages 15–20 μg/dl (6). The physiological efficacy of the cort treatment was evident in the thymus and adrenal weights. Thymus weight was significantly reduced in the cort compared with the control rats (1.01 ± 0.07 vs. 1.32 ± 0.07 mg/kg body wt, respectively, P < 0.01). Similarly, adrenal weight was less in cort vs. control rats (0.09 ± 0.01 vs. 0.14 ± 0.01 mg/kg body wt, respectively, P < 0.01). After anesthesia and surgery, plasma cort increased to 33.9 ± 2.8 μg/dl in the rats not treated with cort (n = 14, control and time control groups combined; P < 0.01 relative to morning values in conscious control rats by between-subjects 1-way ANOVA). Plasma cort did not increase significantly in cort-treated rats in response to anesthesia and surgery, averaging 14.3 ± 5 μg/dl in the seven cort rats in which it was measured (P = 0.43 relative to morning values in conscious cort rats by between-subjects 1-way ANOVA). Figure 1 shows average plasma cort values for experiments in which all three plasma samples were assayed. All the values from cort-treated rats were combined. Values for plasma cort in the time control rats decreased during the experiment (P = 0.02) as the effect of surgical stress diminished with time and the cort that was released in response to the anesthesia and surgery caused feedback inhibition of additional cort secretion (6). In contrast, control rats treated with the cort type II receptor antagonist Mifepristone did not show this decrease in plasma cort with time (P = 0.20); presumably, this was due to the blockade by the antagonist of feedback inhibition of cort release.

Baseline values for MAP, HR, RSNA, and baroreflex function curves were compared using combined data from all control (n = 14) and cort-treated rats (n = 12). Cort increased baseline MAP relative to control rats (128 ± 1 vs. 115 ± 2 mmHg, P < 0.01). HR tended to be higher in the cort rats (424 ± 8 beats/min) than in control rats (389 ± 9 beats/min), but the difference was not significant (P = 0.057). There was no difference in baseline RSNA as a percentage of maximum between the two groups (74.6 ± 2.9 vs. 73.9 ± 4% for control vs. cort, P = 0.83). A comparison of absolute values for RSNA could not be made, because the data are normalized. The baseline baroreflex function curves for control and cort-treated rats are shown in Fig. 2. Cort increased the midpoint of the baseline baroreflex function curve from 124 ± 3 mmHg in control rats to 139 ± 3 mmHg in cort-treated rats, shifting the curve to the right (P < 0.01). In addition, cort decreased the gain coefficient of the baseline baroreflex function curve from 0.172 ± 0.026 in control rats to 0.103 ± 0.008 in cort-treated rats (P = 0.027).

Values for MAP, HR, and RSNA during the experiment are provided for each experimental group in Table 1. Mifepristone significantly decreased arterial pressure in control and cort rats. Arterial pressure did not change significantly in the time control group. In the cort + PE rats, MAP was maintained at baseline by an infusion of phenylephrine or inflation of an occluder. There were no changes in HR or RSNA over the course of the experiment in any experimental group.

The baroreflex function curves for control + Mifepristone rats are shown in Fig. 3. In control rats,
Mifepristone significantly decreased the midpoint of the baroreflex function curve by 3 h, shifting the curve to the left (P < 0.014, Figs. 3 and 4, B). The gain coefficient of the baroreflex function curve was not significantly affected by administration of Mifepristone in these rats (P > 0.50, Figs. 3 and 4, A). Blockade of the acutely elevated plasma cort in control rats could have explained the decrease in midpoint after administration of Mifepristone. However, there was no significant correlation between plasma cort during the baseline period and either the baseline curve midpoint (r² = 0.002) or the decrease in the curve midpoint observed 3 h after Mifepristone (r² = 0.01).

In cort-treated rats, Mifepristone decreased the midpoint of the baroreflex function curve by 2 h (P < 0.01) and, in contrast to control rats, Mifepristone also increased the gain coefficient of the baroreflex function curve within 2 h (P = 0.011, Figs. 4 and 5). Because these changes in the baroreflex function curve with Mifepristone occurred comitantly with a reduction in MAP, it was important to determine if the changes in the baroreflex function were dependent on the reduction in pressure. Therefore, an additional experimental group was studied in which cort-treated rats were given Mifepristone, then pressure was maintained at baseline by the intravenous infusion of phenylephrine (n = 4) or the inflation of an abdominal aortic occluder (n = 1). The baroreflex function curve parameters for the gain coefficient and arterial pressure midpoint are provided in Fig. 4, and the curves are illustrated in Fig. 6. The gain coefficient was significantly increased at 2 h (P = 0.011); however, the increase in gain at 3 h did not reach statistical significance (P = 0.071). The arterial pressure midpoint was significantly reduced by 3 h (P = 0.012). In Fig. 6 it can be seen that the curves at 2 and 3 h after Mifepristone are

Table 1. Average values for mean arterial pressure, heart rate, and renal sympathetic nerve activity during the baseline period and 2 and 3 h after Mif or vehicle

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 h</th>
<th>3 h</th>
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<tbody>
<tr>
<td><strong>Mean arterial pressure, mmHg</strong></td>
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<tr>
<td>Cort + Mif</td>
<td>127 ± 2</td>
<td>114 ± 3⁰</td>
<td>110 ± 3⁰</td>
</tr>
<tr>
<td>Cort + PE</td>
<td>129 ± 1</td>
<td>127 ± 1</td>
<td>127 ± 2</td>
</tr>
<tr>
<td>Control + Mif</td>
<td>116 ± 2</td>
<td>107 ± 7⁰</td>
<td>104 ± 5⁰</td>
</tr>
<tr>
<td>Time control</td>
<td>114 ± 3</td>
<td>112 ± 4</td>
<td>112 ± 5</td>
</tr>
<tr>
<td><strong>Heart rate, beats/min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cort + Mif</td>
<td>426 ± 11</td>
<td>420 ± 10</td>
<td>430 ± 9</td>
</tr>
<tr>
<td>Cort + PE</td>
<td>421 ± 11</td>
<td>404 ± 8</td>
<td>420 ± 9</td>
</tr>
<tr>
<td>Control + Mif</td>
<td>385 ± 6</td>
<td>373 ± 12</td>
<td>384 ± 7</td>
</tr>
<tr>
<td>Time control</td>
<td>392 ± 17</td>
<td>399 ± 19</td>
<td>397 ± 21</td>
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<tr>
<td><strong>Renal sympathetic nerve activity, %</strong></td>
<td></td>
<td></td>
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<tr>
<td>Cort + Mif</td>
<td>70 ± 4</td>
<td>78 ± 8</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>Cort + PE</td>
<td>79 ± 6</td>
<td>63 ± 12</td>
<td>73 ± 12</td>
</tr>
<tr>
<td>Control + Mif</td>
<td>73 ± 5</td>
<td>74 ± 10</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>Time control</td>
<td>76 ± 3</td>
<td>68 ± 6</td>
<td>64 ± 9</td>
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Values are mean ± SE. PE, phenylephrine. Cort, corticosterone; Mif, mifepristone. *P < 0.05 relative to baseline.
actually quite similar. The results from the experiment using the occluder were similar to the rest of the animals in the group. The gain coefficient increased from a baseline of 0.0815 to 0.1053 at 2 h and then decreased to 0.0711 at 3 h. Similarly, four of five rats in this group had a value for the gain coefficient that was lower 3 h after Mifepristone than 2 h after the antagonist. The pressure midpoint in the experiment with the occluder decreased from 144 mmHg during the baseline period to 134 mmHg 3 h after Mifepristone. The midpoint was not decreased at 2 h after Mifepristone (146 mmHg), as was true for another rat in this group. Figures 4 and 7 illustrate that the baroreflex control of RSNA did not change significantly in the time control group after administration of vehicle (P = 0.65 for gain coefficient and P = 0.15 for pressure midpoint). The range of RSNA from minimum to maximum (P1) and the minimum RSNA (P4) did not change significantly during the experiment in any experimental group (Table 2).

**DISCUSSION**

Glucocorticoids are essential for normal cardiovascular function, but when present in excess they produce hypertension (13). Glucocorticoids are commonly prescribed for a variety of clinical indications. Additionally, endogenous production of glucocorticoids can be increased by stress or by glucocorticoid-secreting tumors. There is also growing evidence that glucocorticoids contribute to the pathogenesis of essential hypertension (5, 43, 51, 52, 54). However, the mechanisms of glucocorticoid-induced hypertension remain uncertain, and the effects of glucocorticoids on neural control of the circulation are poorly understood. The baroreceptor reflex is a primary component of neural mechanisms of arterial pressure regulation (46). Therefore, the present study was performed to test the hypothesis that glucocorticoids modulate baroreceptor reflex control of RSNA. Treatment of rats with cort increased baseline arterial pressure from 115 ± 2 to 128 ± 1 mmHg, without significantly affecting baseline HR. Cort also decreased the gain and increased the mid-point of the baroreflex function curve for RSNA (Fig. 2). Administration of the glucocorticoid type II receptor antagonist Mifepristone to cort-treated rats increased the gain and decreased the midpoint of the baroreflex function curve for RSNA and decreased arterial pressure (Figs. 4 and 5 and Table 1). Thus blockade of the glucocorticoid type II receptors in cort-treated rats shifted the baroreflex function curve for RSNA back toward values observed in the control rats. Importantly, Mifepristone increased the gain and decreased the midpoint of the baroreflex function curve for RSNA in cort-treated rats even when arterial pressure was maintained constant throughout the experiment (Figs. 4 and 6 and Table 1). In control rats, Mifepristone also decreased arterial pressure and the baroreflex function curve midpoint (Figs. 3 and 4 and Table 1). However, in control rats, Mifepristone had no effect on the gain of
the baroreflex function curve, and the effect on the midpoint was not as rapid and was more variable compared with that observed in the cort-treated rats. Therefore, these results demonstrate for the first time that chronic elevations in cort increase the midpoint and decrease the gain of baroreceptor reflex control of RSNA by a mechanism that is, in part, independent in its effects to increase arterial pressure.

In these experiments, plasma cort concentration was increased by the established method of subcutaneous administration of cort pellets (1, 39). Under normal conditions, the average plasma cort concentration averaged over 24 h is in the range of 5–7 μg/dl. The cort-treated rats in this study had plasma levels clamped at 12 μg/dl, or about two times the normal value. This is lower than values for cortisol (the primary glucocorticoid in humans) reported for patients with Cushing’s Syndrome (55). Therefore, the cort treatment used in this study produced plasma glucocorticoid concentrations within the range observed clinically. However, the acute anesthesia and surgery increased plasma cort concentration in the control rats to 34 ± 3 μg/dl. Importantly, a similar acute increase in cort was not observed in cort-treated rats, because the exogenous administration of cort inhibited the acute increase in cort secretion that was seen in the control rats (6). Therefore, the effects of glucocorticoid receptor blockade observed in the cort-treated rats could not have been due to the blockade of effects of acutely elevated cort. Glucocorticoid receptor blockade was achieved using the glucocorticoid receptor antagonist Mifepristone. The dose chosen has been previously used in this laboratory and is in the range of doses reported by others (15, 39).

Table 2. Average values for P1 and P4 during the baseline period and 2 and 3 h after Mif or vehicle

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 h</th>
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<tbody>
<tr>
<td><strong>Range of renal sympathetic nerve activity (P1)</strong></td>
<td></td>
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<tr>
<td>Cort + Mif</td>
<td>100.8 ± 0.7</td>
<td>101.8 ± 0.7</td>
<td>101.4 ± 12.5</td>
</tr>
<tr>
<td>Cort + PE</td>
<td>101.9 ± 1.9</td>
<td>79.9 ± 6.8</td>
<td>104.4 ± 14.3</td>
</tr>
<tr>
<td>Control + Mif</td>
<td>101.0 ± 1.1</td>
<td>86.5 ± 10.7</td>
<td>93.4 ± 10.7</td>
</tr>
<tr>
<td>Time control</td>
<td>100.4 ± 0.7</td>
<td>99.2 ± 8.6</td>
<td>101.1 ± 12.6</td>
</tr>
<tr>
<td><strong>Minimum renal sympathetic nerve activity (P4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cort + Mif</td>
<td>−0.7 ± 0.6</td>
<td>−0.2 ± 0.5</td>
<td>−0.7 ± 1.0</td>
</tr>
<tr>
<td>Cort + PE</td>
<td>−3.7 ± 1.5</td>
<td>−2.1 ± 1.5</td>
<td>−0.8 ± 0.8</td>
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<tr>
<td>Control + Mif</td>
<td>−1.8 ± 1.1</td>
<td>−0.0 ± 1.3</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>Time control</td>
<td>0.3 ± 0.8</td>
<td>−0.2 ± 1.0</td>
<td>−0.5 ± 0.7</td>
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</tbody>
</table>

Values are means ± SE.

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Fig. 6. A: average baroreflex function curves (mean ± SE) for RSNA in cort rats given Mif at the end of the baseline period. Arterial pressure was then maintained at baseline throughout the experiment. B: curves constructed from average parameter values. For clarity, only the baroreflex curves determined during the baseline period (●) and 3 h after Mif (x) are shown in A. ●. Values for arterial pressure and RSNA just before the baseline and 3-h curves.

Fig. 7. A: average baroreflex function curves (mean ± SE) for time control rats given vehicle at the end of the baseline period. B: curves constructed from average parameter values. For clarity, only the baroreflex curves determined during the baseline period (●) and 3 h after Mif (x) are shown in A. ●. Values for arterial pressure and RSNA just before the baseline and 3-h curves.
these techniques are associated with problems. Inflation of the occluder reduces renal perfusion pressure and thus stimulates the renin-angiotensin system, and angiotensin decreases the gain of baroreflex function (2). Thus use of the occluder to maintain pressure could mask effects of Mifepristone to increase the gain and decrease the midpoint of the baroreflex function. The use of an infusion of phenylephrine could have the opposite effect. Imaizumi et al. (18) reported that intravenous infusion of phenylephrine for several minutes increased the gain of the baroreceptor reflex curve. The doses of intravenous phenylephrine infused during baroreflex function determination are not clearly stated. However, the infusion of phenylephrine used for intravenous infusion in those experiments increased MAP by \(\sim 50–75\) mmHg. In the present study, Mifepristone decreased arterial pressure in cort rats from \(127 \pm 2\) to \(110 \pm 3\) mmHg, a difference of just 17 mmHg. Thus the phenylephrine infusion in the present study would have increased arterial pressure by only an approximate average of 17 mmHg and was presumably a much lower dose than that used by Imaizumi et al. Furthermore, Imaizumi et al. reported that the effect of phenylephrine was greatest at low carotid sinus pressures. In contrast, in the present study Mifepristone combined with a phenylephrine infusion had the greatest effect on baroreflex function at higher carotid sinus pressures (Fig. 6). Finally, other investigators have failed to demonstrate an effect of intravenous phenylephrine to increase the slope of the baroreceptor reflex (2). Overall, it would appear that in these experiments the infusion of phenylephrine was preferable to the use of the occluder. Importantly, the data from the one experiment with the occluder were similar to the data from the four experiments using the phenylephrine infusion.

In control rats, Mifepristone significantly decreased arterial pressure within 2 h and lowered the baroreceptor reflex midpoint within 3 h. Two possible mechanisms can account for this reduction in midpoint in the control rats. First, in contrast to the effects of Mifepristone in cort-treated rats, it is possible that these effects of glucocorticoid receptor blockade in control rats were due to blockade of actions of acutely elevated cort. However, there was no significant correlation between plasma cort levels during the baseline period and the effect of Mifepristone on the shift in the midpoint in the control rats. Thus the data do not strongly indicate that there is an acute effect of cort on the baroreflex function in these experiments. Second, it is possible that the shift in the midpoint of the baroreflex function curve in the control rats was due to the reduction in arterial pressure after Mifepristone administration to control rats. The likelihood of this possibility is increased by the fact that the reduction in arterial pressure, which was significant at 2 h (Table 1), preceded the decrease in the midpoint, which was not significant until 3 h (Fig. 4B). It is important to note from Fig. 3A, that although the shift in the calculated midpoint is statistically significant, the variability in the data brings into question its physiological significance. Therefore, the relative importance of the two possible mechanisms in mediating the decrease in midpoint was not examined. In the present study, Mifepristone was administered by subcutaneous injection, thus eliminating the possibility of detecting very rapid actions of the antagonist on cardiovascular control. Intravenous administration was originally attempted, but all vehicles tested had significant effects on arterial pressure and RSNA. The appropriate way to determine the rapid effects of cort on baroreflex function would be to perform experiments in conscious rats with basal cort levels and then intravenously administer a controlled amount of cort.

The results of the present experiments suggest that the actions of cort on arterial pressure and on the midpoint and gain of the baroreceptor reflex function curve can be reversed more rapidly than they are established. Even after anesthesia and surgery increased baseline cort in the control rats, the comparison of baseline baroreflex curves demonstrated an increase in arterial pressure and modulation of baroreflex curve in the cort-treated rats relative to the control rats. Yet, administration of Mifepristone to cort-treated rats returned arterial pressure and baroreflex function back toward that of the baseline values observed in control rats within 2 h. One possible explanation is that cort increases the synthesis of a protein, for example a neurotransmitter receptor subunit, with a very short half-life or one that is subject to rapid membrane turnover. Another possibility is that cort increases the synthesis of a receptor mediating the rapid actions of cort. It would take hours to days to increase receptor concentrations sufficiently to see an effect, but blocking the upregulated receptors could rapidly reverse the effect. Others have reported that Mifepristone reduced or blocked rapid effects of glucocorticoids on neuronal activity (4, 33).

The effect of cort on baseline RSNA cannot be determined from the results of the present study. Other studies have reported effects of cort on resting sympathetic nerve activity. Acute central administration of cortisol increased baseline RSNA in rats (49) and increased the RSNA response to stimulation of the RVLM after endotoxin-induced hypotension in rabbits (22). Prenatal glucocorticoid treatment potentiated the RSNA response to birth in preterm sheep (42). In contrast, acute or chronic (7 day) treatment of humans with prednisone that either decreased or tended to decrease arterial pressure also decreased lumbar sympathetic nerve activity (12, 27). No mechanism for the decrease in arterial pressure produced by the prednisone was suggested. Dodt et al. (9) showed that a 3-h infusion of hydrocortisone in humans decreased arterial pressure but did not lower resting muscle sympathetic nerve activity. However, because nerve activity increased in the control subjects, they concluded that hydrocortisone decreased nerve activity. Sherrer et al. (37) reported that 48 h of dexamethasone treatment in humans changed neither resting blood pressure nor resting muscle sympathetic nerve activity. In the present experiments, cort increased baseline MAP and
tended to increase HR, although the effect on HR did not reach statistical significance ($P = 0.057$). There was no difference in baseline RSNA between control and cort-treated rats. However, RSNA is normalized in each animal to 100%, and comparison of absolute values for RSNA between groups is not possible. [Note that in the 4-parameter logistic function, each parameter is independent. Therefore, the gain index (P2) and midpoint (P3) are independent of the range normalization and can be compared between groups.] Treatment with Mifepristone decreased arterial pressure at both 2 and 3 h in both control and cort-treated rats, but had no significant effects on baseline HR or RSNA. It is possible that an effect of glucocorticoid receptor blockade on baseline values for HR or RSNA would have been observed at a later time point. Clearly, additional studies are required to determine the effects of glucocorticoids on resting sympathetic nerve activity. Important differences may be found between sympathetic nerves innervating specific vascular beds and/or other tissues.

In the study by Sherrer et al. (37), dexamethasone pretreatment eliminated the effect of insulin-induced hypoglycemia to increase muscle sympathetic nerve activity. The increase in muscle sympathetic nerve activity in control subjects was accompanied by muscle bed vasodilation that was also eliminated by glucocorticoid pretreatment. Similarly, Davis et al. (8) reported that pretreatment with cortisol blunted the sympathoexcitatory effects of insulin-induced hypoglycemia. The results from the present study demonstrate that 2–3 wk treatment with glucocorticoids can decrease the gain and increase the midpoint of baroreceptor reflex control of RSNA. There is a widely proposed hypothesis, based largely on measurements of plasma catecholamines, that glucocorticoids have an acute effect to restrain the response of the sympathetic nervous system to stress (25). The attenuation of sympathetic nerve activity responses by glucocorticoids is generally thought to be protective, eliminating a positive feedback effect of stress on sympathetic outflow. However, in the case of insulin-induced hypoglycemia, when excitation of muscle sympathetic nerve activity is accompanied by vasodilation (37), the blunting or elimination of sympathetic excitation is not beneficial. Similarly, with the baroreceptor reflex, attenuation of reflex control of sympathetic nerve activity by glucocorticoids could be detrimental by contributing to the maintenance of hypertension that is produced by glucocorticoids or by other mechanisms.

**Perspectives**

This research demonstrates for the first time that glucocorticoids modulate baroreflex control of RSNA. The cort treatment used produced plasma concentrations of glucocorticoids within the range seen clinically in humans (55). Hypertension remains a leading risk factor for cardiovascular disease, morbidity, and mortality. The mechanisms leading to the development of essential hypertension are still being discovered. It has been demonstrated that glucocorticoids are required for the development of genetic hypertension in rats (17) and that abnormalities of glucocorticoid receptors, metabolism, and function are found in essential hypertension in humans (5, 43, 51, 52, 54). Furthermore, chronic stress, an accepted risk factor for hypertension, produces elevated glucocorticoids. Recently, it has become evident that brief prenatal exposure to glucocorticoids results in both elevated glucocorticoids and increased risk for hypertension in adulthood (41). Thus the effects of glucocorticoids to decrease the gain and increase the pressure midpoint of the baroreflex function curve could provide an important contribution to the etiology of human essential hypertension from both a genetic and environmental perspective.

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**REFERENCES**

GLUCOCORTICOIDS MODULATE THE BARORECEPTOR REFLEX


