Glucocorticoids act in the dorsal hindbrain to increase arterial pressure

Deborah A. Scheuer,1 Andrea G. Bechtold,1 Sylvan S. Shank,1 and Susan F. Akana2

1Department of Pharmacology, University of Missouri, Kansas City, Missouri 64108; and 2Department of Physiology, University of California, San Francisco, California 94143-0444

Submitted 27 August 2003; accepted in final form 22 September 2003

Scheuer, Deborah A., Andrea G. Bechtold, Sylvan S. Shank, and Susan F. Akana. Glucocorticoids act in the dorsal hindbrain to increase arterial pressure. Am J Physiol Heart Circ Physiol 286: H458–H467, 2004. First published September 25, 2003; 10.1152/ajpheart.00824.2003.—Glucocorticoid receptors (GRs) are present at a high density in the nucleus of the solitary tract (NTS), an area of the dorsal hindbrain (DHB) that is critical for blood pressure regulation. However, whether these receptors play any role in the regulation of blood pressure is unknown. We tested the hypothesis that glucocorticoids act in the DHB to increase arterial pressure using two experimental strategies. In one approach, we implanted pellets of corticosterone (Cort) or sham pellets onto the DHB over the NTS. Compared with rats with sham pellets, rats with DHB Cort pellets had an increased (P < 0.05) mean arterial pressure (111 ± 2 vs. 104 ± 1 mmHg) and heart rate (355 ± 9 vs. 326 ± 5 beats/min) after 4 days. In the second approach, we implanted subcutaneous Cort pellets to increase the systemic Cort concentration and then subsequently implanted pellets of the GR antagonist mifepristone (Mif; previously RU-38486) or sham pellets onto the DHB. Two days of DHB Mif treatment reduced (P < 0.05) mean arterial pressure in those rats with elevated plasma Cort levels (118 ± 2 vs. 108 ± 1 mmHg for sham vs. Mif DHB pellets). Cort and Mif pellets placed on the dura had no effects on arterial pressure or heart rate, ruling out systemic cardiovascular effects of the steroids. The DHB Cort treatment had no effects on plasma Cort concentration or adrenal weight, indicating that the contents of the DHB Cort pellet did not diffuse into the systemic circulation or into the forebrain areas that regulate plasma Cort concentration in concentrations sufficient to produce physiological effects. Immunohistochemistry for the occupied GRs demonstrated that the Cort and Mif from the DHB pellets were delivered to the DHB with minimal diffusion to the ventral hindbrain or forebrain. We conclude that glucocorticoids act in the DHB to increase arterial pressure.

corticosterone; nucleus of the solitary tract; brain; hypertension

GLUCOCORTICOIDS ARE IMPORTANT for the maintenance of normal baseline arterial pressure in both rats and humans. Adrenal insufficiency or blockade of type II glucocorticoid receptors (GRs) can lower blood pressure (18, 22, 61). The effects of adrenalectomy to lower blood pressure can be reversed by glucocorticoid, but not mineralocorticoid, replacement (61). It is also established that elevated glucocorticoids produce dose-related increases in arterial pressure, and sufficiently high levels of glucocorticoids, due to either exogenous administration or endogenous overproduction, cause hypertension in both experimental animals and humans (22, 43, 44, 54). Long-term administration of glucocorticoids, commonly prescribed because of their anti-inflammatory and immunosuppressive therapeu tic effects, results in a 20% incidence of iatrogenic hypertension (23). There is also increasing evidence that prolonged mild elevations in glucocorticoids or increased sensitivity to the actions of glucocorticoids promote the development and maintenance of essential hypertension. Several studies have reported elevations in plasma and/or urinary glucocorticoids in essential hypertension (35, 55, 67, 68). Advances in medical imaging have led to the unexpected discovery of small adrenal adenomas, termed “incidentalomas,” in 2–9% of patients undergoing imaging for reasons other than symptoms of cortisol excess. Investigation of this patient population revealed a significant increase in plasma cortisol, impaired feedback inhibition of cortisol release, and a 48% incidence of mild-to-severe essential hypertension (42). The fact that not all studies of hypertensive patients report significant increases in glucocorticoids is likely due to the fact that multiple genetic and environmental factors contribute to the etiology of essential hypertension, such that the causes of hypertension vary among patient populations. Additionally, the protocols used to measure plasma glucocorticoids can influence the results. Glucocorticoid plasma concentrations vary by the time of day, stress level, and food intake, so plasma glucocorticoids must be measured using a controlled and consistent protocol to detect between-group differences (11, 27). Even in the absence of measured elevations in glucocorticoid concentrations, alterations in glucocorticoid metabolism that can lead to increased tissue exposure to glucocorticoids have been reported in essential hypertension (10, 55, 66, 68). Finally, prenatal exposure to elevated glucocorticoids increases both glucocorticoid levels and the risk of hypertension in adulthood (6, 49). Data from experimental animals further support a role for glucocorticoids in the development of hypertension (8, 25, 26, 38, 57, 65).

There are also genetic links between GRs and human hypertension. Watt et al. (67) reported that 27% of a group of patients with essential hypertension whose parents also had essential hypertension were homozygous for a 4.5-kb allele of the GR gene. This allele is also associated with insulin resistance, an increased body mass index, and elevated cortisol levels (41, 62, 67). Other variants of the GR gene have been associated with hypertension and/or coronary artery disease (30, 31).

The mechanism of glucocorticoid-mediated effects on blood pressure remains both controversial and poorly understood. Most work has focused on the effects of glucocorticoids in the periphery (7, 63). These studies demonstrated that glucocorticoids can modulate vascular reactivity to increase peripheral resistance. However, it is not clear that these vascular changes...
alone can account for the hypertensive effects of glucocorticoids. Little is known regarding the effects of glucocorticoids on central nervous system (CNS) regulation of blood pressure. Several investigators have administered glucocorticoid agonists by intracerebroventricular infusion (21, 60) or a single injection (58, 64) and measured arterial pressure, but the results have been inconsistent. No studies have combined chronic delivery of glucocorticoids or GR antagonists to specific areas in the CNS with the measurement of blood pressure or heart rate.

We selected the dorsal hindbrain (DHB) as the focus of the present study because of its key role in blood pressure regulation (56). For example, the baroreceptor reflex is an essential mechanism of blood pressure regulation that is mediated by a pathway that includes DHB structures (56), and systemic elevations in glucocorticoids attenuate the buffering capacity of the baroreceptor reflex (46, 48, 50–52). Furthermore, GRs are present in the DHB, particularly in the nucleus of the solitary tract (NTS), and the catecholaminergic neurons of the NTS contain one of the highest densities of GRs in the brain (1, 16, 24).

The present study used a new technique, developed in our laboratory, of chronic localized application of the endogenous glucocorticoid corticosterone (Cort) or the GR antagonist mifepristone (Mif) to the DHB to test two interrelated hypotheses. The first hypothesis was that chronic elevations in DHB glucocorticoid levels increase arterial pressure. The second hypothesis was that chronic blockade of DHB GRs would decrease arterial pressure in rats with glucocorticoid-induced elevations in arterial pressure. Glucocorticoids bind to two distinct receptor types: type I or mineralocorticoid receptors (MRs), which also bind aldosterone, and type II or GRs, which are selective for glucocorticoids (39). We focused on the actions of Cort mediated by the GR, because we studied effects of increases in Cort and the high-affinity MRs are almost fully occupied at even low levels of Cort (32).

METHODS

Data were obtained from 72 male Sprague-Dawley rats purchased from Charles River Laboratories at an initial weight of 250–275 g. The rats were housed at the University of Missouri-Kansas City and given free access to food and water. The Institutional Animal Use and Care Committee of the University of Missouri-Kansas City approved all procedures.

Hindbrain pellets. Pellets of Cort (Sigma or ICN Biomedicals) or Mif (Sigma) were made by gently melting the steroid, pouring it onto a rubber surface, and carving the hardened steroid into a pellet of the approximate dimensions of 2 × 1.8 × 1.2 mm (length × width × height). Sham pellets were made of Silastic (Kwik-sil, World Precision Instruments) or cholesterol (Sigma). The pellets weighed between ~3 and 4 mg.

Surgical procedures. All surgical procedures were performed using aseptic techniques under a surgical plane of anesthesia as indicated by the absence of a withdrawal reflex to pinch of the hindpaw and of any reflex response to surgical manipulation. For the first surgical procedure, rats were anesthetized with inhaled isoflurane (Aerrane, Henry Schein), and systemic increases in Cort were produced by subcutaneous implantation of two Cort pellets weighing ~100 mg each using a previously described technique (53). Sham surgery was performed in rats that did not receive systemic Cort. Ten to eleven days later, rats were anesthetized with Domitor (0.5 mg/kg ip medetomidine hydrochloride; Pfizer Animal Health, Exton, PA) and ketamine hydrochloride (75 mg/kg ip) (13), and prophylactic antibiotic was administered (Pro-Pen-G; 60,000 U/kg im, Henry Schein). An arterial catheter was implanted into the femoral artery and exteriorized at the neck as previously described (46). Each animal was then positioned in a stereotaxic apparatus with its head ventroflexed at a 60° angle, and a medial incision was made to expose the surface of the dura. A small hole was made through the atlantooccipital membrane to visualize the dorsal surface of the hindbrain including the area postrema. After the pia mater was removed, the pellet was positioned on the midline with approximately two-thirds of its width rostral to the calamus scriptorius. The bottom surface of the pellet was first coated with a thin layer of mineral oil to provide a lipophilic interface between the pellet and the dorsal surface of the brain. After placement on the brain surface, the pellet was covered with a small drop of surgical glue (Vetbond, Henry Schein) to keep it in place, followed by a small drop of silicone gel (Kwik-Sil, World Precision Instruments) to limit diffusion of the pellet contents into the cerebral spinal fluid. After the pellet was in place and covered with glue and silastic gel, a small piece of cellulose membrane (Data Sciences International) was glued over the hole in the atlantooccipital membrane, and the skin incision was sutured closed. To control for systemic diffusion of Cort and Mif from the DHB pellets, in some animals Cort or Mif pellets were implanted on the surface of the dura. A small drop of surgical glue was used to hold the dura pellet in place. Each animal was given Antisedan (1 mg/kg sc atropine sulfate hydrochloride; Pfi floridex, Green Valley Pharmaceutical) to reverse the anesthesia. Separate groups of rats were used for immunohistochemical detection of the occupied GRs to visualize the spread of Cort and Mif from DHB pellets. These rats were adrenalectomized to eliminate endogenous Cort. Adrenalectomy was performed under isoflurane anesthesia through a small dorsal incision. Rats were then given both 0.45% saline and 7% sucrose to drink ad libitum.

Experimental protocol. Each morning for 4 days after the implantation of the DHB pellets and arterial catheters, rats were brought to the laboratory, usually between 8:30 and 9:30 AM, in their home cages. The arterial catheter was connected to a transducer via extension tubing with enough slack so that the animal was free to move about the cage. The signal from the transducer was processed using a bridge amplifier and MacLab data-acquisition system. Mean arterial pressure and heart rate were calculated online. The rats were allowed a minimum of 2 h to acclimate to the laboratory setting, and the cardiovascular data used for analysis were then collected for 1 h. These experiments were performed in seven groups of rats. Four of the seven groups of rats received systemic sham treatment, whereas three groups of rats received systemic Cort treatment. Systemic sham treatment was combined with 1) DHB sham, n = 11; 2) Cort, n = 9; 3) DHB Mif, n = 8; or 4) dura Cort, n = 7. Systemic Cort treatment was combined with 1) DHB sham, n = 12; 2) DHB Mif, n = 8; or 3) dura Mif, n = 6.

We used the systemic Cort plus DHB sham treatment group to determine whether the DHB sham pellet had a nonspecific effect on arterial pressure and heart rate, so this group consisted of rats with three different sham treatments: no pellet (n = 3), a cholesterol pellet (n = 4), and a Silastic pellet (n = 5). The results demonstrated that the type of sham treatment had no effect on the magnitude of the systemic Cort-induced increase in mean arterial pressure. For example, on day 4, mean arterial pressure averaged 117 ± 3, 119 ± 5, and 117 ± 2 mmHg in the no pellet, cholesterol, and Silastic DHB sham plus systemic Cort-treated rats, respectively. Therefore, all other sham pellets were made of Silastic, and the data in the systemic Cort plus DHB sham rats were analyzed as a single group regardless of the nature of the DHB sham treatment.

Plasma Cort measurements. In rats used for cardiovascular measurements, blood samples (200 μl each) were obtained from the arterial catheter on the evening of the third day and the morning of the fourth day after DHB pellet implantation. All samples were centrifuged at 4°C, and the plasma was stored at −20°C before being assayed. Plasma Cort was determined as previously described using a commercially available kit (ImmuChem double-antibody Cort 1125
Rats used for measurement of cardiovascular parameters weighed an average of 315 ± 5 g on the day systemic Cort pellets were implanted or sham surgery was performed, with no significant differences in body weight among experimental groups. On the day DHB or dura pellets and catheters were implanted, rats with systemic Cort treatment weighed significantly less (296 ± 8 g) than rats who had undergone sham surgery for systemic treatment (380 ± 5 g). There were no significant effects of DHB or dura Cort or Mif pellets on body weight relative to DHB sham pellets.

In rats with normal systemic Cort levels, DHB Cort significantly increased mean arterial pressure and heart rate relative to DHB sham treatment after 4 days (Fig. 1, P < 0.05). In contrast, the same Cort pellet placed on the dura had no effect on mean arterial pressure or heart rate, indicating that DHB Cort was not altering these variables due to leakage into the periphery. DHB Mif had no effect on arterial pressure or heart rate, suggesting that DHB GRs do not modulate baseline arterial pressure and heart rate under resting conditions during the day when glucocorticoid levels are near their nadir.
In rats with DHB sham treatment, systemic Cort treatment significantly increased mean arterial pressure ($P < 0.05$; Fig. 2A). DHB Mif significantly reduced mean arterial pressure in rats with systemic Cort treatment ($P < 0.05$). Mif pellets implanted on the dura had no effect on mean arterial pressure. There were no differences in heart rate among the groups (Fig. 2B) except that heart rate was significantly elevated in rats with systemic Cort plus DHB sham treatment on day 3.

Plasma Cort exhibits a diurnal rhythm, peaking at 15–20 µg/dl in the evening and having a nadir of 1–3 µg/dl in the morning. In the present study, all systemic sham pellet rats had normal morning plasma Cort concentrations, and all rats with systemic Cort treatment had elevated morning Cort levels (Fig. 3A). In the evening, DHB Mif increased the plasma Cort concentration in both systemic sham- and systemic Cort-treated rats. However, Mif placed on the dura did not (Fig. 3B). This indicates that Cort normally acts at GRs in the DHB to inhibit the hypothalamic-pituitary-adrenal axis during the peak of the diurnal concentration of glucocorticoids but not during the trough.

Adrenal weight is extremely sensitive to exogenous Cort. Akana et al. (3) have demonstrated that a low dose of exogenous Cort significantly decreases adrenal weight without producing a measurable increase in plasma Cort. This effect of Cort to reduce adrenal weight is mediated by Cort-induced feedback inhibition of adrenocorticotropic hormone, a hormone that stimulates proliferation of the adrenal cortex in addition to glucocorticoid secretion. In the present study, systemic Cort significantly decreased adrenal weight ($P < 0.05$ relative to systemic sham and DHB sham rats), whereas DHB pellets had no effects on adrenal weight (Fig. 4). These data show that the content of the DHB Cort pellet does not leak systemically to produce physiological effects. Interestingly, the same amount of Cort placed outside the brain on the surface of the dura produced a small, but significant, reduction in adrenal

Fig. 2. Mean arterial pressure (A) and heart rate (B) in rats with systemic Cort or sham treatment were measured for 4 days after implantation of DHB or dura pellets. Syst Cort significantly elevated arterial pressure relative to Syst sham treatment in rats with DHB sham pellets. DHB Mif, but not dura Mif, significantly reduced arterial pressure in rats with Syst Cort treatment. Heart rate was significantly elevated in rats with Syst Cort plus DHB sham treatment only on day 3. *$P < 0.05$ relative to the Syst sham + DHB sham group; †$P < 0.05$ relative to the Syst Cort + DHB sham group. Numbers of animals per data point are as follows: Syst sham + DHB sham, $n = 11$ on days 1 and 2 and $n = 9$ on days 3 and 4; Syst Cort + DHB sham, $n = 12$ on days 1–4; Syst Cort + DHB Mif, $n = 8$ on days 1–3 and $n = 7$ on day 4; and Syst Cort + Dura Mif, $n = 5$ on day 1 and $n = 6$ on days 2–4.

Fig. 3. Plasma Cort concentration in the morning (A) and evening (B) in all groups of rats. Syst Cort increased morning but not evening plasma Cort, whereas DHB Mif increased plasma Cort in the evening but not in the morning. *$P < 0.05$ relative to Syst sham + DHB sham rats.

*Fig. 2A, B*
weight, providing further evidence that the steroids in the DHB pellets are not diffusing out of the brain in sufficient quantities to produce physiological actions.

We performed immunohistochemistry of the occupied GRs to demonstrate that Cort and Mif are being delivered to the DHB from the DHB pellets and to estimate the extent of diffusion of the steroid beyond the DHB. Figure 5 shows a series of photomicrographs showing the DHB at the level of the area postrema from rats treated with a DHB sham, Cort, or Mif pellet for 4 days and from an adrenal-intact, stressed rat (positive control). The low-power photomicrographs (total magnification ×40) illustrate the rostral-caudal level at which the high-power photomicrographs were obtained. There was no specific staining for the GR observed in the area postrema. The high-power photomicrographs (total magnification ×200) illustrate specific staining for occupied GRs in the NTS in rats with Cort and Mif pellets and in the positive control, with no specific staining in the NTS of the DHB sham pellet rat. Figure 6 shows composite photomicrographs (magnification ×200) of the CA1 area of the hippocampus (left) and ventral hindbrain at the level of the area postrema (right). In both areas, only very limited or no nuclear staining was observed in the DHB-treated rats, whereas strong nuclear staining was observed in the positive control.

DISCUSSION

Comparison with previous studies. The results from these experiments demonstrate that chronic elevations in DHB glucocorticoid levels increase arterial pressure and that chronic blockade of DHB GRs decreases arterial pressure in rats with glucocorticoid-induced elevations in blood pressure. Existing information regarding the effects of glucocorticoids on CNS regulation of blood pressure is sparse and is based almost entirely on the results of intracerebroventricular administration of glucocorticoid agonists and antagonists (21, 58, 60, 64). Tonolo et al. (60) reported a small reduction in arterial pressure with intracerebroventricular infusion of an intermediate dose of dexamethasone (a selective GR agonist). However, Gomez-Sanchez et al. (21) observed no effect of a low dose of intracerebroventricular Cort infusion on baseline arterial pressure, although the increase in arterial pressure due to intracerebroventricular aldosterone was attenuated. Other studies reported that single injections of higher doses of Cort increased arterial pressure immediately (58) or 24 h later (64), and Takahashi et al. (58) also observed an immediate increase in renal sympathetic nervous activity. Single injections of Mif, at doses probably insufficient to antagonize GRs in vivo (34), produced small reductions in arterial pressure of highly variable onset and duration (64). Our results are not in agreement with some of these previous studies that have suggested that activation of GRs in the CNS leads to a reduction in arterial pressure. However, none of the previous studies measured baseline Cort in conscious animals, and only one provided peripheral Cort replacement (64) to control for changes in peripheral Cort secretion. None of these studies provided any estimation of the extent of drug delivery to the CNS. This is important because Cort and Mif are very lipophilic and have limited solubility in cerebral spinal fluid. All but one study (58) used the indirect tail-cuff method to measure blood pressure, which introduces an element of stress. The present study overcomes many of the difficulties with the previous work, demonstrating that glucocorticoids act in the DHB to increase arterial pressure.

Time course of effects of Cort and Mif. DHB Cort pellets required 4 days to significantly increase arterial pressure and heart rate, but DHB Mif required only 48 h to significantly decrease pressure in rats with systemic elevations in Cort, suggesting the possibility that Cort diffused beyond the DHB to exert its effects. However, our data do not support this explanation for the delayed effects of Cort. First, our immunohistochemical data indicate that the Cort is not diffusing to any large extent beyond the DHB. Second, the differential time course is consistent with our previous findings using systemic Cort and Mif administration. In experiments using radiotelemetry to measure arterial pressure, several days were required to reach a maximum increase in pressure in systemic Cort-treated rats, yet we have reported that Mif affected baroreflex function after several hours (unpublished observations and Refs. 46–48 and 53). Several possible mechanisms could account for these observations. The more rapid reversal of Cort effects by Mif could be explained by inhibition of a protein with a rapid turnover, such as a neurotransmitter receptor. The longer onset latency for the Cort-induced increase in pressure could be due to the requirement for an additional protein with a longer half-life.

Antagonizing actions of glucocorticoids with Mif. The GR antagonist Mif also blocks progesterone receptors (37). However, we have previously shown that adrenalectomy eliminates the effect of Mif on baseline arterial pressure and heart rate, indicating that in male Sprague-Dawley rats it is acting as a selective GR antagonist (46). Furthermore, in the present study, DHB Mif decreased baseline arterial pressure in rats with elevated systemic Cort but not in rats with normal systemic Cort levels, suggesting that Mif is selectively blocking the effects of Cort. It is possible that the actions of Cort in the DHB are also mediated in part by MRs. 11β-Hydroxysteroid dehydrogenase 2, an enzyme that limits the access of Cort to MRs by metabolizing Cort (15), is present in the NTS but does not appear to be entirely colocalized with MRs (39, 40).
Potential mechanisms of action for glucocorticoids within the DHB. Three structures in the DHB participate in cardiovascular regulation: the NTS, the area postrema, and the dorsal motor nucleus of the vagus (DMN). Within the DHB, we found no evidence that GRs are present in the area postrema but consistently observed intense staining for GRs in the NTS of the positive control rats, in agreement with previous studies (1, 16, 24). The DMN is positive for GR immunostaining (1). In cats, vagal efferents projecting to the heart from the DMN appear to primarily modulate contractility, whereas cardiac vagal efferents controlling heart rate originate in the more ventral nucleus ambiguus (20). If this is true in rats as well, glucocorticoids acting within the DHB to modulate cardiovascular control are most likely acting at GRs in the NTS, with some contribution from the DMN. However, further experiments are required to determine the site of action of Cort within the DHB.

Baroreceptor and other visceral afferents terminate in the NTS, where the information regarding prevailing arterial pressure is processed and integrated with information from other sensory afferents and central projections (12). Efferent projections from the NTS mediate reflex control of sympathetic nerve activity to the vasculature as well as sympathetic and vagal control of heart rate (4). We have previously reported that systemic increases in Cort elevate the midpoint and reduce the gain of the baroreceptor reflex. DHB Cort increased both arterial pressure and heart rate, suggesting the possibility that it produced alterations in baroreflex function similar to systemic Cort. In fact, we have reported preliminary findings that DHB Cort increases the midpoint of baroreflex control of heart rate (5). However, the idea that changes in baroreflex function can alter long-term regulation of blood pressure is controversial. Arterial baroreceptor denervation (SAD) rapidly increases resting arterial pressure and arterial pressure variability (56).
Several days after SAD, although arterial pressure variability remains high, average arterial pressure returns to near normal, a fact that has been interpreted by many investigators as evidence that the arterial baroreceptor reflex does not play a role in the long-term regulation of arterial pressure. However, it is now accepted that the CNS has neuroplasticity, and the data can be interpreted to mean there are central pathways that can compensate for the absence of arterial baroreceptor afferent input (9). The existence of baroreceptor “resetting” has also been used as evidence that the arterial baroreceptor reflex does not participate in long-term regulation of arterial pressure. Resetting refers to the process whereby the midpoint of the baroreceptor reflex adjusts to the prevailing pressure both acutely and chronically. However, as pointed out by Sun (56), the development and maintenance of established hypertension requires resetting or some other change in function. Otherwise, the reflex could prevent or significantly attenuate even sustained increases in arterial pressure. Direct evidence that the arterial baroreceptors can initiate and sustain an increase in arterial pressure has recently been provided by Thrasher (59), who reported that continuous unloading of the arterial baroreceptors in dogs for 7 days produced a sustained hypertension. It is possible that prolonged modulation of baroreceptor-related neuronal activity by glucocorticoids in the NTS could also increase arterial pressure. Alternatively, the glucocorticoids could be acting in DHB neurons that are unrelated to baroreflex function. For example, glucocorticoids could activate ascending projections from the NTS that, in turn, modulate the activity of descending projections to the rostral ventral lateral medulla. Further studies are required to determine how activation and blockade of DHB GRs modulate baseline arterial pressure and the role of DHB GRs in neural control of the circulation in response to changes in blood pressure and other perturbations. Additional experiments are also needed to de-

**Fig. 6.** *Left:* photomicrographs of the CA1 area of the hippocampus at approximately −3.5 mm from the bregma. *Right:* sections of the hindbrain at the level of the ventral lateral medulla at the level shown in Fig. 5. All images are at a total magnification of ×200 and magnification bars equal 100 μm. The calibration bar in the top right photomicrograph has been enhanced for improved visibility. The experimental treatments are DHB sham, Cort, and Mif pellets and a positive control (from top to bottom).
terminate whether the effects of DHB GR on arterial pressure regulation are sustained for a longer time than was examined in the present study.

Spread of Cort and Mif from DHB pellets. The experiments were performed using a new method of local application of Cort and Mif to the DHB. We have presented several pieces of evidence that the Cort and Mif from the DHB pellets is not producing effects due to diffusion of steroid into the periphery. First, pellets placed on the dura underneath the overlying muscles had no effects on arterial pressure or heart rate. Second, Cort pellets placed on the DHB did not increase the morning plasma Cort concentration, as would have been expected if the Cort was diffusing into the peripheral circulation. Third, DHB Cort did not decrease adrenal size, as would have been expected if the Cort from the DHB had diffused systemically. The high sensitivity of adrenal size to increases in exogenous systemic Cort has been reported previously (3) and was demonstrated in this study by the reduction in adrenal size in the rats with systemic Cort and the rats with systemic sham plus dura Cort pellets. Fourth, DHB Mif, but not dura Mif, increased the evening (peak) plasma Cort concentration. Others (17) have reported that systemic administration of Mif in humans disinhibits the hypothalamic-pituitary-adrenal axis during the peak, but not the trough, of plasma cortisol secretion. This is the first demonstration that selective blockade of DHB GRs can produce a similar disinhibition of glucocorticoid secretion. The fact that the same type of pellet placed on the dura did not alter the plasma Cort concentration indicates that the effect of the DHB Mif pellet to increase Cort secretion was not systemically mediated.

Immunohistochemistry for the occupied GRs demonstrated that Cort and Mif from the DHB pellets were binding to GRs in the DHB. This technique has been used in previous studies (2, 29) and is useful to demonstrate the presence of either Cort or Mif in CNS regions that contain GRs. Neurons in the ventral lateral medulla express GRs and are important for the control of sympathetic nerve activity (16, 19, 24). Therefore, diffusion of Cort or Mif from the DHB to the ventral lateral medulla could potentially account for their effects on arterial pressure and heart rate. However, we observed primarily cytoplasmic and background staining in the ventral lateral medulla of adrenalectomized rats with DHB sham, Cort, or Mif pellets, in contrast to the nuclear staining in the positive controls. There did appear to be some light nuclear staining in the hippocampus in the adrenalectomized rats with DHB Cort or Mif pellets (see Fig. 6). With the exception of some of the immunohistochemistry experiments, experiments were performed in adrenalectomized rats with normal or elevated plasma Cort levels, and it is extremely unlikely that in adrenalectomized rats Cort or Mif diffusing from the DHB pellets could occupy forebrain receptors in sufficient quantity to produce physiological effects, especially considering that the in vivo potency of Mif is low, requiring approximately a 1:1 ratio of Cort to Mif to produce physiological antagonism (34). The absence of any effects of DHB Cort pellets on the plasma Cort concentration, and the absence of an effect of DHB Mif pellets on the morning plasma Cort concentration, strengthens this conclusion, because Cort influences the activity of the hypothalamic-pituitary-adrenal axis at multiple forebrain sites (32).

There were multiple indications that the mere placement of the 3- to 4-mg pellets on the DHB, regardless of pellet composition, did not, in and of itself, produce changes in arterial pressure. First, baseline arterial pressure in systemic sham plus DHB sham rats averaged between 101 and 104 mmHg, well within the normal range for conscious rats. Second, in rats with systemic Cort plus DHB sham treatment, baseline arterial pressure in rats with Silastic or cholesterol pellets did not differ from rats with no DHB pellet (data presented in METHODS). Finally, the gross histology of the DHB in rats that had pellets appeared normal (Fig. 5). Thus physiological and anatomic data show that the DHB pellets provide a functional means to deliver Cort or Mif selectively to the DHB.

The data presented here demonstrate for the first time that glucocorticoids can act within the DHB to increase arterial pressure. This finding expands the current understanding of the mechanisms of glucocorticoid-induced increases in blood pressure. Understanding the cellular and molecular mechanisms mediating the effect of glucocorticoids in the DHB could provide new avenues for the treatment of high blood pressure in some patient populations. It is likely that glucocorticoids act at other sites in the CNS to regulate blood pressure, and this should also be investigated.

ACKNOWLEDGEMENTS

The authors thank Dr. Mary F. Dallman (University of California-San Francisco) and Dr. Terry N. Thrasher (University of Maryland) for the intellectual input provided in these experiments.

GRANTS

This study was supported by the American Heart Association, Heartland Affiliate.

REFERENCES


HINDBRAIN GLUCOCORTICOID RECEPTORS AND BLOOD PRESSURE

H465

AJP-Heart Circ Physiol • VOL 286 • JANUARY 2004 • www.ajpheart.org


