Vasopressin Response in Collecting Ducts of Rats Resistant to Mineralocorticoid Hypertension

William B. Jeffries, Shari McArdle, Charles Bockman, Peter W. Abel, and William A. Pettinger

In previous studies we found that vasopressin stimulation of both cyclic AMP (cAMP) formation in cortical collecting tubules (CCT) and sodium reabsorption in isolated perfused kidneys was markedly exaggerated in rats with mineralocorticoid hypertension. In the present study, we tested the response (cAMP accumulation) of cortical and outer medullary collecting tubules (OMCT) to vasopressin in two rat models that are resistant to deoxycorticosterone acetate (DOCA)-induced hypertension, the Wistar-Furth strain and NaCl-deficient rats. The blood pressure of normal outbred Wistar rats rose to hypertensive levels (systolic pressure more than 165 mm Hg) during a 5-week treatment with DOCA (10 mg/week) and 1% saline to drink. Significant hypertrophy of the heart and kidneys was also observed. Vasopressin (10−8 M)–induced cAMP formation was enhanced 3.4-fold in the CCT (OMCT unchanged) of hypertensive rats compared with normotensive controls. Significant hypertrophy (as indexed by tubule diameter) of the CCT but not the OMCT was also observed in DOCA-salt hypertensive rats. Restriction of dietary NaCl (0.13% in chow, tap water to drink) completely prevented DOCA-induced hypertension, organ and CCT hypertrophy, and enhancement of vasopressin-stimulated cAMP formation in the CCT. In Wistar-Furth rats, DOCA-salt treatment did not alter blood pressure or cause significant organ hypertrophy. However, DOCA-salt treatment enhanced vasopressin-stimulated cAMP formation by 4.1-fold in CCT of Wistar-Furth rats, with significant tubular hypertrophy in the CCT but not the OMCT. We conclude that DOCA-induced hypertension and changes in CCT function are dependent on excess dietary NaCl. It is unlikely that the alterations in CCT function are the result of hypertension since they are present in DOCA-salt–treated Wistar-Furth rats that remained normotensive. The mechanism for DOCA resistance in the Wistar-Furth strain is extrinsic to vasopressin stimulation of adenylyl cyclase in the CCT. (Hypertension 1991;17:63–71)
rat CCT segments, an action enhanced by prior in vivo DOCA treatment. DOCA-salt treatment increases the antinatriuretic effect of V2-receptor stimulation in the isolated perfused kidney. This effect is present before the onset of severe hypertension, and its magnitude increases with continued DOCA-salt treatment. Thus, we have hypothesized that enhancement of V2-receptor responsiveness in the CCT promotes sodium retention and contributes to the development of hypertension in the DOCA-salt-treated rat.

The purpose of the present study was to investigate this hypothesis further by examining the response to vasopressin in the CCT of rats that are resistant to DOCA-induced hypertension. We chose two models for these experiments, the Wistar-Furth strain of rats and salt-restricted rats. The Wistar-Furth strain is reportedly resistant to adrenal regeneration and DOCA-salt forms of hypertension, despite highly elevated serum deoxycorticosterone levels. The mechanism for resistance to these mineralocorticoid forms of hypertension is unknown. We hypothesize that this mechanism involves interference with DOCA-induced amplification of the renal response to vasopressin.

Normal rats placed on a sodium-restricted diet do not develop hypertension during DOCA treatment, provided that they are not uninephrectomized. These two models allow us to determine whether the altered response to vasopressin is the result of elevated blood pressure or is more fundamentally involved in hypertension etiology and may clarify the role of dietary sodium in the development of altered vasopressin response.

Methods

Animals

Male 10-week-old outbred Wistar and inbred Wistar-Furth rats (Harlan Sprague Dawley, Indianapolis, Ind.) were used in these studies.

Protocol 1

Wistar outbred rats were placed on NaCl-deficient (0.13%) chow (TD 82049, Teklad, Madison, Wis.) and were randomly assigned to one of four groups: 1) low salt (olive oil vehicle injections plus tap water to drink), 2) high salt (vehicle injections plus 1% NaCl to drink), 3) DOCA low salt (5 mg/kg DOCA s.c. twice weekly plus tap water), and 4) DOCA-salt (DOCA injections plus 1% NaCl). Systolic blood pressure was measured every 3-4 days by tail-cuff plethysmography. The rats were trained during the week before the study to familiarize them to the blood pressure measurement procedure. After 5 weeks, the rats were anesthetized with pentobarbital sodium (50 mg/kg), and the left kidneys were removed under anesthesia for microdissection and the rats were killed by exsanguination. The heart and right kidneys were weighed as described above.

Microdissection Studies

Outer medullary collecting tubules (OMCT) and CCT segments were obtained as previously described. The superior mesenteric artery was ligated and a PE-50 polyethylene cannula was inserted into the abdominal aorta below the left renal artery. The aorta was then clamped above the left renal artery and the left kidney was flushed with a slow injection of 5 ml cold Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) equilibrated with a 95% O2-5% CO2 mixture. This buffer contained the following (in mM): 118 NaCl, 4.8 KCl, 24 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, 11.0 dextrose, 0.026 NaEDTA, and 2.5 CaCl2. This wash was followed by an infusion of 10 ml of ice-cold buffer containing 2 mg/ml collagenase (fraction I, Sigma Chemical Co., St. Louis, Mo.) and 10 mg bovine serum albumin (BSA, fraction V, Sigma). Corticomedullary slices were prepared and incubated at 30°C for 30 minutes in the KRB-BSA collagenase buffer, continually gassed with a 95% O2-5% CO2 mixture. After incubation, the slices were rinsed several times with a total of 80 ml ice-cold modified Hank's solution (pH 7.4) containing the following (mM): 137 NaCl, 5.4 KCl, 4.2 NaHCO3, 0.34 Na2HPO4, 0.44 KH2PO4, 1.0 MgSO4, 0.1% glucose.

The microdissection of the CCT and OMCT segments was performed under a stereomicroscope in an ice-cold modified Hank's solution (pH 7.4) containing the following (mM): 137 NaCl, 5.4 KCl, 0.34 Na2HPO4, 0.44 KH2PO4, 1.0 CaCl2, 0.5 MgCl2, 0.5 MgSO4, 1.2 1-methyl-3-isobutylxanthine, 20 HEPES, 5.5 glucose, and 0.05% BSA. Collecting tubule segments were identified by previously established criteria. The CCT segments used were dissected from the region just distal to the initial collecting tubule (i.e., the portion that leads into the medullary ray). OMCT segments were from the outer stripe of the outer medulla. Tubule length was measured with an eyepiece reticle. One to four segments (total length 1.3 mm) were transferred in 20 µl of modified Hank's solution to a siliconized tube for cAMP accumulation studies. Tubes containing CCT or OMCT segments were then incubated for 2 minutes in the presence of

Protocol 2

Wistar and Wistar-Furth rats were randomly assigned to DOCA-salt (5 mg DOCA twice weekly plus 1% NaCl solution to drink) or control (olive oil vehicle injections plus tap water) groups (n=4-7 per group). All rats received standard rat chow that contained a "normal" salt content (approximately 1% NaCl by weight). Blood pressure was measured once weekly by tail-cuff plethysmography as described above. After 5 weeks, the left kidneys were removed under anesthesia for microdissection and the rats were killed by exsanguination. The heart and right kidneys were weighed as described above.
added buffer alone (n=4 tubes/rat) or arginine vasopressin (10^{-8} M, 4 tubes/rat). The incubation was terminated by addition of 50 µl of 10% trichloroacetic acid, and cAMP content was measured by radioimmunoassay as previously described.\textsuperscript{16} The mean cAMP concentration in controls and vasopressin-stimulated segments was obtained for each rat, which was then used to calculate group means.

Some of the tubules on each day (n=4-8) were placed in a 96-well plastic culture plate and were visualized with phase contrast optics on a Nikon Diaphot inverted microscope and were photographed (x160) for later measurement of tubule size and examination of gross morphology, as described by El Mernissi et al.\textsuperscript{19} Mean CCT diameter was measured for each segment starting at the initial branch point along a distance of approximately 1 mm distal to the branch. From five to 15 measurements were taken of the tubule diameter from each segment by a technician who was unaware of the treatment protocol. Measurements were taken only for those portions of the tubule that were in the plane of focus in the photographs. The measurements were calibrated by comparison with daily photographs taken of the 50- µm divisions of a hemocytometer. Group means were calculated and statistical differences among groups were analyzed.

Statistics

Differences among treatment groups were compared with the Newman-Keuls multiple comparison test using the PHARM/PCS computer software.\textsuperscript{20} Differences among group means were considered significant if the calculated value of \( p \) was less than 0.05.

Results

Blood Pressure

DOCA-salt treatment caused blood pressure to rise in outbred Wistar rats to hypertensive levels after 10 days (Figure 1). Sodium restriction completely prevented hypertension caused by DOCA injections (Figure 1). No significant effect of a high or low salt intake alone was seen on blood pressure in outbred Wistar rats during the 5 weeks of observation.

Organ Hypertrophy

DOCA-salt treatment caused significant cardiac and renal hypertrophy in outbred Wistar rats (Figure 3), and the gross hypertrophic effect of DOCA treatment was prevented by salt restriction. No significant differences in organ weights were found between rats on a high or low salt intake (Figure 3). DOCA-salt treatment in outbred Wistar rats caused a significant increase in organ weight compared with rats on a normal salt diet (Figure 4). No significant renal or cardiac hypertrophy was observed in DOCA-salt-treated Wistar-Furth rats (Figure 4).

Cyclic AMP Accumulation in Collecting Tubules

Vasopressin treatment (10^{-8} M) significantly increased cAMP content of CCT segments from rats not receiving DOCA injections (Figure 5). DOCA-salt treatment enhanced vasopressin-stimulated cAMP accumulation in the CCT of outbred Wistar rats (Figure 5). NaCl restriction completely prevented the DOCA-induced augmentation of the response to vasopressin in the CCT. There was no effect of high or low dietary NaCl alone on the V2-receptor response in the CCT (compare Figures 5 and 6). No differences were observed among groups in cAMP accumulation in the OMCT. Interestingly, DOCA-salt treatment enhanced the response to vasopressin to a similar degree in the CCT of Wistar-Furth and outbred Wistar rats (4.1- versus 3.4-fold), despite the absence of DOCA-induced hypertension.
FIGURE 3. Bar graphs showing effect of deoxycorticosterone acetate (DOCA) injections and dietary NaCl content on heart and kidney weight in outbred Wistar rats. Experimental groups are same as in Figure 1. —Na, 0.13% NaCl chow plus tap water to drink; +Na, 0.13% NaCl plus a 1% NaCl solution to drink. ***p<0.001 compared with all other groups.

FIGURE 4. Bar graphs showing effect of deoxycorticosterone acetate (DOCA)-salt treatment on heart and kidney weight in Wistar-Furth (W-F) and outbred Wistar (Wist) rats. ***p<0.001 compared with corresponding Wistar control group.

FIGURE 5. Bar graphs showing effect of dietary NaCl and deoxycorticosterone acetate (DOCA) on basal and vasopressin (AVP)-stimulated cyclic AMP (cAMP) formation in the cortical (CCT) and outer medullary (MCT) collecting tubules of outbred Wistar rats. Each bar represents mean cAMP content per millimeter tubule per group, n=4-6). Individual means for each rat were obtained in quadruplicate. —Na, 0.13% NaCl chow plus tap water to drink; +Na, 0.13% NaCl plus a 1% NaCl solution to drink. **p<0.01 for DOCA +Na groups vs. all other groups (Newman-Keuls test).

and gross renal hypertrophy (Figure 6). The maximal response to vasopressin was not changed in the OMCT of either strain, confirming our earlier observation that this response to DOCA is anatomically specific for the CCT.10

Collecting Tubule Diameter

The diameters of CCT harvested from DOCA-salt hypertensive Wistar rats were significantly greater than normotensive controls (Figures 7 and 8). This is presumably due to the hypertrophic effect of DOCA on basolateral membranes of principal cells.21 Salt restriction reduced but did not eliminate the hypertrophic effects of DOCA (Figure 7). There was no difference between CCT diameters in Wistar rats on a low or high salt diet alone.

The CCT of Wistar-Furth control rats were smaller (p<0.01) than those of corresponding outbred Wistar control rats (Figure 7). However, CCT diameters in Wistar-Furth rats also significantly increased after 5 weeks of DOCA-salt treatment (Figures 7 and 9). No change in tubule size was apparent in OMCT of hypertensive versus normotensive rats (not shown).

Discussion

Vasopressin, acting at V2-receptors, stimulates both cAMP generation and sodium reabsorption in...
FIGURE 6. Bar graphs showing effect of deoxycorticosterone acetate (DOCA)-salt treatment on basal and vasopressin (AVP)-stimulated cyclic AMP (cAMP) formation in the cortical (CCT) and outer medullary (MCT) collecting tubules of Wistar-Furth (W-F) and outbred Wistar rats. Numbers in parentheses represent the fold change over corresponding AVP-stimulated value in control (olive oil + tap water) group. **p<0.01 for AVP-stimulated values in DOCA-salt vs. untreated rats of the same strain.

FIGURE 7. Bar graphs showing deoxycorticosterone acetate (DOCA)-induced changes in cortical collecting tubule (CCT) diameter. Upper panel: Outbred Wistar rats with a high (1% saline to drink) or low (0.13% NaCl in chow) NaCl intake. Lower panel: Comparison of response of outbred Wistar rats to Wistar-Furth (W-F) rats. Control rats in these latter studies were fed a chow containing 1% NaCl with tap water to drink. Bars represent mean diameters of CCT segments measured from photomicrographs of tubules from rats in the different protocols as described in Methods. Number of rats in study was 4–6 per group; number of tubules measured was 4–8 per rat. **p<0.01 for comparisons noted.

Jeffries et al  DOCA Resistance and Vasopressin Response 67

Jeffries et al  DOCA Resistance and Vasopressin Response 67

Jeffries et al  DOCA Resistance and Vasopressin Response 67

Dietary NaCl and Deoxycorticosterone Acetate-Induced Changes in Vasopressin 2-Receptor Responsiveness

Our results demonstrate that a low sodium diet prevented the onset of hypertension in rats treated with DOCA. Similar findings with respect to blood pressure in two-kidney rats were reported by Cox. In the present study, DOCA-induced increases in CCT diameter and the cAMP response to vasopressin were dependent on the presence of excess dietary salt, which suggests that these events may be secondary to sodium entry into the CCT. Other basolateral events that are influenced by mineralocorticoids, such as enhancement of Na⁺, K⁺-ATPase activity, have been shown to be dependent on apical sodium entry as the triggering event.

In a previous report we found that DOCA injections with tap water to drink augmented the response to vasopressin in the CCT (measured after 2 weeks of DOCA). Based on these results, we tentatively concluded that DOCA, independent of NaCl intake, was responsible for this effect. However, in that study all rats were uninephrectomized and were fed standard rat chow, which has an NaCl content of approximately 1%. In the present study, we used a salt-deficient diet (0.13% NaCl), which completely blunted the DOCA-induced enhancement of the cAMP response to arginine vasopressin. Dietary salt intake in the absence of DOCA had no effect on the CCT response to arginine vasopressin. Thus, the dietary intake of NaCl from ordinary rat chow is sufficient to permit hypertension and DOCA-induced changes in adenyl cyclase activity. Because a NaCl-deficient diet (present study) prevented these changes, we now conclude that the combination of excess dietary salt and DOCA treatment is necessary for both induction of hypertension and the enhanced response to vasopressin in CCT.

Wistar-Furth Rats

DOCA-salt treatment did not alter blood pressure in Wistar-Furth rats, a finding similar to the observations of Sciotti and Gallant, who first reported the resistance of the Wistar-Furth strain to hyperten-
FIGURE 8. Photomicrographs showing deoxycorticosterone acetate (DOCA)-induced hypertrophy of cortical collecting tubule (CCT) from outbred Wistar rats. Top panel: Phase-contrast photomicrograph of an unstained CCT microdissected from a rat ingesting 0.13% NaCl chow plus 1% saline to drink (high salt). Bottom panel: CCT from a rat that received a DOCA-salt regimen for 5 weeks. Magnification ×160.

sion induced by DOC-pivalate–salt treatment. Our results differ from that previous study in that we did not observe significant organ hypertrophy; Sciotti and Gallant reported significant increases in kidney weight and a nonsignificant trend toward cardiac hypertrophy over the course of treatment. However, in their study rats were uninephrectomized and treated for 10 weeks (versus 5 weeks in our study), by which time blood pressure had risen slightly (approximately 10%). It is possible that our rats would have developed hypertrophy and mild blood pressure elevation had we used higher doses of DOCA for longer periods of time.

The mechanism for DOCA resistance in Wistar-Furth rats is unknown. These animals have normal resting blood pressure and develop renovascular (two-kidney, one clip) hypertension similar to Sprague-Dawley rats. Plasma deoxycorticosterone concentrations in outbred rats and Wistar-Furth rats are similarly elevated during DOCA-salt treatment, prompting the notion that these animals are resistant to the target organ effects and not the normal disposition of DOCA. We therefore hypothesized that these rats are resistant to deoxycorticosterone-induced augmentations in V2-receptor–stimulated cAMP formation in the CCT, which may in turn contribute to their resistance to hypertension. However, this now appears not to be the site of resistance since the augmenting effects of DOCA-salt on V2-receptor–induced cAMP formation are intact in DOCA-salt–treated Wistar-Furth rats. DOCA-induced hypertrophy of the CCT, which is associated
with increased Na\(^+\), K\(^+\)-ATPase activity\(^{24}\) was also apparent in Wistar-Furth rats. Our results suggest that augmentation of V2-receptor function is not the result of hypertension, since it is present in Wistar-Furth rats that remained normotensive during DOCA-salt treatment.

Thus, the dissociation between V2-receptor augmentation and hypertension in Wistar-Furth rats suggests that these two events may be unrelated. However, a possible renal role for vasopressin in the onset of DOCA hypertension cannot yet be ruled out since this form of hypertension is exacerbated by uninephrectomy and high salt intake and is vasopressin-dependent. It is possible that there is a renal defect in Wistar-Furth rats in steps distal to V2-receptor activation of adenylyl cyclase (protein kinase A, phosphodiesterase, and sodium and water channels) that prevents hypertension from occurring after DOCA-induced augmentation of cAMP formation. Further, there could be distal lesions or metabolic defects (central nervous system, blood vessels, see above) that prevent hypertension in Wistar-Furth rats despite the development of pathogenic renal abnormalities.

Two likely possibilities for the site of resistance to mineralocorticoid hypertension in Wistar-Furth rats are the central nervous system and the resistance vessels. Brain lesioning studies have shown that the central nervous system may play a role in mineralocorticoid hypertension. For example, experimental lesions of the area postrema prevent hypertension\(^{25}\) while leaving peripheral responses...
vascular hyporesponsiveness to DOCA is secondary to another initiating event that is absent in the Wistar-Furth rat.

Tubular Hypertrophy and Vasopressin 2-Receptor Response

As noted above and in other studies, DOCA treatment causes hypertrophy of some distal tubular segments. In our studies (with one exception), it appeared that the response to vasopressin varied with tubular size (compare Figure 7 to Figures 5 and 6). The lone exception was in the rats that received DOCA with dietary NaCl deprivation. In these rats, CCT size was significantly increased, but the response to vasopressin was similar to control rats. These findings suggest that these responses to DOCA may be mediated by different mechanisms. Further studies are needed to address this hypothesis.

The functional significance of the relation between tubule size and vasopressin response is a complex issue. Other investigators (Reference 19 and L. Kinter, personal communication, May 1990) have noted that when arginine vasopressin–stimulated cAMP accumulation is normalized as a function of membrane protein content, DOCA-induced increases in the response are not seen. However, such normalization may not be appropriate since the increases in membrane protein after mineralocorticoid treatment are confined to the basolateral membrane of the principle cells. Thus, the tubular urine passes through the same length of CCT in DOCA-treated versus control rats, but the response to antidiuretic hormone is increased along this length of tubule. In functional studies, in vivo DOCA treatment increases arginine vasopressin–stimulated sodium reabsorption in perfused rat CCT segments and in the rat isolated perfused kidney. We therefore suggest that the best way to quantify biochemical changes in the CCT is to express data per unit tubule length, not per milligram membrane protein, since the former method best correlates with functional data.

In conclusion, we have demonstrated that the following DOCA-induced changes in outbred Wistar rats are regulated by dietary NaCl intake: hypertension, cardiac and renal hypertrophy, changes in V2-receptor–mediated cAMP accumulation in CCT, and alterations in CCT morphology. Thus, it is possible that altered V2-receptor responsiveness plays a role in the development of DOCA-salt hypertension in this strain. In the Wistar-Furth strain of rats, DOCA-salt treatments did not induce hypertension or significant organ hypertrophy; however, enhanced V2-receptor responsiveness and CCT hypertrophy were observed. Thus, it appears that mineralocorticoid resistance to hypertension in this strain is unrelated to vasopressin-stimulated cAMP production in the CCT. DOCA resistance may either occur at a step distal to V2-receptor–stimulated cAMP generation or is due to an altered response at another target organ.

Acknowledgments

We thank Rachel Fallet and Jim Orr for technical assistance.

References


**KEY WORDS** • vasopressins • deoxycorticosterone • kidneys • mineralocorticoid hypertension • Wistar–Furth Rats
Vasopressin response in collecting ducts of rats resistant to mineralocorticoid hypertension.
W B Jeffries, S McArdle, C Bockman, P W Abel and W A Pettinger

Hypertension. 1991;17:63-71
doi: 10.1161/01.HYP.17.1.63
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/1/63

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/