Impaired Cardiovascular Reflexes Precede Deoxycorticosterone Acetate–Salt Hypertension

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Abstract We hypothesized that impaired cardiopulmonary reflexes but not altered baroreceptor reflexes precede deoxycorticosterone acetate (DOCA)–salt hypertension. Uninephrectomized rats were given either DOCA and 0.9% NaCl as drinking water, 0.9% NaCl alone, or tap water. We measured mean blood pressure, heart rate, and renal sympathetic nerve activity. After 8 days, mean blood pressure was not different in DOCA-salt and control rats. Volume-sensitive cardiopulmonary reflexes were tested by intravenous volume loading with saline (10% body weight in 15 minutes), which decreased renal sympathetic nerve activity without changing mean blood pressure or heart rate. This response was blunted in DOCA-salt rats. Chemosensitive cardiopulmonary reflexes were tested by 15-minute infusions of the serotonin 5-HT, agonist phenylbiguanide, which decreased renal sympathetic nerve activity without changing mean blood pressure or heart rate. Sustained decreases in renal sympathetic nerve activity occurred during phenylbiguanide infusion in controls but were blunted over time in DOCA-salt rats. The arterial baroreflex responses to graded infusions of methoxamine and nitroprusside were analyzed by sigmoidal curve fitting. There were no differences in gain of renal sympathetic nerve activity or heart rate between the groups. Thus, DOCA-salt rats exhibit impaired cardiopulmonary reflexes before the onset of hypertension; the volume-sensitive reflexes are more severely affected than chemosensitive reflexes. The arterial baroreceptor reflex is unaltered. The decreased sensitivity of cardiopulmonary reflexes may contribute to DOCA-salt hypertension.

Key Words • sympathetic nervous system • rats, inbred SHR • pressoreceptors • hypertension, mineralocorticoid • receptors, serotonin

Altered central sympathetic outflow and decreased reflex control of peripheral sympathetic nerves are thought to be associated with established hypertension1-4; however, the distinction between cause and effect is unclear.5-6 The sympathetic nervous system could influence blood pressure control not only by its effect on the regulation of peripheral resistance but also by controlling volume homeostasis via efferent renal sympathetic nerve activity (RSNA).7,8 It is well established that RSNA influences tubular water and sodium reabsorption independent of changes in intrarenal and systemic hemodynamics.2,9 An increase in RSNA augments sodium and water reabsorption, consequently decreasing urinary flow and salt excretion. A decrease in RSNA leads to opposite effects. Thus, an impairment of RSNA regulation could alter volume homeostasis and thereby contribute to the development of hypertension.10

The activity of renal sympathetic nerve fibers is specifically controlled by cardiopulmonary reflexes. In the rat, prolonged stimulation of volume-sensitive cardiopulmonary reflexes with isotonic volume expansion inhibits RSNA but not the efferent activity of sympathetic fibers controlling peripheral resistance.5,11

The aim of the present study was to elucidate the putative role of these cardiopulmonary reflexes in the development of hypertension. We investigated cardiovascular reflexes in uninephrectomized rats treated with deoxycorticosterone acetate (DOCA)–salt before the rats developed hypertension to exclude any effect of high blood pressure per se on these reflexes. The DOCA-salt model was chosen because of its sodium and volume dependency, its clearly defined prehypertensive phase, and the obvious lack of genetic differences between DOCA-salt and control animals.10,12

Our main hypothesis was that the control of RSNA by cardiopulmonary reflexes is impaired before the onset of hypertension in DOCA-salt–treated unilaterally nephrectomized rats. Because it had been reported that a high dietary sodium intake decreased the sensitivity of volume-sensitive cardiopulmonary reflexes in rats,13 we used two control groups in the investigation of cardiopulmonary reflexes: unilaterally nephrectomized rats that received either tap water or isotonic saline for drinking.

The arterial baroreceptor reflex (ABR) is the major determinant of total efferent sympathetic outflow, including RSNA.14,15 We therefore investigated the function of the ABR and evaluated it by means of a four-parameter logistic model.16 We hypothesized that the sensitivity of the baroreceptor reflex might be increased in prehypertensive DOCA-salt rats compared with controls, as described in other models of prehypertensive and hypertensive animals.1,6

Cardiopulmonary reflexes are stimulated not only by changes in cardiac filling pressure but also by chemical agents.17 These chemosensitive reflexes are often referred to as the Bezold-Jarisch reflex.18 Endogenously occurring substances stimulating the Bezold-Jarisch reflex are prostaglandins and serotonin 5-HT,
receptors. We studied serotonin 5-HT3-sensitive cardiopulmonary reflexes in prehypertensive DOCA-salt rats to demonstrate that cardiopulmonary reflexes with different sensoric properties are differently affected in early DOCA-salt hypertension. We hypothesized that volume-sensitive cardiopulmonary reflexes are much more severely impaired than serotonin 5-HT3-sensitive reflexes.

Methods

Preparations

Male Sprague-Dawley rats (Ivanovas, Kisslegg, Germany) underwent right unilateral nephrectomy at 7 to 9 weeks of age. Three weeks were allowed for recovery and compensatory left renal hypertrophy before DOCA-salt or the control treatments were begun when the rats weighed approximately 250 g. The DOCA-salt treatment consisted of the implantation of DOCA pellets containing 50 mg DOCA (Innovative Research of America) under the skin of the right flank during methohexital (30 mg/kg IP) anesthesia (Breviniyal, Eli Lilly & Co). The animals were fed a standard rat diet (no. C-1000, Altromin) containing 0.2% sodium by weight and were allowed access to either isotonic saline (DOCA and control 1) or tap water (control 2). The rats were randomly assigned to one of the three groups. Eight days later on the day of the experiment rats were equipped with one femoral arterial and two venous catheters under methohexital anesthesia. After the venous catheter was inserted, anesthesia was continued with a maintenance infusion of methohexital (80 μg/kg g per minute). This regimen was the standard anesthesia used in our experiments. An electrode to measure RSNA was implanted as described above. The protocol was approved by the local governmental agency (Regierung von Mittelfranken, Ansbach, Germany) in accord with recommendations of the American Physiological Society.

Renal Sympathetic Nerve Recording

Renal sympathetic nerve recording was performed as described previously.\(^5,^{11,19}\) Briefly, the left kidney was exposed through a flank incision, and a renal nerve bundle was dissected and placed on a bipolar stainless steel electrode (Cooner Wire Co). RSNA was amplified (×10 000 to ×50 000) and filtered (high pass, 100 Hz, low pass, 1000 to 3000 Hz) using a Grass P511 band-pass amplifier and a Grass HIP511 high-impedance probe. The amplified and filtered signal was channeled to an audio amplifier, loudspeaker (Grass model AM8 audio monitor) was used for auditory evaluation, as well as a rectifying voltage integrator (Grass model 7P10). The integrated voltage signals were displayed on the polygraph.

All data were additionally stored on a computer system with the aid of an analog-to-digital convertor (DT 2861, Data Translation Inc) and appropriate software (Brain Wave Systems) for later evaluation. The quality of the RSNA signal was assessed by its pulse synchronous rhythmicity and examination of the magnitude of the decrease in recorded RSNA during ganglionic blockade with the short-acting ganglionic blocker trimethaphan (10 mg/kg IV) and after suppression with an injection of methoxamine (10 μg IV). When an optimal signal was observed, the recording electrode was fixed to the nerve bundle with a silicone adhesive (Sil-Gel 604, Wacker-Chemie). The electrode cable was then secured to the abdominal trunk muscles by a suture.

Drugs

Phenylbiguanide and methoxamine were purchased from Sigma Chemical Co, and sodium nitroprusside was obtained from Pfizer. Trimethaphan camsylate (Arfonad, F. Hoffmann-La Roche) was provided by Roche Laboratories. All drugs except for the previously prepared trimethaphan were dissolved in saline and prepared anew for every experiment.

Experimental Protocols

Protocol 1

Three groups (n=6) of rats implanted with a DOCA pellet as described above and three groups (n=6) of controls receiving tap water were equipped with respective catheters after either 4, 8, or 12 days (n=12). Blood pressure was continuously recorded to obtain baseline data on the development of DOCA-salt–induced hypertension. The experiments, as all other experiments, were performed with rats under methohexital anesthesia as described above.

Protocol 2

On day 8, six rats from each treatment group (DOCA-salt, control 1 [salt], and control 2 [tap water]) were equipped with venous and arterial catheters. A catheter was also inserted into the left ventricle to record baseline values of left ventricular end-diastolic pressure (LVEDP). We did not measure LVEDP continuously during the experiments to assess the sensitivity of cardiopulmonary reflexes because left ventricular catheterization may harm the aortic valve, thus altering the hemodynamics of the left ventricle.\(^30\)

For all other experiments the rats were equipped as described above (one arterial and two femoral venous catheters, renal nerve electrode). The arterial catheter was connected to a Statham P23 Db transducer for continuous recording of blood pressure and heart rate (HR). After surgery, anesthetized animals were allowed 1 hour for stabilization and equilibration before the experiment was begun. A maintenance infusion of methohexital (80 μg/kg g per minute) was given through one of the venous femoral catheters. The second venous catheter was used for administration of drugs.

Protocol 3

Nine anesthetized animals from each of the three groups were infused with a 0.9% saline load of 10% body weight for 15 minutes to stimulate volume-sensitive cardiopulmonary reflexes.

Protocol 4

Nine anesthetized animals from each of the three groups were infused with a 15-minute, 32-μg/min infusion and a 10-μg bolus injection of the serotonin 5-HT3 receptor agonist phenylbiguanide to stimulate serotonin 5-HT3-sensitive cardiopulmonary reflexes. The order of treatment (bolus or infusion) was randomized.

Rats of all three groups were killed; the hearts were excised, dried, and weighed.

Protocol 5

Six rats treated with DOCA-salt and six control rats on tap water were artificially ventilated. Blood pressure, HR, and RSNA were recorded 30 minutes before and 80 minutes after bilateral cervical vagotomy.

Protocol 6

Rats for baroreceptor investigations were taken from the DOCA-salt group (n=9) and the tap water control group (n=8). The following maneuvers were used to stimulate the ABR. A graded intravenous infusion of sodium nitroprusside was administered to lower mean arterial blood pressure (MAP) to approximately 40 mm Hg over a period of 4 to 5 minutes. The infusion was stopped, and MAP, HR, and RSNA were allowed to recover for 45 minutes. Then a graded intravenous infusion of methoxamine was begun to increase MAP to 200 mm Hg over a period of 4 to 5 minutes. During the infusions of either sodium nitroprusside or methoxamine, MAP, HR, and RSNA data were stored on the hard disk of a computer at a...
rate of 10 Hz with the data-acquisition system mentioned above.

**Baroreceptor Reflex Evaluation**

The details of the procedures to stimulate the baroreceptor reflex are outlined under the description of protocol 6 above. Stored HR and RSNA data were plotted against MAP. The resulting sigmoidal curves were analyzed (CURVEFIT for Windows 1.0, SIGMAPLOT 4.0; Jandel Scientific) with a four-parameter logistic regression equation:

\[
y=a+b/(1+\exp(d(x-c))
\]

where \(y\) is the change in HR or RSNA and \(x\) is MAP. The four parameters are the lower plateau \((a)\), range of change in \(y\) \((b)\), midrange of the curve \((c)\), and slope coefficient \((d)\).

The maximal gain at the midrange of the curve (ie, when MAP=c) was calculated as

\[
\text{Gain}_{\text{max}} = -d \cdot b / 4
\]

The four parameters generated by the logistic regression equation for each experiment were averaged respectively and used to generate group curves. For single comparisons between the model parameters of the two groups, the \(r\) test was used, and a value of \(P<.05\) was considered statistically significant.

**Data Analyses for All Other Experiments**

Integrated RSNA was expressed as microvolts integrated over 1-second intervals. The background noise level was recorded as postmortem activity (average of 30 minutes) and was subtracted from the measured nerve activity. Because of the limitations of comparing values from multifiber sympathetic nerves between animals, the data are expressed as percent change from baseline values.

The data were statistically analyzed with ANOVA and Newman-Keuls post hoc test using a CSS statistical software package (StatSoft Inc). Only a priori fixed comparisons were tested. Statistical significance was defined at a value of \(P<.05\). All data are given as mean±SEM.

**Results**

Body weights at the time of the experiments were 285±15 g for DOCA-salt rats, 295±19 g for salt controls, and 292±8 g for tap water controls.

**Protocol 1**

Baseline MAP values in the different groups under methohexital anesthesia were after 4 days: DOCA-salt, 104±5 mm Hg; salt controls, 95±7 mm Hg; and tap water controls, 100±8 mm Hg. After 8 days: DOCA-salt, 102±8 mm Hg; salt controls, 98±7 mm Hg; and tap water controls, 104±8 mm Hg. After 12 days: DOCA-salt, 151±11 mm Hg; salt controls, 103±9 mm Hg; and tap water controls, 98±6 mm Hg. Thus, after 12 days MAP was significantly elevated in the DOCA-salt group compared with the two control groups.

The main difference observed was a markedly attenuated RSNA inhibition during volume expansion with isotonic saline in the DOCA-salt group compared with the two control groups.

**Protocol 2**

Baseline LVEDP values on day 8 were DOCA-salt, 9.3±0.6 mm Hg; salt controls, 5.2±0.4 mm Hg; and tap water controls, 4.9±0.6 mm Hg. The LVEDP values in DOCA-salt rats were significantly higher than in the two control groups.

**Protocol 3**

Fig 1 shows the results of the saline loading experiments. MAP baseline values were DOCA-salt, 104±6 mm Hg; salt controls, 98±6 mm Hg; and tap water controls, 102±6 mm Hg. HR values were 340±28 beats per minute (bpm) in DOCA-salt rats, 328±10 bpm in salt controls, and 335±20 bpm in tap water controls. The asterisks in Fig 1 represent significant differences between the groups, not significant changes from baseline. The main differences observed were a marked attenuation in RSNA inhibition during volume expansion with isotonic saline in the DOCA-salt group compared with the two control groups.

**Protocol 4**

Figs 2 and 3 show the results of the bolus and 15-minute infusions of phenylbiguanide. MAP baseline values were DOCA-salt, 102±4 mm Hg; salt controls, 97±6 mm Hg; and tap water controls, 98±6 mm Hg. HR values were 340±32 bpm for DOCA-salt rats, 347±10 bpm for salt controls, and 342±28 bpm for tap water controls. The asterisks in Fig 2 again represent significant differences between the groups rather than significant changes from baseline. Inhibition of RSNA was significantly less with phenylbiguanide infusion (Fig 2) in the DOCA-salt group than in the two control groups.
Fig 2. Line graphs show responses of mean arterial blood pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) to 15-minute intravenous infusions of phenylbiguanide (32 μg/min) in prehypertensive deoxycorticosterone acetate (DOCA) rats, salt controls, and tap water controls. Responses of RSNA to phenylbiguanide infusions were initially preserved and blunted over time in DOCA rats. Asterisks represent differences between groups.

Initially, HR decreased more in DOCA-salt rats; however, these rats showed no change in MAP.

Heart weights were not significantly different among the groups: DOCA-salt rats, 310±20 mg/100 g body wt; salt controls, 298±17 mg/100 g body wt; and tap water controls, 305±10 mg/100 g body wt.

Protocol 5
Fig 4 shows the results of the experiments with bilateral cervical vagotomy. MAP baseline values were DOCA-salt, 104±8 mm Hg; and tap water controls, 99±9 mm Hg. HR values were DOCA-salt, 360±32 bpm; and tap water controls, 372±28 bpm. Bilateral vagotomy did not affect any of the measured parameters during the time period of the experiments.

Protocol 6
The Table and Figs 5 and 6 show the results of the baroreceptor experiments. No difference in gain could be detected between the groups with respect to control of HR or RSNA. The upper plateau increase in RSNA was significantly greater in DOCA-salt rats compared with the other groups, and then only with marked decreases in MAP.

Discussion
The renal sympathetic nerve influences natriuresis and therefore volume and blood pressure homeostasis. RSNA is tightly controlled by cardiovascular reflexes that react to cardiac filling pressure, arterial blood pressure, and endogenous chemical substances such as serotonin. We evaluated the reflex control of renal nerves in prehypertensive DOCA-salt-treated rats. If these reflexes play a role in the development of high blood pressure, one would expect impaired reflex function occurring before the onset of hypertension. In contrast, changes in reflex function occurring in established hypertension are more likely to be a consequence of the elevated blood pressure.

The most important finding of our study was that the reflex control of RSNA by volume-sensitive cardiopulmonary receptors was markedly impaired in prehypertensive DOCA-salt-treated rats. During volume expansion, suppression of RSNA in DOCA-salt rats was only half that of the suppression occurring in either salt or tap water control rats. The implantation of DOCA pellets under the skin induced a very steep but reproducible increase in MAP after 8 days of the DOCA-salt regimen. However, on day 8 we were not able to detect an increase in blood pressure in these rats in any of the protocols.

Impaired volume-sensitive cardiopulmonary reflexes could directly contribute to the development of hypertension. For instance, impaired suppression of RSNA in response to a volume load will lead to a prolonged or impaired excretion of the excess volume. The natriuresis necessary to excrete the volume may not occur in the presence of inappropriately high RSNA. Ultimately, further salt and volume retention could result, thereby contributing to increased blood pressure.

The notion that RSNA plays a role in the development of DOCA-salt hypertension is supported by data from denervation experiments. Renal denervation de-
lays the onset of high blood pressure in DOCA-salt rats.\textsuperscript{12,27} The fact that blood pressure eventually rises even in denervated rats does not argue against a role of the renal nerves because renal renervation may occur.\textsuperscript{9} The results showing a critical role of the efferent sympathetic nervous system in DOCA-salt hypertension are also consistent with a role for RSNA in this model of volume-dependent hypertension.\textsuperscript{12,27}

LVEDP was increased in prehypertensive DOCA-salt rats compared with both control groups. One could argue that the impaired response to acute volume loading might be a consequence of elevated cardiac filling pressure. Continuous stimulation of volume-sensitive cardiopulmonary reflexes might produce a state of depressed RSNA, which is resistant to further inhibitory influences. However, the lack of an increase of RSNA after vagotomy demonstrates that there was no persistent inhibitory input by vagal afferents. Furthermore, the results of our baroreceptor investigation also argue against the hypothesis of a persistent suppression of the renal nerves. After arterial baroreceptor loading, RSNA could be suppressed by the same degree in DOCA-salt and control rats. These data suggest that the resting activity of the renal nerve was not suppressed in DOCA-salt rats. However, a resetting of the peripheral and

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<th>Summary of Data on Arterial Baroreceptor Reflex Control of Renal Sympathetic Nerve Activity and Heart Rate in Prehypertensive DOCA-Salt-Treated Rats and Tap Water Controls</th>
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DOCA indicates deoxycorticosterone acetate; RSNA, renal sympathetic nerve activity; and HR, heart rate. Values are mean±SEM; n is number of rats. Lower plateau and range are percent change from baseline; midrange, millimeters of mercury.

*P<.05 compared with tap water controls.
abnormality occurring only at extreme blood pressure reductions is not likely to influence the development of hypertension in DOCA-salt rats.

In borderline hypertensive rats fed either a low or high sodium diet, a complete resetting of the baroreceptor reflex occurred only in animals on a high sodium diet, which exhibited a slightly elevated blood pressure. No change in range or sensitivity could be observed with respect to the cardiac or peripheral sympathetic portion of the baroreceptor reflex. Reports of baroreceptor control of RSNA in spontaneously hypertensive rats are inconsistent. The reflex was found normal by some researchers and abnormal by others. However, if hypertension is prevented, baroreceptor reflex control of HR remains normal. Furthermore, a variety of antihypertensive agents were able to restore baroreceptor reflex control of HR and RSNA to normal as long as blood pressure was effectively lowered. In prehypertensive rabbits with renovascular hypertension, the baroreceptor reflex control of HR and RSNA proved to be more sensitive compared with that in control animals.

These heterogeneous data do not permit general conclusions. However, the cited experiments generally suggest that the changes in the sensitivity of baroreceptor function do not play a pivotal role in the development of hypertension but rather are caused by the hypertension itself.

Cardiopulmonary reflexes are not only stimulated by changes in cardiac filling pressure but also by chemical agents. These chemosensitive reflexes are often referred to as the Bezold-Jarisch reflex. Cardiopulmonary reflexes with different sensoric properties might be affected differently in the development of DOCA-salt hypertension. An endogenously occurring substance stimulating the Bezold-Jarisch reflex is serotonin via 5-HT3 receptors.

Circulating levels of serotonin are very low. Increased levels of serotonin can be observed in areas with endothelial damage. We did not investigate specific morphological changes of the heart such as coronary artery damage. However, we assume that significant endothelial lesions in these rats are unlikely at this early stage of DOCA-salt hypertension. We regard as equally unlikely a substantial increase in aggregating platelets releasing serotonin that could alter or desensitize 5-HT3 receptors on cardiac afferent fibers.

During bolus injection of the 5-HT3 serotonin agonist phenylbiguanide, the cardiovascular responses and inhibition of RSNA did not differ between DOCA-salt rats and control animals as opposed to phenylbiguanide infusion. A prolonged stimulation of serotoninergic 5-HT3 receptors for 15 minutes exhibited similar initial responses compared with bolus injection; however, after 4 minutes the RSNA in DOCA-salt animals began to recover toward baseline and was no longer inhibited compared with controls. The prolonged phase of this serotonin-sensitive 5-HT3 reflex is characterized by selective renal sympathoinhibition, without any changes in MAP and HR. A likely explanation for the reduced renal sympathoinhibition in DOCA-salt rats by serotoninergic cardiopulmonary reflexes could be an involvement of central nervous mechanisms in the development of DOCA-salt hypertension as outlined above. Interestingly, these putative central abnormalities did not influence the reflex during short-term stimulation.
Our results suggest that neither the arterial baroreceptor nor cardiopulmonary serotonin-sensitive reflexes are likely to play a role in the development of DOCA-salt hypertension in rats. In contrast, the observed loss of sensitivity of volume-sensitive cardiopulmonary reflexes with respect to RSNA might be genuinely involved in the pathogenesis of high blood pressure in the DOCA-salt model. The impaired control of RSNA by volume-sensitive cardiopulmonary reflexes in prehypertensive Dahl salt-sensitive rats supports the possible importance of these reflexes.5,48

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References


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