PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 58–63 (1984)

# Contributions of Vasopressin and Other Pressor Systems to DOC-Salt Hypertension in Rats (41766)

PETER F. MENTO, HSUEH-HWA WANG, AND WILBUR H. SAWYER

*Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032*

*Abstract.* The roles of arginine vasopressin (AVP), the sympathetic nervous system, and the renin–angiotensin system in maintaining elevated blood pressure in established DOC-salt hypertension in rats were studied by injection of specific antagonists of these systems. The specific AVP antagonist dPVDAVP decreased blood pressure by 19 ± 3 mm Hg in hypertensive rats and 6 ± 2 mm Hg in control rats. In a different group of rats ganglionic blockade with chlorisondamine also caused a greater decrease in blood pressure in DOC-salt rats compared to controls (99 ± 6 vs 58 ± 4 mm Hg, respectively). In rats with autonomic ganglia blocked subsequent vasopressin antagonism decreased blood pressure 29 ± 4 mm Hg in DOC-salt rats and 14 ± 2 mm Hg in control rats. Converting enzyme inhibition with captopril in rats with autonomic ganglia blocked caused a lesser decrease in blood pressure in DOC-salt rats than in controls (8 ± 2 vs 14 ± 2 mm Hg, respectively). These results indicate that both AVP and the sympathetic nervous system contribute to the maintenance of DOC-salt hypertension. The renin–angiotension system appears to be relatively less important.

Arginine vasopressin (AVP) is important in the development of DOC-salt hypertension in rats. AVP accelerates the appearance of this form of hypertension (1) and homozygous Brattleboro rats with diabetes insipidus do not develop hypertension in response to DOC-salt treatment, unless they are also treated with AVP (2, 3). There is, however, some question as to whether AVP contributes directly to the maintenance of established hypertension. Mohring *et al.* (4) found that an antiserum specific for AVP acutely reduced blood pressure in DOC-salt rats, but studies using recently developed specific AVP antagonists gave conflicting results. For example, Crofton *et al.* (2) found that the specific antagonist dPVDAVP decreased blood pressure in DOC-salt rats. Rabito *et al.* (5) reported that dPVDAVP had no effect on blood pressure in the rats in their study. Rascher *et al.* (6) found that the specific AVP antagonist $d(CH_2)_5Tyr(Me)AVP$ did not decrease blood pressure in DOC-salt rats, but it did significantly increase cardiac output.

In the present study we examined the direct role of AVP in blood pressure maintenance in conscious DOC-salt rats by injecting the specific AVP antagonist dPVDAVP. We also estimated the relative contributions of AVP, the sympathetic nervous system, and the renin–angiotensin system by sequential blockade of these systems with appropriate antagonists.

**Materials and Methods.** DOC-salt hypertension was produced in Sherman rats of both sexes weighing about 200 g. A total of 38 males and 32 females were used and approximately equal numbers of males and females were included in each experimental protocol. They were anesthetized with pentobarbital and the left kidney was removed through a flank incision. Penicillin (60,000 units) was given im to prevent infections. One week was allowed for recovery from surgery. Weekly injections of deoxycorticosterone (Percorten Pivalate, Ciba), 30 mg/kg sc, were then started. These rats were given standard laboratory rat chow *ad libitum* and 1% NaCl for drinking water. Another group of rats served as controls. These rats were subjected to similar surgical procedures without the removal of the kidney. Weekly injections of the suspension solution described for Percorten, consisting of 12 mg/ml carboxymethylcellulose, 10.5 mg/ml methylcellulose, 1 mg/ml Tween 80, and 8 mg/ml NaCl, were given in appropriate volumes per weight (1.2 ml/kg). Control rats were also given rat chow *ad libitum* but had tap water to drink. Most rats were studied after 5 weeks of Percorten or vehicle treatment although a few rats were studied at 4 or 6 weeks.

After the treatment periods rats were anes-



0037-9727/84 $1.50

thetized with ether and the left carotid artery and jugular vein were cannulated with PE-50 tubing. The cannulas were brought out under the skin to the back of the rat's head and exteriorized through small skin incisions. The cannulas were then cut short, filled with concentrated heparin solution (1000 units/ml in 0.9% NaCl), and plugged with the cut-off ends of straight pins. Penicillin (60,000 units) was given im to prevent postoperative infections. After recovery from surgery, the animal was studied in the conscious state. A minimum of 4 hr was allowed for recovery. Some rats were studied one to 2 days after surgery. On the day of the experiment the cannulas were connected to longer lengths of PE-50 tubing. The arterial cannula was connected to a Statham P23Dc pressure transducer and pulsatile blood pressure was recorded on a Grass Model 7 or Model 7D polygraph. During all experiments the rats were conscious and the surroundings were kept quiet to minimize fluctuations in blood pressure due to disturbing the rat. All drug injections were made into the venous cannula.

In the first series of experiments the pressor effects of AVP were blocked with the specific antagonist dPVDAVP. This antagonist does not affect the pressor response to other substances such as norepinephrine and angiotensin II (2, 4). A dose of 50 μg of dPVDAVP was used and the effectiveness of the blockade was tested with 10 ng of AVP. Twenty minutes was allowed for recovery from the test dose of AVP before dPVDAVP was given. After 5 min the effectiveness of the blockade was tested with a second dose of AVP.

In the second series of experiments the sympathetic nervous system, AVP, and the renin–angiotensin system were sequentially blocked with chlorisondamine (1 mg/kg), $d(CH_2)_5$Tyr(Me)AVP (10 μg), and captopril (1 mg/kg). A different AVP antagonist was used in these experiments because of limited supplies of dPVDAVP and the subsequent availability of the more potent antagonist $d(CH_2)_5$Tyr(Me)AVP. Chlorisondamine was always given first. In some rats $d(CH_2)_5$-Tyr(Me)AVP was the second and captopril the last blocker used, while in others the order of these two antagonists was reversed. The results were the same with either order of injection and the data were combined for analysis.

The following drugs were used for these experiments: angiotensin II (Hypertensin, Ciba), chlorisondamine (Ecolid, Ciba), ether (Mallinckrodt), sodium heparin (Sigma), penicillin G (Longicil, Fort Dodge Laboratories), pentobarbital (Diabutal, Diamond). Arginine vasopressin, dPVDAVP ([1-deaminopenicillamine, 4-valine, 8-D-arginine]vasopressin, Manning *et al.* (7)), and $d(CH_2)_5$Tyr(Me)AVP ([1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(*O*-methyl)tyrosine]arginine vasopressin, Kruszynski *et al.* (8)) were generously supplied by Dr. M. Manning, Medical College of Ohio, Toledo. Captopril was the generous gift of Dr. Z. P. Horovitz, Squibb Institute, Princeton, New Jersey.

Statistical comparisons between group means were done using a paired or unpaired *t* test, where appropriate (9). When comparisons of several means were needed, an analysis of variance was done first to test for overall significant differences. Comparisons of individual means was then done, if necessary, using the method of the least significant difference (10). Differences were considered to be significant for $P < .05$.

**Results.** The 10-ng test dose of AVP raised mean arterial blood pressure 26 ± 3 mm Hg and this pressor response was reduced by 85–95% after 50 μg of dPVDAVP. Responses to dPVDAVP were recorded as the maximal decrease in blood pressure, which occurred 1 to 2 min after injection. The 50-μg dose of dPVDAVP caused a small decrease in blood pressure in the control group (6 ± 2 mm Hg, n = 12) and a significantly greater decrease in the DOC-salt group (19 ± 3 mm Hg, n = 11)[1]. The responses of individual rats to the 50-μg dose of dPVDAVP are shown in Fig. 1. It might be argued that in rats with higher starting blood pressure the dose of dPVDAVP might not be sufficient to completely antagonize the pressor effects of circulating AVP. To test this possibility, an additional 50-μg dose of dPVDAVP was given to two of the DOC-salt rats. There was no additional de-

[1] Although the male DOC-salt rats as a group had higher mean resting blood pressure than the female rats (165 ± 5, $N = 5$ vs 143 ± 5 mm Hg, $N = 6$, respectively, $P < 0.001$), male and female rats did not differ in their responses to the AVP antagonist (18 ± 4 vs 21 ± 3 mm Hg, respectively).

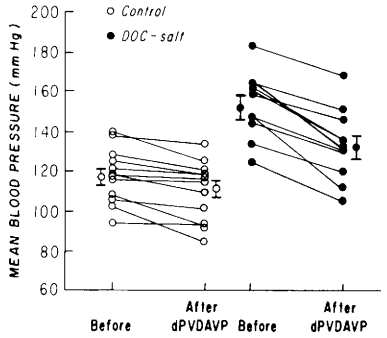


FIG. 1. Responses of individual rats to the 50-μg dose of dPVDAVP. The mean blood pressure ± standard error is also shown for both groups of rats.

crease in blood pressure, suggesting that the 50-μg dose of dPVDAVP was sufficient to antagonize circulating AVP.

The results of the sequential blockade of the sympathetic nervous system, AVP, and the renin–angiotensin system are shown in Table I. After the injection of chlorisondamine the maximal fall in blood pressure was significantly greater in DOC-salt rats than in controls. Mean blood pressure was acutely reduced to about the same level in both groups (64 ± 3 vs 58 ± 2 mm Hg, respectively). After sufficient time (20 to 30 min) for other pressor systems to compensate for this large decrease in blood pressure, the DOC-salt rats still had significantly higher resting blood pressure than control rats (90 ± 5 vs 70 ± 3 mm Hg, respectively, $P < .02$). The subsequent antagonism of AVP by $d(CH_2)_5Tyr(Me)AVP$ and blockade of the renin–angiotensin system by captopril showed a different pattern in the two groups of rats. In the control group both antagonists caused the same average decrease in blood pressure. In contrast, in DOC-salt rats, the decrease in blood pressure after $d(CH_2)_5Tyr(Me)AVP$ was significantly greater than the decrease after captopril.

**Discussion.** The present experiments indicate that AVP contributes to the maintenance of DOC-salt hypertension in rats. The specific AVP antagonist dPVDAVP decreased blood pressure by 19 ± 3 mm Hg in conscious DOC-salt rats. Crofton *et al.* (2) reported a value of 27 ± 5. It is not clear why Rabito *et al.* (5) and Rascher *et al.* (6) found that AVP antagonists did not decrease blood pressure in DOC-salt rats. It is possible that this is due to differences in DOC-salt treatment protocol with respect to dose or length of treatment. The protocol of Crofton *et al.* (2) is closest to that used in the present study: Percorten 30 mg/kg once a week in unilaterally nephrectomized rats. In contrast, Rascher *et al.* (6), gave DOCA (5 mg/kg) twice a day for 7 days and then Percorten (15 mg/kg) three times a week for a total of 4 weeks of treatment. This group of investigators did not perform unilateral nephrectomy as part of the treatment. Rabito *et al.* (5) implanted one strip of DOCA–silicone rubber (100 mg/kg) sc in unilaterally nephrectomized rats. The length of treatment was not specified but the rats studied showed signs of malignant hypertension. It may be, therefore, that in these latter two studies hypertension had progressed into a later stage in which AVP no longer makes a direct pressor contribution.

Data from the present experiments indicate that although AVP contributes to resting blood pressure in DOC-salt rats, it is of relatively minor importance. If AVP were a major contributing factor in maintaining hypertension in DOC-salt rats then we would expect to observe a strong correlation between starting blood pressure and the decrease in blood pres-

TABLE I. EFFECTS OF SEQUENTIAL BLOCKADE OF PRESSOR SYSTEMS ON BLOOD PRESSURE (BP) IN CONTROL AND DOC-SALT RATS[a]

| | | | Mean change in BP after: | | |
|---|---|---|---|---|---|
| | *N* | Baseline BP | Chlorisondamine | Cyclo[b] | Captopril |
| Control | 12 | 125 ± 5 | 58 ± 4 | 14 ± 2 | 14 ± 2 |
| DOC-salt | 12 | 157 ± 7* | 99 ± 6* | 29 ± 4* | 8 ± 2* |

[a] Changes are given as the maximal response after injection of each drug. All values are means ± SE.
[b] Cyclo = $d(CH_2)_5Tyr(Me)AVP$.
* $P < 0.001$ compared to control.

sure in response to AVP antagonism. Figure 2 shows a plot of these two variables for the DOC-salt group. It is clear from this graph that AVP contributes about 20 mm Hg of the blood pressure elevation no matter what the level of hypertension. Also, blood pressure does not return to normal in most of these rats after the administration of an AVP antagonist. This indicates that AVP is not the major factor responsible for maintaining elevated blood pressure in this model.

The importance of the sympathetic nervous system in DOC-salt hypertension has been shown by several studies (11–15). Our results support the contention that increased sympathetic activity plays an important role in the maintenance of DOC-salt hypertension. Blockade of the autonomic ganglia in DOC-salt and control rats lowers blood pressure to about the same level. Matsuguchi and Schmid (19) and Takeda and Bunag (20) also reported that ganglionic blockade lowered blood pressure to about the same level in DOC-salt and control rats. From this observation we might expect to find a strong correlation between starting blood pressure and the decrease in blood pressure in response to blockade of the autonomic ganglia. Figure 3 shows a plot of these variables for both control and DOC-salt rats. The correlation is very good for both groups of rats as indicated by the high correlation coefficients, suggesting that in both groups of rats the sympathetic nervous system is a major determinant of resting blood pressure.

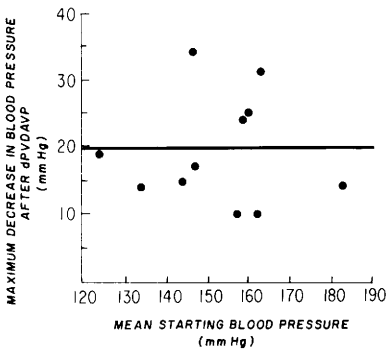


FIG. 2. Decreases in blood pressure after AVP blockade with dPVDAVP (50 μg) as a function of starting pressure, in DOC-salt rats. Each point represents the response of one rat. The least squares regression line is also shown. ($r = -0.016$, $P > 0.4$, $y = -0.008x + 20.6$)

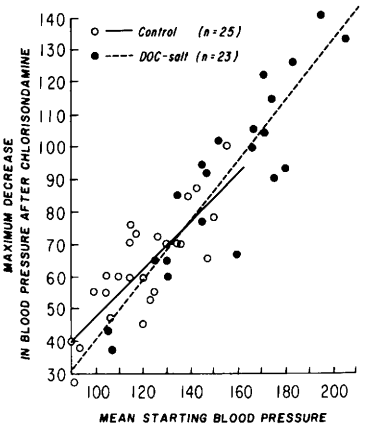


FIG. 3. Decreases in blood pressure after chlorisondamine as a function of starting blood pressure in control and DOC-salt rats. Each point represents the result in one rat. The least-squares regression lines are also shown. (Control: $r = 0.81$, $P < 0.01$, $y = 0.74x - 26.5$; DOC-salt: $r = 0.91$, $P < 0.01$, $y = 0.92x - 51.1$)

After ganglionic blockade and sufficient time for other pressor systems to compensate for the reduced blood pressure, the DOC-salt rats are then able to maintain blood pressure at a level about 20 mm Hg higher than that in the control rats (90 ± 5 vs 70 ± 3 mm Hg, respectively). In control rats, AVP and the renin–angiotensin system are about equally important as back-up systems under conditions of decreased blood pressure, as blood pressure decreased equally when either system was blocked. The order of blockade of these systems was not important. Gavras *et al.* (22) reported that AVP and the renin–angiotensin system are important pressor systems in the absence of sympathetic function, although they gave no estimate of their relative importance. Also, Andrews and Brenner (23) found that in anesthetized, water-deprived rats AVP and the renin–angiotensin system contribute to resting blood pressure even in the presence of intact sympathetics.

In DOC-salt rats with autonomic ganglia blocked, AVP antagonism causes a greater decrease in blood pressure than in controls. In contrast, blockade of the renin–angiotensin system with captopril causes a smaller decrease in blood pressure than in controls under similar conditions. Thus, in DOC-salt hypertension AVP appears to be a more important pressor system than the renin–angiotensin

system under conditions of reduced blood pressure. It has been reported that circulating levels of renin are markedly decreased under resting conditions in DOC-salt hypertension (24) and that converting enzyme inhibition has no effect on blood pressure (25). Our results indicate that even under conditions of reduced blood pressure the renin–angiotensin system is less important as a pressor system than in normal rats. Whether this is due to a change in the mechanisms of the release of renin, the conversion of angiotensin I to angiotensin II, a decreased vascular sensitivity to angiotensin II or some other mechanism is impossible to determine from the experiments reported herein.

After blockade of AVP, the sympathetic nervous system and the renin–angiotensin system mean blood pressure in DOC-salt and control rats is similar (61 ± 3 vs 58 ± 3, respectively). This finding suggests that the effects of these three pressor systems taken together are sufficient to maintain hypertension in DOC-salt rats. It does not appear to be necessary to postulate the contribution of other factors to explain high blood pressure in this hypertensive model.

In summary, the results of our experiments indicate that although increased sympathetic activity appears to be the major contributing factor for the maintenance of hypertension in DOC-salt rats, circulating AVP also contributes to the elevation of blood pressure in this model. In addition, AVP functions as an important back-up pressor system in these rats under conditions of reduced blood pressure and sympathetic dysfunction. In contrast, the renin–angiotensin system appears to be less important both under resting conditions and under conditions of reduced blood pressure.

The authors wish to thank Dr. M. Manning for the generous supplies of AVP and AVP antagonists, and Dr. Z. P. Horovitz for the generous supply of captopril. This work was supported by USPHS Grants HL 12738, GM 07182, and AM 01940.

1. Friedman SM, Friedman CL, Nakashima M. Accelerated appearance of DCA hypertension in rats treated with Pitressin. Endocrinology **67**:752, 1960.
2. Crofton JT, Share L, Shade RE, Lee-Kwon WJ, Manning M, Sawyer WH. The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. Hypertension **1**:31, 1979.
3. Berecek KH, Murray RD, Gross F, Brody MJ. Vasopressin and vascular reactivity in the development of DOCA hypertension in rats with hereditary diabetes insipidus. Hypertension **4**:3, 1982.
4. Mohring J, Mohring B, Petri M, Haack D. Vasopressor role of antidiuretic hormone in the pathogenesis of malignant DOC hypertension. Amer J Physiol **232**:F260, 1977.
5. Rabito SF, Carretero OA, Scicli AG: Evidence against a role of vasopressin in the maintenance of high blood pressure in mineralocorticoid and renovascular hypertension. Hypertension **3**:34, 1981.
6. Rascher W, Lang RE, Taubitz M, Meffle H, Unger Th, Ganten D, Gross F. Vasopressin-induced increase in total peripheral resistance in deoxycorticosterone acetate hypertensive rats is buffered by the baroreceptor reflex. Clin Sci **61**:153s, 1981.
7. Manning M, Lowbridge J, Stier CT Jr, Haldar J, Sawyer WH. [1-Deaminopenicillamine, 4-valine, 8-D-arginine] vasopressin, a highly potent inhibitor of the vasopressor response to arginine vasopressin. J Med Chem **20**:1228, 1977.
8. Kruszynski M, Lammek B, Manning M, Seto J, Haldar J, Sawyer WH. [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-methyl)-tyrosine]arginine vasopressin and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine vasopressin, two highly potent antagonists of the vasopressor response to arginine vasopressin. J Med Chem **23**:364, 1980.
9. Snedecor CW, Cochran WB. Statistical Methods. Ames, Iowa State Univ Press, 7th ed, 1980.
10. Kleinbaum DG, Kupper LL. Applied regression analysis and other multivariable methods. North Scituate, Mass, Duxbury Press, 1978.
11. deChamplain J, Krakoff LR, Axelrod J. Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. Circ Res **23**:479, 1968.
12. deChamplain J, Krakoff LR, Axelrod J. Catecholamine metabolism in experimental hypertension in the rat. Circ Res **20**:136, 1967.
13. Reid JK, Zivlin JA, Kopin IJ. Central and peripheral adrenergic mechanisms in the development of deoxycorticosterone-saline hypertension in rats. Circ Res **37**:569, 1975.
14. deChamplain J, Ameringen MR. Regulation of blood pressure by sympathetic nerve fibers and adrenal medulla in normotensive and hypertensive rats. Circ Res **31**:617, 1972.
15. Ayitey-Smith E, Varma DR. An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats. Brit J Pharmacol **40**:175, 1970.
16. Clarke DE, Smookler HH, Barry H. Sympathetic nerve function and DOCA-NaCl induced hypertension. Life Sci **9**:1097, 1970.
17. Mueller RA, Thoenen H. Effect of 6-hydroxydopamine hydrobromide and adrenalectomy on the de-

velopment of deoxycorticosterone-NaCl hypertension in rats. Fed Proc **29**:546, 1970.
18. Provoost AP, DeJong W. Differential development of renal, DOCA-salt, and spontaneous hypertension in the rat after neonatal sympathectomy. Clin Exp Hypertens **1**:177, 1978.
19. Matsuguchi H, Schmid PG. Pressor response to vasopressin and impaired baroreflex function in DOC-salt hypertension. Amer J Physiol **242**:H44, 1982.
20. Takeda K, Bunag D. Augmented sympathetic nerve activity and pressor responsiveness in DOCA hypertensive rats. Hypertension **2**:97, 1980.
21. Mohring J, Kintz J, Schoun J. Studies on the role of vasopressin in blood pressure control of spontaneously hypertensive rats with established hypertension (SHR, stroke-prone strain). J Cardiovasc Pharmacol **1**:593, 1979.
22. Gavras H, Hatzinikolaou P, North WG, Bresnahan M, Gavras I. Interaction of the sympathetic nervous system with vasopressin and renin in the maintenance of blood pressure. Hypertension **4**:400, 1982.
23. Andrews CE Jr, Brenner BM. Relative contributions of arginine vasopressin and angiotensin II to maintenance of systemic arterial pressure in the anesthetized water-deprived rat. Circ Res **48**:254, 1981.
24. Gavras H, Brunner HR, Laragh JH, Vaughan ED, Koss M, Cote LJ, Gavras I. Malignant hypertension resulting from deoxycorticosterone acetate and salt excess. Circ Res **36**:300, 1975.
25. Douglas BH, Langford HG, McCaa RE. Response of mineralocorticoid hypertensive animals to an angiotensin I converting enzyme inhibitor. Proc Soc Exp Biol Med **161**:86, 1979.

---

Received July 12, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted September 28, 1983.