Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension

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MÖHRING, JAN, BÄRBEL MÖHRING, MARIA PETRI, AND DORIS HAACK. Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension. Am. J. Physiol. 232(3): F260-F269, 1977 — During the onset of malignant hypertension (MH) in rats treated with deoxycorticosterone trimethylacetate (DOC), plasma arginine vasopressin (AVP) concentrations increase tenfold as a consequence of hypovolemia and hyperosmolality. In benign hypertensive (BH) rats, plasma AVP is increased threefold in comparison with control animals. Plasma renin is markedly suppressed in both BH and MH animals. In MH rats, biologically active AVP antiserum lowers blood pressure (BP) transiently to normal or subnormal levels; in BH rats, a small BP-lowering effect of the AVP antiserum is seen. (Biologically active angiotensin II antiserum does not lower BP in MH rats.) The relationship between the height of BP and plasma AVP concentration in DOC hypertensive rats indicates, when compared with that relationship in diabetes insipidus rats infused with AVP, a marked enhancement of the vasopressor effect of AVP. These findings and the earlier observation of vasopressin-induced vascular damage by Byrom (F. B. Byrom, The Hypertensive Vascular Crisis, London: Heinemann, 1969) strongly suggest that ADH is involved as a vasopressor hormone in the pathogenesis of malignant DOC hypertension.

sodium; potassium; renin; renal failure; stroke

COULD VASOPRESSIN, i.e., the antidiuretic hormone (ADH), play a role in the pathogenesis of malignant DOC hypertension similar to that ascribed to the renin-angiotensin system in the pathogenesis of malignant renal hypertension? Answering this question has been the aim of the studies to be presented.

In both malignant renal and malignant deoxycorticosterone (DOC) hypertension the organism becomes volume depleted (3, 14, 30). In spite of decreasing blood volume, blood pressure remains high, indicating that vasopressor systems are activated. It has been predicted by systems analysis that in malignant hypertension volume depletion will activate vasopressor systems to an extent that blood pressure will remain high (16). In malignant renal hypertension increasing plasma renin and angiotensin concentrations induce progressive vasoconstriction, whereby high blood pressure levels are maintained (3, 9, 30). However, in malignant DOC hypertension the activity of the renin-angiotensin system remains markedly suppressed (14). Therefore, in this latter situation vasoconstrictors other than the renin-angiotensin system should become activated, such as the sympathetic nervous system or vasopressin, or both.

Although no direct evidence has been presented so far to support the notion that endogenous vasopressin could act as a vasopressor hormone, Szczepanska-Sadowska (38) and Cowley et al. (8) have recently presented indirect evidence that vasopressin induced vasoconstriction might play an important role even in normal blood pressure regulation. We did consider such a possibility for the pathophysiological situation of malignant DOC hypertension in rats. Here hypovolemia (14) and hyperosmolality (observation from an unpublished study) develop, both of which are the physiological stimuli for vasopressin release (11). It has been repeatedly demonstrated since the early work of Byrom (ref. 4, p. 57-58) that pharmacological doses of vasopressin might rapidly induce vascular damage (22, 25).

MATERIALS AND METHODS

General experimental protocol. Male Wistar rats (WU strain, Ivanovos) weighing 200-230 g were placed in individual cages in a room with constant temperature (23 ± 1°C) and humidity (60 ± 5%) that was lighted automatically from 6 A.M. to 6 P.M. The animals were fed a commercial diet (ssniff) containing 100 meq sodium/kg and 210 meq potassium/kg. Fresh demineralized water was given every 2 days. After 5-7 days the rats were submitted to unilateral nephrectomy and were subsequently offered, instead of water, 1% NaCl as drinking fluid. Deoxycorticosterone trimethylacetate (DOC) was injected subcutaneously at a weekly dosage of 12.5 mg to one group of animals (n, 76), while the other rats (n, 24) were injected with 0.5 ml of 0.9% NaCl, the vehicle only. Systolic blood pressure (BP) was recorded once or twice a week by tail plethysmography under light ether anesthesia. Body weight, saline and food intake were measured every 2nd or 3rd day at 10 A.M., and when the experiment progressed daily measurements were done.

Experiment I. When, during the 4th-7th wk after the start of DOC treatment, signs of malignant hypertension (MH) had become apparent in some of the rats for at least 3 days, i.e., systolic BP values of 170-190 mmHg or above and weight loss (for details, see RESULTS), these animals, as well as rats that did not exhibit such signs, i.e., benign hypertensive (BH) rats, and the control animals were decapitated at 9 A.M. (blood sampling was performed in 38 hypertensive and in 12 control animals). Blood was collected in heparinized glass capillaries and
in heparinized plastic tubes. The collected blood was centrifuged, and the hematocrits and plasma sodium and urea concentrations were determined; osmolalities were measured by freezing-point depression (Knauer osmometer). Arginine vasopressin concentrations were estimated in 2 ml of plasma by a recently developed radioimmunoassay (26). After blood collection, the kidneys were excised, fixed in 10% Formalin, and thin paraffin sections were stained with periodic acid-Schiff and examined.

**Experiment II.** Seven control, seven BH, and seven MH rats were submitted to the same protocol as in experiment I, except that blood sampling was performed in a different way: the animals were anesthetized with ether and 1 ml of blood was taken from the cut tail for the measurement of hematocrit and plasma renin concentration (33). Then a catheter was placed in the jugular vein for further blood sampling, and plasma sodium and potassium concentrations were determined.

**Experiment IIIa.** In order to assess a possible vasoconstrictor effect of plasma arginine vasopressin (AVP) in rats with benign and malignant DOC hypertension, rabbit sera containing specific AVP antibodies were used. Prior to such studies, the biological activity of these antiserums [antiserum “Erwin,” “Kaspar,” and “Oskar” according to the names of the immunized rabbits (26)] were evaluated. The biochemical characteristics of AVP antibody Erwin were as follows: titer, 1/6,000; $K_a = 0.9 \times 10^{-11}$ 1/mol; cross reactivity with lysine vasopressin 100%, with oxytocin < 0.01%, with arginine vasotocin < 0.01%, with angiotensin I < 0.0001%, and with angiotensin II < 0.0001%. The antiserum Kaspar had a titer of 1/3,750 and the $K_a$ equaled $1.3 \times 10^{-11}$ 1/mol; the respective values for AVP antiserum Oskar were 1/9000 and $K_a = 0.7 \times 10^{-11}$ 1/mol. The antiserum II antiserum had a titer of 1/160,000, and the $K_a$ equaled $1 \times 10^{-11}$ 1/mol. Rats with hereditary hypothalamic diabetes insipidus (DI) (39) were nephrectomized under ether anesthesia, and one PE-10 catheter was placed in the left femoral artery while two other PE-10 catheters were inserted into the left femoral vein. The catheters emerged at the neck. Three to four hours after surgery the arterial catheter was connected to a Statham transducer (P23Db), and the BP was recorded continuously in the conscious animal which could move freely in its cage and did so. Then the following experiments were performed:

1) To two DI rats, which received no further treatment, 0.4 ml of the AVP antiserum Erwin was injected. 2) To four DI rats an infusion of arginine vasopressin (kindly supplied by Dr. Eva Sedlakova, Academy of Science, Prague) was infused at a rate of 37.5 or 60 rig/h (about 150-260 ng/kg per h), which increased BP by 35 and 45 mmHg. When the BP had stabilized, 0.2 ml of an angiotensin II antiserum was injected. After the BP had returned to the preinjection level for 15 min, 0.2 ml of the AVP antiserum Erwin was given.

**Experiment IIIb.** In 15 rats exhibiting malignant DOC hypertension, in 9 rats with benign DOC hypertension, and in 5 control rats one PE-10 catheter was placed in the femoral artery and another catheter in the femoral vein. Two or three hours after surgery, the arterial catheter was connected to a Statham transducer; 20 min later the experiment was started by injecting the various rabbit sera. Of the AVP antiserum Erwin, 0.2 ml were injected into six MH rats, and 0.4 ml into five other MH animals. Two BH rats were given 0.2 ml of the antiserum Erwin, and seven BH rats as well as five control animals were injected with 0.4 ml of this AVP antiserum. Four of the MH rats and two of the control rats were given 0.4 ml angiotensin II antiserum either before or after the injection of the AVP antiserum. In addition, 0.4 ml of an angiotensin I antiserum (titer 1/1,000) was injected into two MH rats. Finally, 0.4 ml serum of a normal rabbit was given to three of the MH rats, to four of the BH rats, and to three of the control animals. Angiotensin I and II antisera as well as the control serum were injected either before or after the injection of the AVP antiserum. To two of the four remaining MH rats 0.4 ml of the AVP antisera Kaspar and Oskar was given.

At the end of these studies, the animals were anesthetized with ether, the kidneys were excised and fixed in 10% Formalin for histological examination.

**Statistics.** All values in the text and figures are means ± SE. The significance of difference between mean values was evaluated by the Student $t$ test. Regression equations and correlation coefficients were calculated by the method of least squares.

**RESULTS**

In all rats treated with DOC blood pressure increased progressively. In most of those rats in which aortic BP reached a level of 170-190 mmHg during the 4th–7th experimental wk weight started to decline and the general condition deteriorated in most of these rats. In the other hypertensive animals body weight continuously increased, as in untreated control rats. In 34 of the 41 hypertensive rats that exhibited weight loss (which reflects sodium and water depletion according to the balance studies of Gavras et al. (14)), signs of malignant nephrosclerosis were found, such as arteriolar sclerosis, fibrinoid insudation of the vascular wall, and fibrinoid necrosis. However, the degree of histological alterations varied remarkably, being moderate in some of these animals. Furthermore, during the daily handling period it was observed that in most of the weight-losing rats the eyes became remarkably pale. Eighteen of the 41 weight-losing rats showed signs of a cerebral vascular...
crisis, such as twitching movements, uncoordinated motor activity, convulsions, etc. [described in detail by Byrom (4)]; four other rats died during the dark period. All these observations demonstrated that in the weight-losing animals the course of high blood pressure had become malignant. Therefore, these animals are called malignant hypertensive rats, while the other hypertensive animals are referred to as benign hypertensive rats.

In five hypertensive rats, in which BP was as high as in MH rats, but in which body weight did not fall continuously during the days prior to blood sampling, arteriolosclerosis in the kidney was pronounced, but no fibrinoid inclusions or fibrinoid necrosis could be detected after repeated examination. Since these animals seem to represent a group of "borderline cases" with respect to malignant hypertension, they will be described separately at the end of this section. In addition, the data of four rats, in which at the day prior to blood collection BP was rather low in comparison with the other MH rats, and which exhibited most severe signs of malignant hypertension, will also be summarized separately.

**Experiment 1.** One or two days before the end of the studies, systolic BP levels of the MH rats were higher (range, 170-200 mmHg) than in the BH animals (range, 130-165 mmHg), as may be seen from the data presented in Fig. 1. Body weight fell in the MH rats, while in the BH animals daily weight gain was almost similar to that of the controls. Saline intake was significantly increased in the BH animals when compared with control rats (Fig. 1), while food intake was similar. In the MH rats, saline intake was further increased during the last 3 days prior to blood collection and food intake was reduced (Fig. 1).

In Fig. 2 a typical case report is given for an MH rat and compared with the case report of a BH animal. It may be seen that in both animals the BP increased progressively after the start of DOC administration at day 0. In rat 7, BP leveled off between 160 and 170 mmHg during the 5th-6th experimental wk, body weight increased steadily, and saline and food intake showed minor fluctuations. In the other animal (rat 12), BP increased to higher levels, and when it had reached a range of 180-190 mmHg, daily weight gain stopped and then started to fall. During the first 3 days of weight loss, food intake remained constant, but subsequently also declined. Such a reduction of food intake by at least 3 g had been observed in 8 of the 15 MH rats included in Fig. 1. When weight started to decline, saline intake slightly decreased in 12 of the 15 MH rats by 10 to 25 ml for 2-10 days. At day 33 of the experiment shown in Fig. 2, the MH animal started to recover. Daily weight gain and food intake normalized, and saline intake increased remarkably (such an increase was seen in four of the six MH rats in which a similar recovery occurred within the period of observation). During the last 2 experimental days, rat 12 (Fig. 2) started to lose weight again, i.e., a second malignant phase became apparent. Food intake again showed a tendency to decline, but now saline intake remained increased (this was seen in four of the six MH rats exhibiting a second malignant phase).

As can be seen in Fig. 3, the plasma arginine vasopressin concentrations of the MH rats were increased tenfold in comparison with the control animals. The plasma AVP concentrations of MH rats decapitated during a second malignant phase (six rats) fell into the range of those values obtained from MH rats during the first malignant phase (nine rats). In the BH animals, plasma AVP concentrations were increased threefold (Fig. 3).

Osmolality was moderately elevated in the BH animals as compared with the control rats ($P < 0.05$). In the MH rats it increased further (Fig. 3; $P < 0.01$). Plasma sodium concentrations were 139.9 $\pm$ 0.7 meq/liter in controls, 141.8 $\pm$ 0.7 meq/liter in BH rats ($P < 0.02$ in comparison with controls), and 146.6 $\pm$ 1.5 meq/liter in MH animals ($P < 0.01$). Plasma urea concentrations of these three groups were $7.7 \pm 0.2$ mM, $7.1 \pm 0.2$ mM ($P < 0.05$), and $10.0 \pm 0.9$ mM ($P < 0.01$), respectively. Hematocrit values were $44.1 \pm 0.5\%$ in controls, $44.2 \pm 0.3\%$ in the BH rats, and $42.0 \pm 1.7\%$ in the MH animals. However, in those nine MH rats sacrificed during the first malignant phase, i.e., during the onset of the malignant course of DOC hypertension, the hematocrits were significantly higher than in BH and control rats ($46.3 \pm 0.8\%, P < 0.01$). In each of the other six animals, hematocrit values were below the lowest normal value measured in control rats (i.e., below 42.2%), the mean value being $35.3 \pm 2.2\%$ (range, 26.1-41.4%).

When the values of plasma AVP concentrations were plotted against the respective values of plasma osmolality for all groups studied (i.e., control rats, benign and malignant hypertensive rats, and the borderline cases) a statistically significant correlation was obtained ($r = 0.52; P < 0.001$). Such a correlation was also found between plasma AVP and sodium concentrations ($r = 0.64; P < 0.001$) and between blood pressure and plasma AVP ($r = 0.67; P < 0.001$). However, within each of the experimental groups no such correlations existed. In fact, in the group of MH rats plasma AVP could be rather low although hematocrit, osmolality, and plasma
Sodium were relatively high (e.g., 4.6 pg/ml, 50.8%, 318 mosmol/kg, and 150.1 meq/liter); and the opposite pattern could be also found (e.g., 11.5 pg/ml, 45.0%, 305 mosmol/kg, and 143.7 meq/liter).

Five rats with DOC hypertension in which kidney arteriolosclerosis was pronounced but in which no fibrinoid insudation or necrosis could be detected after repeated examination were regarded as borderline cases with respect to malignant hypertension. Systolic BP was 180 ± 4 mmHg (range, 165-190 mmHg). During the last 5-12 days of the study weight increased or decreased from day to day (maximum ± 5 g), the mean daily weight gain being ± 1.7 ± 0.1 g; saline intake was 44.7 ± 5.0 ml/day, and food intake 17.1 ± 1.0 g/day. Hematocrit was 43.9 ± 0.1%, plasma sodium concentration 143.3 ± 1.1 meq/liter, plasma urea concentration 8.1 ± 0.7 mM, plasma osmolality 300.2 ± 1.1 mosmol/kg, and plasma AVP concentration 6.7 ± 0.7 pg/ml (P < 0.01, as compared with control or MH rats).

In four MH rats that exhibited the most severe signs of a cerebral vascular crisis, BP was difficult to measure (as in other MH rats) at the day prior to blood sampling; it ranged between 90 and 135 mmHg, while it was 173 ± 7 mmHg 3-5 days before. Weight fell by 6.0 ± 2.1 g during the last 3 days of the study; saline intake was 35.7 ± 8.0 ml/day and food intake 12.3 ± 2.2 g/day. In these four animals, hematocrit values were 47.9, 53.5, 51.3, and 48.6%, plasma sodium concentrations 143.7, 140.5, 143.7, and 140.5 meq/liter, plasma urea concentrations 10.5, 5.5, 8.9, and 10.0 mM, plasma osmolalities 314, 318, 284, and 294 mosmol/kg, and plasma AVP concentrations 4.4, 0.9, 4.4, and 4.1 pg/ml.

**Experiment II.** Plasma renin concentrations were markedly reduced in BH and MH rats, the mean values...
being $5.4 \pm 0.4$ µg AL/ml per h for seven BH animals and $5.8 \pm 0.3$ µg AL/ml per h for seven MH rats, compared with $43.6 \pm 3.8$ µg AL/ml per h for seven control rats. Plasma sodium concentrations were $141.6 \pm 0.5$ in controls, $142.9 \pm 0.8$ in BH rats, and $145.5 \pm 0.7$ meq/liter in MH animals ($P < 0.01$ as compared with BH rats); plasma potassium concentrations were $4.30 \pm 0.11$, $3.49 \pm 0.06$ and $3.56 \pm 0.15$ meq/liter ($P < 0.01$ for BH and MH rats compared with controls).

Experiment IIIa. In the two nephrectomized diabetes insipidus rats that were given no infusion, the injection of 0.4 ml of AVP antiserum Erwin did not affect BP. In 11 other DI rats the infusion of AVP at a rate of 140–260 ng/kg per h increased mean BP from $92 \pm 2$ mmHg (range, 85–100 mmHg) to $133 \pm 14$ mmHg (range, 105–150 mmHg), the smallest increase being 15 and the greatest 50 mmHg. In seven of these rats, plasma AVP was measured when the BP had reached a plateau, and the concentrations varied between 115 and 157 pg/ml (mean, $136 \pm 6$ pg/ml). In the four other rats, the injection of 0.2 ml of AVP antiserum Erwin induced a fall of BP of 10–25 mmHg (mean $16 \pm 3$ mmHg). Within 2 min after the injection BP started to decline and reached a minimum within the next 2–6 min. Then BP started to rise again, and it reached the preinjection level 10–15 min after the injection of the antiserum. (A typical experiment is presented in Fig. 4.) In two other DI rats the injection of 0.4 ml of AVP antiserums Kaspar and Oskar did not affect BP. Similarly, after the injection of 0.4 ml of serum obtained from a normal rabbit in two of the above DI rats BP remained unchanged (Fig. 4).

In two DI rats the infusion of angiotensin II at a rate of 0.45 and 0.6 µg/h increased BP by 35 and 45 mmHg, respectively. Within 1 min after the start of the 0.2-ml injection of angiotensin II antiserum, the BP started to fall, and it rapidly reached a minimum 25 and 40 mmHg below the preinjection level. Within 2–4 min the BP returned to preinjection level (Fig. 4). When, in these two animals, 0.2 ml of AVP antiserum was injected, the BP remained unaffected (Fig. 4).

Experiment IIIb. In each of the 11 MH rats that were injected with 0.2 or 0.4 ml of the biologically active AVP antiserum Erwin, the BP started to decline within 1–3 min after the start of the injection (Fig. 5); within the subsequent 2–6 min, the BP reached a minimum and started to increase again, reaching the preinjection level in 25–100 min (mean, $52 \pm 8$ min in the 11 MH rats studied). After the injection of 0.2 ml of AVP antiserum, mean BP fell by $62 \pm 5$ mmHg ($n$, 6; Fig. 6), and after the injection of 0.4 ml by $82 \pm 8$ mmHg ($n$, 5; $P < 0.03$ when compared with the mean value after 0.2 ml). After injection of 0.4 ml of angiotensin II antiserum (four MH rats), 0.4 ml of angiotensin I antiserum (two MH rats), and 0.4 ml of serum obtained from a normal rabbit (three MH rats), the BP remained virtually unchanged. In each of two other MH rats, the injection of 0.4 ml of the two biologically inactive AVP antiserums Kaspar and Oskar did not affect BP.

The injection of 0.2 ml of AVP antiserum Erwin to two BH rats did not affect the BP. But the injection of 0.4 ml of this antiserum to seven BH rats induced a transient fall of mean BP, which ranged from 15 to 35 mmHg.

![FIG. 4. Evaluation of biological activity of antiserum. Effect of vasopressin (AVP) or angiotensin II (Ang II) antiserum on blood pressure of 2 individual rats with diabetes insipidus, which were infused either with arginine-vasopressin (upper panel) or with angiotensin II (lower panel). For further details see text.](http://ajprenal.physiology.org/)

![FIG. 5. Effect of vasopressin (AVP) or angiotensin II (Ang II) antiserum on blood pressure of a conscious unrestrained rat exhibiting a benign course of DOC hypertension (rat 40) and of a rat with malignant DOC hypertension (rat 49).](http://ajprenal.physiology.org/)
In recent balance studies, the onset of malignant hypertension in rats with unilateral renal artery stenosis and an untouched contralateral kidney has been further analyzed (27, 30, 32). Based on this analysis and on similar clinical observations we have extended the vicious circle concept of Wilson and Byrom. When blood pressure increases to a critically high range, renal salt and water loss ensues, resulting in hypovolemia; subsequently, the renin-angiotensin system is markedly activated, whereby high blood pressure levels are maintained and a vicious circle situation develops. Due to progressive vasoconstriction and hemoconcentration in the presence of high blood pressure, microcirculation and vascular permeability are altered to an extent that finally induces the chain of events that result in vascular damage. Consistent with this concept was the crucial observation that under a self-selection regimen rats with malignant renal hypertension took large amounts of saline whereby they compensated for renal salt and water loss; subsequently, all signs of malignant hypertension either did not develop or they nearly or completely disappeared, although blood pressure levels were finally as high as before (32). In addition to these observations, which clearly indicated that a sudden salt and water loss triggered the onset of malignant hypertension, it could be demonstrated that by activation of the renin-angiotensin system subsequent to salt and water depletion high blood pressure levels were maintained. In rats with unilateral renal artery stenosis, the competitive angiotensin II antagonist saralasin lowered blood pressure more effectively the higher the plasma renin concentrations were (15, 23); and similar results have been obtained with pepstatin, an inhibitor of the renin-angiotensinogen reaction (unpublished observations).

Obviously, the concept outlined above cannot be ascribed to the pathogenesis of those forms of malignant hypertension in which the renin-angiotensin system is not activated (3, 13, 24). This is particularly the case in malignant DOC hypertension of rats. Here, in fact, the renin-angiotensin system remains markedly suppressed, as Gavras et al. have recently shown (14) and as has been confirmed in the present studies. Furthermore, the angiotensin II antagonist saralasin (14) as well as a biologically active angiotensin II antiserum (present studies) did not lower BP in this pathophysiological situation. Since BP levels remained high in spite of decreasing blood volume, vasopressor systems other than renin-angiotensin should have become activated. Based on the findings of an unchanged renal norepinephrine content and on the absence of an activation of the suppressed renin-angiotensin system in rats with malignant DOC hypertension, Gavras et al. (14) concluded "absence of positive evidence for a role of vasoconstrictor substances in the pathogenesis of vascular disease." The findings made in the present studies render it unnecessary to draw such a "conclusio per exclusionem".

The experiments presented in this communication followed the experimental protocol and findings of Gavras et al. (14). In balance studies, these authors had shown that, similarly to malignant renal hypertension...
The findings reported in this communication confirm that experimental approach.

In most of the animals in which BP increased to a range of 170–190 mmHg, weight loss occurred during the period of observation, i.e., during the 4th–7th wk after the start of DOC treatment. It was only in these animals that signs of a vascular crisis were observed, such as malignant nephrosclerosis or stroke, and some of these animals died. The fact that such signs of a vascular crisis were not found in all of the weight-losing rats (and in none of the borderline cases) indicates that the chain of events resulting in vascular damage was most likely triggered subsequent to the onset of sodium and water loss.

The course of malignant DOC hypertension appeared to be phasic (14). After a period of weight loss, during which signs of a vascular crisis became apparent, the animals could recover with respect to daily weight gain, food intake, and general condition. But after some days elapsed, a further malignant phase developed (see Fig. 2). This is exactly the same feature which has been described for rats with malignant renal hypertension (27). In addition, in both pathophysiologic situations renal function is impaired, as indicated by an increase of plasma urea concentration (13, 32, 41). Furthermore, volume depletion was reflected in an increase of hematocrit in both malignant renal and malignant DOC hypertension (14, 30). But in DOC hypertensive rats it was only during the onset of the malignant course that hematocrit was found to be elevated. When hematocrit was measured during the later course of malignant DOC hypertension, it was always found reduced. Such a reduction in hematocrit of the rats with malignant DOC hypertension has been demonstrated to be due to microangiopathic hemolytic anemia (13, 41), and it is found in spite of volume depletion (14). Since in the present studies hematocrits were always determined during an apparent malignant phase, i.e., during a phase of sodium and water loss, the reduced hematocrits of the animals exhibiting a second malignant phase most likely did not reflect hypervolemia. In contrast to malignant DOC hypertension, we have only occasionally observed such a reduction of packed cell volume in malignant renal hypertension of rats (unpublished observation).

Negative sodium balance of rats exhibiting a malignant course of DOC hypertension (14) was not reflected in a reduction of plasma sodium concentration, as is the case in malignant renal hypertension (30, 32). In fact, plasma sodium concentration increased. This difference could be explained to a certain extent by the difference between the experimental protocols. While malignant renal hypertensive rats could drink water only in order to compensate for volume depletion and, thereby, could "dilute" their extracellular sodium, rats with malignant DOC hypertension could drink only 1% saline, i.e., a drinking solution containing 171.1 meq sodium/liter. However, this explanation would only be valid if DOC hypertensive rats augment their saline intake during the onset of malignant hypertension, when they start to lose sodium and water. But this has not been observed in most of the animals; and some of the rats even had a reduced saline intake, when blood sampling was performed and elevated plasma sodium concentrations were measured. Therefore, animals with malignant DOC hypertension seem to lose more water than sodium. Provided that extrarenal water loss did not change substantially, this implies that in comparison with benign hypertensive rats the urine should have become less concentrated. Such a possible impairment in the concentrating capability of the kidney has been related to the increasing BP per se in rats with renal and spontaneous hypertension (37).

The increase in plasma sodium concentration was the main factor for increasing plasma osmolality during the onset of malignant DOC hypertension. Together with hypovolemia the hyperosmolality should have stimulated vasopressin release (11), thereby increasing plasma vasopressin concentration in rats with malignant DOC hypertension. The rise of plasma vasopressin concentration, as well as the concomitant increase of plasma sodium concentration, might have counterbalanced the stimulating effect of hypovolemia on renal renin release (40). Thus, the activity of the renin-angiotensin system remained markedly suppressed. Furthermore, the increased plasma concentration of vasopressin might have increased renal vascular resistance, and thereby reduced the GFR (36), and, thus, induced a rise of plasma urea concentration. Arteriolar and glomerular damage might have contributed to the rise of plasma urea in those malignant hypertensive rats where it was present.

In rats exhibiting a benign course of DOC hypertension, plasma vasopressin concentration was also increased in comparison with normotensive control rats, but by far less than in malignant hypertensive animals. That increase could have been the consequence of a moderate rise of plasma sodium concentration as well as of plasma osmolality, which have been observed in the present studies. Since vasopressin release seems already to be stimulated on the first day of DOC administration (28), this finding indicates that plasma vasopressin concentration could be elevated throughout the course of DOC hypertension. Thus, the pattern of change in plasma vasopressin concentration closely resembled the change of plasma renin and angiotensin II concentration observed during the development of renal hypertension in rats (30–32). This increase of plasma vasopressin concentration could be of great pathogenetic significance, not only during the malignant course but also during the development of DOC hypertension in rats, since Friedman et al. (12) noticed either no or a markedly attenuated BP increase in lesion-induced diabetes insipidus rats treated with DOC for 3 wk.

Based on the findings of an increase in plasma vasopressin concentration in rats exhibiting a benign course of DOC hypertension and of a further increase in rats with malignant DOC hypertension, an attempt has...
been undertaken in the present studies to clarify whether vasopressin exerted vasoconstriction in both of these situations. For this purpose a vasopressin antiserum was used, which proved to be biologically active. "Biological activity" has been defined as the capability of a vasopressin antiserum to lower BP in rats with hypothalamic diabetes insipidus in which BP was raised by vasopressin infusion. Such a blood pressure-lowering effect was not seen when BP was raised by the infusion of angiotensin II. Furthermore, BP was not affected by the vasopressin antiserum when no infusion of vasopressin was given.

Only one of the three vasopressin antisera tested proved to be biologically active, although the in vitro characteristics, which had been evaluated at 4°C under equilibrium conditions, and in Tris buffer (26), appeared to be similar. Thus, the similarity of these in vitro characteristics did not necessarily imply similarity to in vivo activities. A possible explanation for this discrepancy could be that, despite similar affinity constants of various antibodies, their initial rate constants of equilibrium, which would determine their acute effects in vivo, might vary considerably, especially at different temperatures (E. Hackenthal, personal communication) But, unfortunately, very little is known about the in vivo kinetics of antibody-antigen associations, i.e., about how antibodies inhibit the effects of hormones in vivo. Recently, it has been shown that, in vivo, angiotensin II antibodies equilibrate within minutes with their antigen and that fractional antibody occupation seems to be remarkably small, when the antigen plasma concentration falls into the range of the affinity constant of the antibody (33). There the degree of antibody occupation would be determined by the antigen concentration in the medium; on the other hand, the biological effect of an antibody would depend critically on its plasma concentration, because this determines the amount of antigen bound and, hence, the inhibitory effect of the antibody. Thus, at low antigen concentrations an inhibitory effect of the antiserum would become apparent with the increasing volume of antiserum injected. Furthermore, for a given plasma concentration of the antibody its inhibitory effect would be enhanced with increasing plasma antigen concentrations; but if antigen concentrations are raised to "supramaximal" levels (as has possibly been done in the experiments in which vasopressin had been infused), the inhibitory effect of the antibody would, for a given antibody plasma concentration, progressively decline. Finally, since the antibody interferes with the steady-state system of secretion rate and metabolic clearance rate of the antigen, these two variables will determine the duration of the inhibitory effect of the antibody, i.e., the time until the original plasma antigen concentrations are reestablished.

The injection of the biologically active vasopressin antiserum in rats with malignant DOC hypertension induced a transient fall of BP to normal or even subnormal levels. (The injection of the two inactive vasopressin antisera, of a biologically active angiotensin II antiserum, of an angiotensin I antiserum with low titer, and of a serum from a normal rabbit did not affect BP in the malignant hypertensive animals). Thus, arginine vasopressin appears to act as a vasopressor hormone in this pathophysiologic situation. Consistent with this conclusion are observations made in some of the MH animals, in which BP had fallen to rather low levels during the days prior to blood sampling. In these rats, plasma vasopressin concentrations were similar to those of BH rate. Furthermore, in those rats that represented a group of borderline cases and in which hypovolemia was not present despite day-to-day variations of body weight (see RESULTS), plasma vasopressin as well as BP levels were higher than in BH animals.

In rats with benign DOC hypertension, the vasopressin antiserum also lowered BP transiently. But the decrease of BP was less by far than in malignant hypertensive rats. This suggests that plasma vasopressin participates in the maintenance of high BP in rats with benign DOC hypertension, but its quantitative contribution seems to be of minor importance in malignant hypertensive animals. In control rats, the injection of 0.4 ml of the vasopressin antiserum never induced a fall of BP. This finding would be consistent with the notion that vasopressin is not involved as a vasoconstrictor hormone in normal blood pressure regulation. However, as outlined above, lack of an inhibitory effect of an antibody should be interpreted with caution.

It could be argued that the injection of the AVP antiserum induced a fall of BP by blocking AVP-mediated antidiuresis and thus causing rapid volume depletion. However, from studies in DI rats (39) it is apparent that such a diuresis might only amount to 0.2 ml per min in animals weighing 300 g, which would equal a reduction of extracellular fluid volume of about 3%/10 min. This figure cannot account for the marked fall of BP observed in the present studies.

Besides the use of antibodies or competitive antagonists, another approach often is used to demonstrate that the measured increase in plasma concentration of a vasopressor hormone caused the given increase of BP: infusing the vasopressor hormone and relating the induced plasma concentrations to the increase of BP (2, 7). Such an approach has been successful in elucidating the role of the renin-angiotensin system in the pathogenesis of renal hypertension (9, 5, 9, 19). Furthermore, such studies have also demonstrated that a marked enhancement of the BP-elevating effect of the given plasma renin or angiotensin II concentrations may occur in various hypertensive states.

From the present studies it becomes apparent that a vasoconstrictor effect of vasopressin in DOC hypertension of rats should also be markedly enhanced. In the diabetes insipidus rats that were infused with vasopressin, BP increased only to levels found in the benign hypertensive animals, although the induced plasma vasopressin concentrations were eventually about 40 times higher. The mechanism underlying such a change in sensitivity has not been elucidated in the present studies, but recent experiments performed by Cowley et al. (8) could provide some explanation. These authors have evaluated the BP-increasing effect of exogenous vasopressin in dogs under varying experimental conditions. When the baroreceptor reflex was interrupted by derer-
viation, the dose-response curve was shifted to the left by a factor of 60–100. When such studies were performed under angiotensin II or noradrenaline infusion (8), the effect was less by far. Thus, the baroreceptor reflex seems to be sensitized, i.e., its feedback gain increased, under vasopressin infusion as compared with angiotensin II or noradrenaline infusion. Finally, Cowley et al. showed that in decapitated dogs that were given an infusion of noradrenaline in order to maintain normal BP levels, the dose-response curve for vasopressin was further shifted to the left, the sensitivity being increased now by a factor of 8,000. Such an increase in the sensitivity to the vasoconstrictor effect of neurohypophyseal hormones by catecholamines (and vice versa) has been repeatedly demonstrated (1, 6) since the early work of Kepinow (20) in 1912.

These observations could be most significant for understanding the vasoconstrictor effect of vasopressin in DOC hypertension of rats. It has been shown that the metabolism of catecholamines is altered in DOC hypertension, the result being a moderate increase in circulating noradrenaline concentration (10, 35). Furthermore, hyperresponsiveness to exogenous vasopressin as well as to noradrenaline has been demonstrated in DOC hypertension of rats (17). Thus, a noradrenaline-mediated increase in the sensitivity of vascular smooth muscles as well as a dampened sensitivity of the baroreceptor reflex could underlie the greatly enhanced vasoconstrictor effect of vasopressin in DOC hypertension of rats. This suggestion implies an interrelated role of vasopressin and the sympathetic nervous system in the pathogenesis of DOC hypertension as well as of its malignant course.

In summary, the findings made in the present studies strongly suggest that in the pathogenesis of malignant DOC hypertension vasopressin might play a role similar to that ascribed to the renin-angiotensin system in malignant renal hypertension. This conclusion is based on the demonstration of increased plasma vasopressin concentrations in malignant DOC hypertension of rats, on the BP-lowering effect of a specific vasopressin antiserum, and on the earlier demonstration of vasopressin-induced vascular damage by Byrom (4). Preliminary reports from our laboratory suggest that the neurohypophysial vasopressor principle might also induce systemic vasoconstriction during the development of malignant renal hypertension (29) and of acute renal failure in rats (18). Finally, other studies have reported that in man urinary excretion rates of vasopressin are increased in essential hypertension (21) and that plasma vasopressin levels are elevated during malignant hypertension (34).

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REFERENCES

16. Gutter, A. C., T. G. Coleman, J. D. Bower, and H. J. Gran-
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