Relative Contributions of Arginine Vasopressin and Angiotensin II to Maintenance of Systemic Arterial Pressure in the Anesthetized Water-Deprived Rat

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SUMMARY We used a structural analogue of arginine vasopressin (AVP) and investigated the role of AVP in the maintenance of mean arterial pressure ($\overline{AP}$) in anesthetized, water-deprived rats. The administration of [1(β-mercapto-β,β-cyclopentamethylene propionic acid) 4-valine-8-D-arginine] vasopressin, d$(CH_2)_5$VDAVP, completely inhibited the 30–40 mm Hg rise in $\overline{AP}$ which normally accompanied the administration of 50 mU exogenous AVP (group 1). Thus, d$(CH_2)_5$VDAVP is a specific antagonist of the vascular effects of AVP. d$(CH_2)_5$VDAVP failed to significantly alter $\overline{AP}$ in water diuretic rats (group 3) and was without effect on urine osmolality during water diuresis or antidiuresis. However, bolus injection of d$(CH_2)_5$VDAVP into water-deprived rats (group 2) prompted an abrupt fall in $\overline{AP}$ from 112 ± 4 to 94 ± 4 mm Hg ($P < 0.001$). This fall in $\overline{AP}$ was transient, with return of $\overline{AP}$ to 110 ± 4 mm Hg within 15 minutes. Administration of saralasin, an angiotensin II antagonist, not only prevented the compensation in $\overline{AP}$, but also significantly magnified the maximal hypotensive response seen following d$(CH_2)_5$VDAVP (group 4). Discontinuing the saralasin allowed $\overline{AP}$ to return to baseline. Bilateral nephrectomy (group 5) also prevented the return of $\overline{AP}$, further implicating endogenous angiotensin II as the specific mediator of the compensation in $\overline{AP}$ following d$(CH_2)_5$VDAVP administration. These studies clearly demonstrate that circulating AVP contributes to the maintenance of $\overline{AP}$ during water deprivation in the anesthetized rat. When this vascular action of AVP is blocked, angiotensin II assumes major responsibility for blood pressure regulation in the antidiuretic state. *Circ Res 48: 254-258, 1981*

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A GROWING BODY of evidence recently has been amassed to suggest that arginine vasopressin (AVP) contributes significantly to the maintenance of mean arterial blood pressure ($\overline{AP}$) in rat and dog (Aisenbrey et al., 1979; Cowley et al., 1980; Laycock et al., 1979; Manning et al., 1977). Such evidence has been obtained in a variety of experimental circumstances in which circulating levels of AVP either were demonstrated or were presumed to be elevated, as during chronic water deprivation (Aisenbrey et al., 1979), graded arterial hemorrhage (Cowley et al., 1980; Laycock et al., 1979), and during the induction phase of glycerol-induced acute renal failure (Hofbauer et al., 1977). A possible pressor role for AVP also has been suggested in rats with two-kidney Goldblatt hypertension (Möhring et al., 1978a), DOCA-induced hypertension (Möhring et al., 1977; Crofton et al., 1979), and spontaneous genetic hypertension in Kyoto-Wistar rats (Möhring et al., 1978b).

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The evaluation of the physiologic role of AVP as a pressor substance has been facilitated by the development of a number of structural analogues of AVP with specific vascular receptor blocking properties (Aisenbrey et al., 1979; Crofton et al., 1979; Manning et al., 1977). These peptides have been shown to selectively block the vasopressor, but not the antidiuretic response to exogenously administered AVP, yet do not interfere with the vasoconstrictor response to other vasoactive hormones (Crofton et al., 1979; Pang et al., 1979). One such peptide analogue was employed in the present study to evaluate the role of AVP in the regulation of systemic arterial blood pressure in rats in which endogenous AVP levels were varied intentionally by extremes of hydration. The interaction between endogenous AVP and the renin-angiotensin system in the regulation of $\overline{AP}$ also was examined.

Methods

General

Experiments were performed in five groups of adult male Sprague-Dawley rats weighing 210–270 g. Rats in group 1 received standard rat pellet chow and tap water until the time of experiment. Rats in groups 2, 4, and 5 were allowed free access to chow and water until 30–40 hours prior to study, at which time both were removed. Rats in group 3 underwent chronic water diuresis induced by the addition of

5% dextrose to distilled water for drinking, beginning 7–14 days prior to study. Twenty-four hours prior to experiment, 0.15% NaCl was added to this drinking solution to further stimulate drinking.

On the morning of the study, all rats were anesthetized with Inactin (100 mg/kg, ip; Byk Gulden Lomberg Chemische Fabrik GmbH) and placed on a heated operating table to maintain body temperature at 37°C. Following tracheostomy, the left jugular vein was catheterized (PE-50) for administration of experimental solutions. The left femoral artery was catheterized (PE-50) and blood pressure recorded by means of an electronic transducer (model P23Db, Statham Instrument Div., Gould) connected to a direct writing recorder (model 7702B, Hewlett-Packard Co.). The left ureter was catheterized (PE-10) for collection of urine and determination of urine osmolality (U_{osm}) by freezing point depression (Fiske OS Osmometer, Fiske Associates).

During the following protocols, rats received a 100-μg iv bolus injection of [1-(β mercapto-β,β-cyclopentamethylene propionic acid)4-valine-8-D-arginine] vasopressin, $d(CH_2)_5$ VDAVP, a structural analogue of naturally occurring AVP. In a pilot study, this dose (100 μg), given as a single bolus, was shown to block for at least 3 hours the 30–40 mm Hg rise in (AP) normally associated with a bolus injection of 50 mU AVP.

The individual protocols were as follows:

Group 1 (n = 5 Rats)

Following a stabilization period of 1 hour from the end of preparative surgery, these rats each received iv bolus injection of 50 μl of chlorobutanol (5 g/liter) in 0.05 N acetic acid which served as vehicle for $d(CH_2)_5$VDAVP injection. This was followed in 15 minutes by an iv bolus injection of 50 mU of AVP (Parke-Davis). Changes in (AP) were recorded continuously. After return of (AP) to baseline (within 30 minutes in all cases), bolus of $d(CH_2)_5$VDAVP (100 μg) was given iv, followed in 15 minutes by a second bolus of AVP (50 mU). Changes in (AP) were recorded before and after all injections.

Group 2 (n = 10 Rats)

Following the stabilization period, rats received a continuous iv infusion of 0.9% NaCl at the rate of 1.08 ml/hr. Blood pressure was measured periodically during an initial 30-minute control period. At 30 minutes, 100 μg of $d(CH_2)_5$VDAVP was given iv. The immediate effect on (AP) was determined by noting the lowest value of (AP) obtained in the first minute after injection. Blood pressure was monitored continuously for the next 44 minutes. At 75 minutes, a bolus injection of 50 mU AVP was given iv, and its effects on (AP) were recorded. U_{osm} was determined on urine collections obtained 30 minutes before and 5–40 minutes after $d(CH_2)_5$VDAVP administration.

Group 3 (n = 5 Rats)

During surgical preparation, a right jugular venous catheter (PE-50) was placed in each of these rats for infusion of 0.85% dextrose in 0.3% NaCl given at the rate of 60 ml/kg per hr to maintain water diuresis. Following the 1-hour postsurgical stabilization period, urine flow rate was determined and the infusion rate of hypotonic fluid was adjusted to equal twice the flow rate measured for the left kidney. An iv bolus of $d(CH_2)_5$VDAVP was given and the immediate effects on (AP) were noted. U_{osm} was determined before and after injection of $d(CH_2)_5$VDAVP.

Group 4 (n = 5 Rats)

To evaluate the role, if any, of the renin-angiotensin system in the recovery of AP after administration of $d(CH_2)_5$VDAVP, each rat in this group was treated in the same manner as described for group 2 except that the angiotensin antagonist, saralasin acetate (P113, Eaton Laboratories), was added to the 0.9% NaCl solution to yield a saralasin infusion rate of 0.3 mg/kg per hr. Changes in AP were recorded throughout the first 30 minutes, after which time a bolus injection of $d(CH_2)_5$VDAVP was given and the immediate and more delayed effects on AP were recorded. Fifteen minutes after injection of the AVP analogue, P113 was discontinued and replaced by 0.9% NaCl solution. Changes in AP were determined over the ensuing 30-minute interval. As in group 2, a bolus injection of AVP (50 mU) was given at 75 minutes. U_{osm} was determined both before and after infusion of $d(CH_2)_5$VDAVP, as in group 2.

Group 5 (n = 3 Rats)

These rats differed from those in groups 2 and 4, in that bilateral nephrectomy was carried out prior to the postsurgical stabilization period. Care was taken to minimize blood loss. One hour after nephrectomy, these rats were treated exactly as were those in group 4, except that measurements of U_{osm} were omitted.

Analytical

Results are expressed as absolute values for $\overline{AP}$ and also as percent of baseline AP values. Statistical significance was evaluated using paired and unpaired t-tests where appropriate. Significance is defined as $P < 0.05$.

Results

Group 1

These rats were employed to evaluate the effectiveness of $d(CH_2)_5$VDAVP as an AVP vascular receptor blocking agent. As shown in Figure 1,

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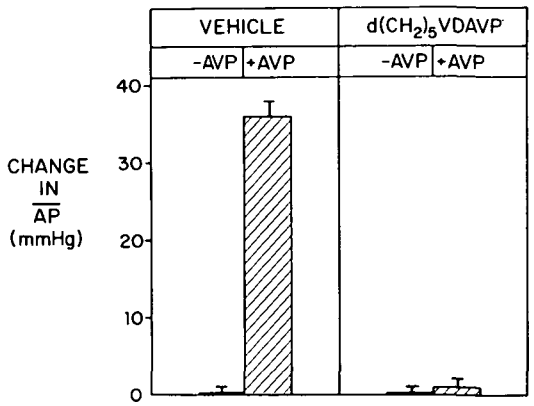

FIGURE 1 *Effect of d(CH₂)₅VDAVP on the pressor response to 50 mU of exogenous AVP (group 1). Vehicle is chlorobutanol (5 g/liter) in 0.05 N acetic acid. Dose of d(CH₂)₅VDAVP is 100 μg (n = 5).*

injection of 50 mU of AVP 15 minutes after infusion of vehicle produced a rise in AP averaging 36 mm Hg ± 2 SEM ($P < 0.001$). Subsequently, d(CH₂)₅VDAVP injection in these same rats failed to affect AP (0 ± 1 mm Hg; $P > 0.5$). Likewise, bolus AVP injection 15 minutes after d(CH₂)₅VDAVP injection produced only a 1 ± 1 mm Hg rise in AP ($P > 0.5$), indicating virtually complete inhibition of the pressor response to exogenous AVP by d(CH₂)₅VDAVP.

Group 2

Baseline AP averaged 112 ± 4 mm Hg during the initial 30-minute period of 0.9% NaCl infusion (Fig. 2). Following d(CH₂)₅VDAVP injection into these water-deprived rats AP fell abruptly in each rat to 94 ± 4 mm Hg or 83 ± 3% of baseline ($P < 0.001$). This reduction was transient, however; AP returned to 110 ± 3 mm Hg at 45 minutes ($P > 0.5$ vs. baseline) and remained stable at this level for the remainder of the experiment. Bolus injection of 50 mU AVP at 75 minutes failed to alter AP (1 ± 1 mm Hg), indicating continued efficacy of d(CH₂)₅VDAVP in antagonizing the pressor response to a bolus injection of AVP.

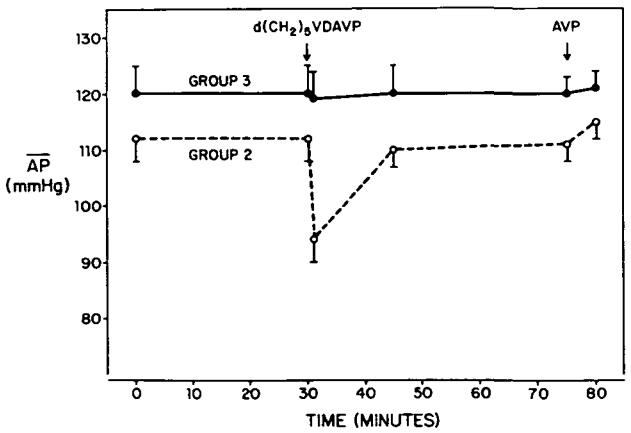

FIGURE 2 *Effect of d(CH₂)₅VDAVP on AP during water deprivation-induced antidiuresis (group 2) and chronic water diuresis (group 3). Group 2 rats received 0.9% NaCl at 1.08 ml/hr, iv. Group 3 rats additionally received 0.85% dextrose in 0.3% NaCl at twice urine flow rate of the left kidney to maintain water diuresis.*

As shown in Table 1, despite a pronounced transient decline in AP, d(CH₂)₅VDAVP failed to alter values for U_{osm}.

Group 3

As shown in Figure 2, bolus injection of d(CH₂)₅VDAVP into these water diuretic rats failed to alter AP significantly (120 ± 5 mm Hg before vs. 119 ± 5 mm Hg; $P > 0.5$) in the initial 1-minute interval after administration. Likewise, as shown in Table 1, average values for U_{osm} remained at markedly hypotonic levels and failed to change significantly in response to d(CH₂)₅VDAVP.

Group 4

Infusion of P113 into these water-deprived rats caused AP to fall, on average, from 114 ± 5 to 94 ± 4 mm Hg, or 83 ± 3% of baseline ($P < 0.025$), within 15 minutes (Fig. 3). By 30 minutes, however, AP recovered to an average value of 103 ± 4 mm Hg, or 90 ± 4% of baseline, a value not statistically different from pre-P113 values ($P > 0.09$). As shown in Figure 3, d(CH₂)₅VDAVP injection at 30 minutes produced an immediate and marked fall in AP to 70 ± 5 mm Hg, 62 ± 4% of baseline, ($P < 0.001$). This fall in AP in P113-treated rats exceeded that induced by d(CH₂)₅VDAVP in group 2 rats ($P < 0.001$). AP changed insignificantly over the next 14 minute period, at which time P113 was discontinued. AP rose dramatically in the ensuing 30 minutes, on average to 107 ± 4 mm Hg, or 95 ± 2% of baseline, a value not statistically different from baseline ($P > 0.5$). As in group 2, bolus injection of AVP at this time failed to significantly alter AP. Values for U_{osm} again were unaffected by d(CH₂)₅VDAVP administration (Table 1).

Group 5

Unlike group 4, these nephrectomized rats showed no change in AP during P113 infusion (Fig. 3). d(CH₂)₅VDAVP injection prompted a 44 ± 5%

TABLE 1 *Effects of d(CH₂)₅VDAVP on Urine Osmolality*

Condition	U_{osm}(mOsm) Group 2 ($n = 10$)	Group 3 ($n = 5$)	Group 4 ($n = 5$)
pre d(CH₂)₅VDAVP	2028 ± 127	91 ± 18	2057 ± 72
post d(CH₂)₅VDAVP	2086 ± 84	89 ± 18	2006 ± 115
P value	>0.5	>0.5	>0.5

Results are expressed as mean ± SEM.

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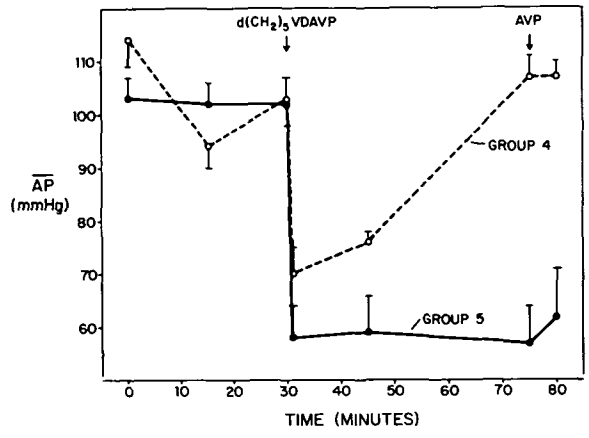

FIGURE 3 *Effect of angiotensin II in mediating the compensation in AP following d(CH₂)₅VDAVP administration. Groups 4 and 5 both received an infusion of P113 at 0.3 mg/kg per hr in 0.9% NaCl at 1.08 ml/hr. At 45 minutes, this solution was changed to 0.9% NaCl at 1.08 ml/hr.*

fall in AP to 58 ± 6 mm Hg ($P < 0.001$). As in group 4, AP changed little in the ensuing 14 minutes of continued P113 infusion. Following discontinuation of P113, no significant change in AP occurred, values averaging 55 ± 4 mm Hg, or 52 ± 4% of baseline, at 75 minutes (Fig. 3). An AVP bolus again caused little change in AP.

Discussion

These studies demonstrate that d(CH₂)₅VDAVP is an effective, specific antagonist of the vascular response to AVP in the rat. As shown in group 1, d(CH₂)₅VDAVP completely inhibited the normal vasoconstrictor response to a pressor dose of exogenous AVP. The lack of effect on AP in group 3 confirms that, in states of low circulating levels of endogenous AVP, d(CH₂)₅VDAVP has little, if any, intrinsic hypotensive or agonist properties. Since d(CH₂)₅VDAVP failed to alter U_{osm} significantly in either antidiuretic or diuretic rats, the present findings also clearly indicate that this peptide selectively inhibits only the vascular action of AVP.

Because of the efficacy and specificity of d(CH₂)₅VDAVP, we used this peptide to investigate the role of endogenous AVP in the maintenance of systemic arterial pressure during water deprivation. As shown in Figure 2, AP fell dramatically following bolus injection of this analogue in antidiuretic rats (group 2), but proved to be without effect in water-diuretic rats (group 3). Thus, the findings suggest that during water deprivation, AP is partially maintained by the action of circulating endogenous AVP. These observations confirm those of Aisenbrey et al. (1979) who showed that, in the conscious water-deprived rat, inhibition of the vascular response to AVP resulted in an abrupt fall in AP.

The current experiments extend the studies of Aisenbrey et al. (1979) in that we have been able to demonstrate an interaction between angiotensin II and AVP in the maintenance of AP during water deprivation. With the renin-angiotensin system intact, inhibition of the vascular response to AVP produced a significant, *but transient*, depression in AP in water-deprived rats (group 2). Interruption of the renin-angiotensin system by competitive antagonism of angiotensin II with P113 (group 4), or by removal of the main source of renin following bilateral nephrectomy (group 5), prevented the compensation in AP that otherwise followed administration of d(CH₂)₅VDAVP. The finding that AP returned to baseline following discontinuation of P113 in group 4 (Fig. 3) implicates angiotensin II as the specific humoral factor mediating the compensation of AP. In addition, interruption of the renin-angiotensin system significantly magnified the maximal hypotensive response noted following the administration of d(CH₂)₅VDAVP. Conversely, when the renin-angiotensin system was inhibited with P113 in group 4 in the absence of AVP analogue, there was a transient fall in AP (Fig. 3). In this circumstance, release of AVP in response to the P113-mediated fall in AP may have mediated this compensation of AP. Whereas an influence of the sympathetic nervous system and other vasoactive systems was not specifically excluded in the present study, our findings clearly indicate that AVP and angiotensin II interact to regulate AP in the anesthetized, water-deprived rat.

That such an interaction exists between AVP and the renin-angiotensin system in the regulation of AP is also suggested by the findings reported by others previously (Johnson et al., 1979; Tagawa et al., 1971; Vandongen, 1975). By varying plasma AVP concentrations within the physiological range, Tagawa et al. (1971) demonstrated a significant inhibition of renal renin release in sodium-deprived dogs. Despite depression of renal renin release by as much as 30% and, presumably, comparable decreases in angiotensin II in these sodium-deprived dogs, AP failed to change significantly. Based on the findings of the current study, AVP may have served to maintain AP in these dogs. The mechanism of inhibition of renin release by AVP has not been elucidated fully, but appears to be a direct vascular effect of AVP on the kidney. In this regard, Vandongen (1975) demonstrated inhibition of renin release by AVP in the isolated perfused rat kidney. Johnson et al. (1979) demonstrated that the vascular effect of AVP mediates the suppression of renin release. In their study, AVP infusion in water-diuretic dogs significantly depressed renal renin release, whereas the infusion of comparable amounts of 1-deamino-D-arginine vasopressin, a structural analogue of AVP with antidiuretic but not vascular activity, failed to alter renal renin release significantly.

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The effects of angiotensin II on AVP release also have been examined. Infusions of angiotensin II have been shown to enhance AVP release in response to osmotic (Shimizu et al., 1973) but not hypovolemic stimuli (Shade and Share, 1975). In addition, supraphysiological pressor infusions of renin or angiotensin II result in little or no increment in AVP release (Bonjour and Malvin, 1970; Claybaugh et al., 1972). Therefore, it seems unlikely that angiotensin II directly influences AVP release in response to hypovolemic stimuli.

Recent reports have underscored the physiological significance of the vasoconstrictor properties of AVP. Cowley et al. (1980) demonstrated that the maintenance of AP following hypotensive hemorrhage in the nephrectomized, spinal areflexic dog was attributable to the rise in plasma AVP concentration. Möhring and coworkers provided evidence that AVP may be an important pressor agent in several models of hypertension (Möhring et al., 1977; Möhring et al., 1978a, 1978b). In addition, the observation of Aisenbrey et al. (1979) on conscious water-deprived rats and the current study on anesthetized water-deprived rats confirm that AVP participates in the maintenance of AP during physiological antidiuresis.

One unexpected observation made during these experiments was that when effects of both AVP and angiotensin II were inhibited in rats in groups 4 and 5, AP fell to levels below 70 mm Hg and showed little tendency to return to higher levels. Thus, a role for AVP- and angiotensin II-independent mechanisms, such as the sympathetic nervous system, in the regulation of AP was not evident. The most probable explanation for this observation is that Inactin anesthesia interfered with the sympathetic response. Inactin is a substituted barbiturate and thus belongs to a class of drugs known to inhibit sympathetic ganglionic activity (Exley, 1954). Whereas no direct measurements of catecholamines or sympathetic nerve activity were made in the present study, it seems likely that the anesthesia necessary for the conduct of these experiments interfered with the sympathetic response. Therefore, these data should not be interpreted as precluding a role for the sympathetic nervous system or other anesthesia-sensitive factors in the regulation of AP in the conscious state.

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