Cardiovascular Effects Associated with Antidiuretic Activity of Vasopressin after Blockade of Its Vasoconstrictor Action in Dehydrated Dogs

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SUMMARY. In view of our previous findings that a specific antidiuretic (V2) agonist, 4-valine-8-D-arginine vasopressin, acutely increased cardiac output and heart rate in dogs, we examined the hypothesis that interaction with V2-like receptors might contribute to the hemodynamic response seen after blockade of the vasoconstrictor (V1) effect of arginine-vasopressin in dehydrated dogs. After 48 hours of water restriction which increased plasma vasopressin to 10.6 ± 2.0 pg/ml, the V1 antagonist 1-(β-mercapto-β,β-cyclopentamethylene propionic acid) 2-(O-methyl)tyrosine arginine-vasopressin, 10 µg/kg, was injected intravenously into six conscious dogs, and the combined V1 + V2 antagonist 1-(β-mercapto-β,β-cyclopentamethylene propionic acid) 2-(O-ethyl)-d-tyrosine, 4-valine arginine-vasopressin, 10 µg/kg, was administered to another six dogs. Mean arterial pressure, cardiac output (electromagnetic flowmeter), and regional blood flows (radioactive microspheres) were measured before and 20–30 minutes after antagonist administration. Mean arterial pressure did not change significantly in either instance. Cardiac output increased by 31.0 ± 7.1% after V1 blockade, but by only 10.8 ± 2.1% following V1 + V2 blockade. Blood flow increased significantly and to a similar extent in the skin, the skeletal muscles, and the fat following both antagonists. Conversely, kidney, arterial liver, and bone blood flow increased only after V1 blockade. In six additional, normally hydrated conscious dogs, it was shown that the V1 + V2 antagonist had no significant hemodynamic effects, a finding previously established for the V1 antagonist. The V1 + V2 antagonist completely prevented the hemodynamic effects associated with administration of the V2 agonist 4-valine-8-D-arginine vasopressin, 200 ng/kg, whereas the V1 antagonist did not. Both antagonists had similar effects on the hemodynamic changes induced by nitroprusside infusion, namely a potentiation of the blood pressure lowering action. These results suggest that part of the hemodynamic response to blockade of the vasoconstrictor action of vasopressin in dehydration is caused by unmasking cardiovascular effects linked to the antidiuretic activity of the arginine-vasopressin molecule. (Circ Res 58: 631–640, 1986)
infusion of the much more selective V1-agonist, 2-phenylalanine-8-ornithine oxytocin, in the presence of the V1-antagonist, nor were they observed during infusion of oxytocin alone. On the other hand, the specific antidiuretic (V2) agonist 4-valine-8-D-arginine vasopressin (VDAVP) also increased cardiac output and decreased peripheral resistance (Schwartz et al., 1985). These effects could not be explained by renal retention of water, since they appeared within minutes of the administration of the V2-agonist and without any sign of volume expansion.

Since V1-receptor blockade in dehydrated dogs increases cardiac output and heart rate (Schwartz and Reid, 1983), and since interaction of AVP analogs with V2-like receptors may elicit, under some conditions, hemodynamic changes of a similar nature (Schwartz et al., 1985), we examined the hypothesis that part of the hemodynamic effects observed after V1-receptor blockade in dehydrated dogs may be due to unmasking interaction with V2-like receptors. This was attempted by comparing the effects of V1-receptor blockade to that of combined V1- and V2-receptor blockade with appropriate vasopressin analogs. It was reasoned that any contribution of V2-receptors could be excluded if the responses to V1 blockade and to combined V1 + V2 blockade were identical.

**Methods**

**Animal Preparation**

A total of 23 male mongrel dogs was used in these studies. In protocols A and C, 18 dogs weighing 20.5 ± 1.0 kg (protocol A) and 20.3 ± 0.9 kg (protocol C) were equipped, under pentobarbital anesthesia (30 mg/kg, iv, supplemented as necessary) and using sterile techniques, with Tygon catheters (Norton Plastics) inserted into the abdominal aorta and the inferior vena cava through iliac vessels. Through a thoracotomy at the 4th intercostal space, an electromagnetic flowmeter was placed around the root of the aorta for cardiac output measurement (without coronary blood flow) and a catheter was inserted into the left atrium for microsphere injections (protocol A). All cables and catheters were tunneled subcutaneously to the intrascapular region of the back and were protected by a jacket. In protocol B, five dogs (body weight 19.1 ± 0.1 kg) were prepared with chronic arterial and venous catheters, as well as a bladder cannula, for timed urinary collections (Cowley et al., 1983). Antibiotics were administered postsurgically for 1 week.

**Collection of Hemodynamic Data**

All experiments were performed with animals in the conscious state after at least 1 week of recovery from surgery. In protocols A and C, mean and pulsatile arterial pressure, cardiac output (by integration of the aortic flow signal), and heart rate were continuously recorded on a multichannel paper oscillograph. In addition, the phasic pressure and flow signals were fed into a DATEL ST-LS12 A/D converter, with a sampling rate of 60 Hz for each waveform. Information was extracted from these waveforms with a PDP-11/03 computer. The program calculated over any desired period the average systolic, diastolic, and mean arterial pressures, as well as stroke volume, cardiac output, heart rate, and total peripheral resistance (ratio of mean arterial pressure and cardiac output). Possible shifts of the aortic flow baseline were corrected by defining as zero the diastolic flow value preceding each ejection. Hemodynamic measurements for protocols A and C were conducted in dogs lying quietly in the recording pen after adequate training, whereas those in protocol B were made with the dogs standing in a sling. Regional blood flows were determined with radioactive microspheres as described previously (Liard, 1981). Briefly, about 10⁶ NEN-TRAC microspheres, 15 μm in diameter, were flushed into the left atrial catheter through an injector vial, while a reference arterial sample was collected at a precisely measured rate of about 10 ml/min. Three isotopes were used in each dog: ⁴⁶Sc, ¹¹³Sn, and ¹⁵³Gd. The animals were killed immediately after the last injection with an overdose of pentobarbital, and organs and tissues were weighed, cut into small pieces, and counted. Organs counted in toto included the heart, lungs, kidneys, gastrointestinal tract, spleen, pancreas, brain, adrenals, thyroid, prostate, gallbladder, testicles, eyes, and mandibular glands. Representative samples were taken from the liver, skin (12 locations), bones (20 locations), skeletal muscle (more than 50 samples, 15 g each, taken in 18 different muscle groups), and omental fat. Counting was performed in a three-channel Auto-gamma Packard spectrometer with appropriate setting for each isotope, and blood flows in tissues were determined from the ratio of the activity found in the reference sample and that measured in the tissue sample.

**Other Measurements**

Blood samples were taken from the aortic catheter. Hematocrit was measured by microcentrifugation, plasma vasopressin concentration was measured by radioimmunoassay (Cowley et al., 1981), and plasma renin activity by radioimmunoassay of the angiotensin I generated during a 1-hour incubation at 37°C (Sealey and Laragh, 1980). Sodium and potassium concentrations were measured by flame photometry in plasma and urine. Osmolality was obtained by vapor-pressure osmetry in plasma and urine. Plasma volume was measured with Evans Blue dye by extrapolation to zero time from samples collected 20, 30, and 40 minutes after injection.

**Experimental Protocols**

**Protocol A**

The dogs were kept in metabolic cages and their urine was collected. Canned food (H/D Prescription Diet, Rijiana) was given in constant quantity in the morning, providing about 600 ml/day of water per day. Sodium chloride was added to the food to provide a total intake of about 210 mmol/day. Water was provided ad libitum during the control period. On the morning of day 0, the dogs were not fed and were brought to the recording pen. Control measurements of hemodynamic (including regional blood flows) and other variables were obtained. The dogs were then fed, but were denied drinking water for the next 48 hours. On day 2, a second set of measurements was obtained; then, one of two analogs of AVP was injected intravenously at a dose of 10 μg/kg. Six dogs

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received the analog 1-(β-mercaptop-β-cyclopentamethylene proprionic acid) 2-(O-methyl)tyrosine arginine-vasopressin, or d(CH₂)₅Tyr(Me)AVP, a vasopressor antagonist synthesized by Kruszynski et al. (1980) and generously supplied by Dr. K. Hofbauer, Ciba-Geigy, Basel, Switzerland. The other six dogs received the analog 1-(β-mercaptop-β-cyclopentamethylene proprionic acid) 2-(O-ethyl)-o-tyrosine, 4-valine-vasopressin, or d(CH₂)₅-D-Tyr(Et)AVP, a combined antidiuretic and vasopressor (V₁ + V₂) antagonist (Manning et al., 1982), generously provided by Dr. M. Manning, Toledo, Ohio, and by Dr. K. Hofbauer, Ciba-Geigy, Basel, Switzerland.

A third microsphere injection was performed between 20 and 30 minutes after antagonist administration, and the dogs were killed.

**Protocol B**

This protocol was designed to check the ability of the antagonist d(CH₂)₅-D-Tyr(Et)AVP to block the renal antidiuretic action of AVP in dogs. The five dogs in this group were kept on a 40 mEq/day sodium intake. On day 0, they were brought to the laboratory and their bladder was emptied. Urine collection was then started for a control period of 30 minutes. Control blood samples were taken for measurement of plasma vasopressin, sodium, and osmolality. An intravenous infusion of hypertonic saline (2.5% NaCl) was started at a rate of 0.5 ml/min, providing 1.25 mEq Na+/min, and was continued for 2 hours. Urine was collected in small aliquots during the whole infusion period, and additional blood samples were taken (see Results). At the end of the 2-hour infusion, the dogs were given water to drink. Then the effect of a bolus injection of AVP, 50 ng/kg, on mean arterial pressure and heart rate was determined. Two days later, the same dogs were subjected to an identical protocol, but the analog d(CH₂)₅-D-Tyr(Et)AVP, 10 μg/kg, was injected intravenously after 1 hour of infusion. Both 50 and 500 ng/kg AVP were injected after the end of the infusion in order to assess the degree of blockade of V₁-receptors provided by this antagonist.

**Protocol C**

This protocol was designed to determine the hemodynamic effects of the antagonist d(CH₂)₅-D-Tyr(Et)AVP in normally hydrated dogs, as well as to assess the ability of this antagonist to block the cardiovascular effects of the antidiuretic agonist VDAVP, generously supplied by Dr. M. Manning, Toledo, Ohio (Sawyer et al., 1974). Finally, the specificity of the hemodynamic effects of the antagonist d(CH₂)₅-D-Tyr(Et)AVP in dehydrated dogs was checked by comparing the response to nitroprusside infusion before and after administration of either the combined V₁ + V₂ or the V₁ antagonist. The six dogs used in protocol C were fasted for 12 hours before any hemodynamic measurements, but received water ad libitum. On day 0, they were placed in the recording pen, and the response to intravenous injection of VDAVP, 200 ng/kg, was determined. After at least 30 minutes, an intravenous infusion of nitroprusside (Nitropress, in 5% dextrose solution) was administered for 5 minutes at a rate of 3 μg/kg per min. After another 2 days, the same sequence was repeated, but the antagonist was used in the V₁ antagonist d(CH₂)₅-Tyr(Me)-AVP, 10 μg/kg.

**Statistical Analysis**

The evaluation of the data was performed on absolute (not percent) values by one-way and two-way analysis of variance for repeated measures and Duncan's multiple range test (Winer, 1971). One-way analysis of variance (ANOVA) was used to determine whether a significant variation across time occurred within each group, and two-way ANOVA was used to compare two different groups. When repeated measurements were performed twice (protocol B) or three times (protocol C) in the same dogs, a two-way ANOVA for repeated measurements with different treatments was performed. Changes were considered significant for P < 0.05. All reported values are means ± SE. In order to facilitate comparison between relative changes in systemic and regional hemodynamics, several results are reported as percent changes.

**Results**

**Protocol A**

We have previously reported the effects of 48-hour water deprivation in an almost identical protocol (Liard et al., 1982). The results obtained in the present study are summarized in Table 1. Arterial hemodynamic and humoral changes induced by a 48-hour period of water restriction in 12 dogs are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Hemodynamic and Humoral Changes Induced by a 48-Hour Period of Water Restriction In 12 Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
</tr>
<tr>
<td>Cardiac output (ml/kg per min)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Total peripheral resistance (U)</td>
</tr>
<tr>
<td>Plasma AVP (pg/ml)</td>
</tr>
<tr>
<td>Plasma renin (ng AI/ml per hr)</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
</tr>
<tr>
<td>Plasma sodium (mEq/liter)</td>
</tr>
<tr>
<td>Plasma potassium (mEq/liter)</td>
</tr>
<tr>
<td>Blood volume (ml)</td>
</tr>
<tr>
<td>Urine osmolality† (mOsm/kg)</td>
</tr>
<tr>
<td>Drinking water intake† (ml/day)</td>
</tr>
</tbody>
</table>

* Significant change from control.
† Control values are based on average value for the 2 days before water restriction. Value after 48 hours of dehydration represents last 24-hour period.
pressure did not change, and cardiac output fell significantly, but the change was smaller than previously observed. We found no significant change in heart rate in these experiments, in contrast to our earlier study. The increases in plasma AVP, renin activity, osmolality, sodium, as well as urine osmolality, conform with our previous findings (Liard et al., 1982).

Regional blood flow measurements during the control period are summarized in Table 2 and were similar to those previously reported from our laboratory (Liard, 1981; Liard et al., 1982). Water restriction for 48 hours significantly reduced flow to the skeletal muscle mass as a whole by 21%. Most other organ blood flows did not change significantly, including myocardial and liver blood flow, which were found to decrease significantly in our previous study (Liard et al., 1982).

The six dogs that received the V₁ antagonist d(CH₂)₅Tyr(Me)AVP after 48-hour water deprivation exhibited hemodynamic changes, as shown in Figure 1. Significant increases in cardiac output and heart rate (47.3 ± 8.5%) took place, whereas total peripheral resistance fell by 25.2 ± 3.6%. Mean arterial pressure did not change. From the values listed in Table 1 under 48-hour dehydration, plasma renin activity increased further by 3.2 ± 1.1 ng AI/ml per hour, and plasma potassium fell significantly by 0.26 ± 0.1 mEq/liter. Plasma sodium, osmolality, and hematocrit did not change significantly. Plasma AVP could not be measured because of significant cross-reactivity of the antagonist in the radioimmunoassay.
Significant increases in regional blood flows took place in the left and right ventricular myocardium (33.1 ± 10.2 and 35.4 ± 12.2% respectively), kidneys (20.3 ± 7.3%), liver (arterial, 76 ± 25.5%), bone (70.8 ± 9.1%) (Fig. 2), omental fat (78.5 ± 19.5%), skin (74.3 ± 25.5%), skeletal muscle (63.4 ± 18.0%) (Fig. 3), and pericardium (31.5 ± 13.9%). Significant decreases were observed in the stomach (34.9 ± 2.2%) and the brain (10.4 ± 4.3%). There were no significant changes in other organs and tissues, notably in the esophagus, the duodenum, the small intestine, the colon, the pancreas, the aortic wall, the thyroid gland and the mandibular gland, all vascular areas that were shown to respond with vasoconstriction to small acute increases in plasma AVP (Liard et al., 1982).

The six dogs that received the combined $V_1 + V_2$ antagonist $d(CH_2)_5-D-Tyr-(Et)VAVP$ after 48-hour water deprivation showed hemodynamic changes summarized in Figure 1. Cardiac output increased significantly, but only by 10.8 ± 2.1%. This increase was significantly less than that following $V_1$ blockade. It merely returned cardiac output to its prede-
### Table 3
Effects of a 2-Hour Infusion of Hypertonic Saline in Five Dogs Receiving, after 60 Minutes of Infusion, either d(CH₂)₅-D-Tyr(Et)VAVP, 10 μg/kg (antagonist), or Vehicle Alone

<table>
<thead>
<tr>
<th>Minutes of infusion</th>
<th>-30-0</th>
<th>0-30</th>
<th>30-60</th>
<th>60-90</th>
<th>90-120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma AVP (pg/ml)</td>
<td>3.6 ± 0.6</td>
<td>10.1 ± 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
<td>293.4 ± 1.0</td>
<td>300.6 ± 1.7</td>
<td>308.2 ± 1.5</td>
<td>313.5 ± 1.0</td>
<td>319.7 ± 0.7</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/liter)</td>
<td>145.2 ± 0.4</td>
<td>149.4 ± 0.6</td>
<td>152.8 ± 0.5</td>
<td>156.1 ± 0.5</td>
<td>159.5 ± 0.6</td>
</tr>
<tr>
<td>Urinary volume (ml/min)</td>
<td>0.14 ± 0.04</td>
<td>0.16 ± 0.02</td>
<td>0.49 ± 0.08</td>
<td>1.07 ± 0.18</td>
<td>1.77 ± 0.23</td>
</tr>
<tr>
<td>Na⁺ excretion (μEq/min)</td>
<td>4.8 ± 1.3</td>
<td>7.6 ± 2.5</td>
<td>64.8 ± 13.5</td>
<td>230 ± 53.8</td>
<td>399 ± 74.1</td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma AVP (pg/ml)</td>
<td>3.7 ± 1.2</td>
<td>10.2 ± 1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
<td>295.8 ± 1.5</td>
<td>304.0 ± 1.6</td>
<td>311.0 ± 1.5</td>
<td>318.8 ± 0.9</td>
<td>325.7 ± 1.5</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/liter)</td>
<td>146.1 ± 0.7</td>
<td>150.4 ± 0.8</td>
<td>153.7 ± 0.7</td>
<td>157.6 ± 0.8</td>
<td>160.9 ± 0.8</td>
</tr>
<tr>
<td>Urinary volume (ml/min)</td>
<td>0.21 ± 0.07</td>
<td>0.27 ± 0.05</td>
<td>0.52 ± 0.11</td>
<td>1.83 ± 0.20*</td>
<td>2.71 ± 0.34*</td>
</tr>
<tr>
<td>Na⁺ excretion (μEq/min)</td>
<td>10.7 ± 5.4</td>
<td>12.2 ± 6.0</td>
<td>80.7 ± 32.1</td>
<td>245 ± 40.4</td>
<td>369 ± 54.8</td>
</tr>
</tbody>
</table>

Values are means ± se. Plasma values have been obtained at the end of the indicated period.

* Value significantly different from that measured after vehicle alone.

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**Protocol B**

Table 3 and Figure 4 summarize the results obtained. There were progressive, significant increases in plasma AVP, osmolality, and sodium during the infusions of hypertonic saline, which did not differ significantly whether the V₁ + V₂ antagonist was administered or not. Sodium excretion increased similarly, whereas urinary volume increased significantly more following administration of d(CH₂)₅-D-Tyr(Et)VAVP (60-90 and 90-120 minutes). Free water clearance became progressively more negative in the time control run (Fig. 4). After antagonist injection, free water clearance was significantly affected and became much less negative or, in a few instances, positive (Fig. 4). At the peak of the effect of the antagonist, which was obtained after approximately 30 minutes, urinary osmolality in the antagonist-treated dogs was 312.2 ± 39 mOsm/kg, and free water clearance was 0.12 ± 0.27 ml/min. Values measured at the corresponding time in the time control run were significantly different, being 612.9 ± 68.4 mOsm/kg and -1.28 ± 0.39 ml/min, respectively. At the end of the infusion, the dogs drank 610 ± 38.5 ml in the control run, and 710 ± 45.9 ml following the antagonist, which was significantly more.

The injection of AVP, 50 ng/kg, increased mean arterial pressure by 25 ± 2.2 mm Hg and decreased heart rate by 53.4 ± 9.6 beats/min in the control run from values of 116 ± 6.6 mm Hg and 120.2 ± 12.1 beats/min, respectively. The administration of the antagonist d(CH₂)₅-D-Tyr(Et)VAVP, 10 μg/kg, had no effect on mean arterial pressure or on heart rate, but after its injection, neither 50 nor 500 ng/kg AVP significantly affected mean arterial pressure. A small decrease in heart rate was still noted...
after both doses of AVP, significant only at the dose of 50 ng/kg (−8.8 ± 2.2 b/min). These findings indicate very effective blockade of the vasoconstrictor activity of AVP. Furthermore, they differ markedly from those associated with administration of the analog d(CH2)5Tyr(Me)AVP, since the injection of AVP, 40 ng/kg, produced a significant increase in heart rate (and cardiac output) after administration of this antagonist (Liard and Spadone, 1984).

Protocol C

The administration of the antagonist d(CH2)5-D-Tyr(Et)AVP, 10 µg/kg, to six conscious dogs had no significant hemodynamic effects. Values for 30 minutes before injection and for 5 to 25 minutes after injection, respectively, were: mean arterial pressure, 83.0 ± 4.8 and 84.1 ± 5.1 mm Hg; cardiac output, 115.3 ± 8.5 and 115.3 ± 8.5 ml/kg per min; heart rate, 82.4 ± 6.5 and 81.7 ± 6.5 beats/min. Total peripheral resistance, stroke volume, peak aortic blood flow, and the maximum derivative of aortic blood flow were unaffected by the V1 + V2 blocker.

Figures 5 and 6 summarize the effects of V1 and V1 + V2 blockade, respectively, on the hemodynamic response to the injection of VDAVP, 200 ng/kg (Fig. 5), and to the infusion of nitroprusside, 3 µg/kg per min (Fig. 6). In untreated dogs, VDAVP induced significant falls in arterial pressure and total peripheral resistance, as well as increases in cardiac output and heart rate. These changes were completely prevented by the V1 + V2 antagonist (Fig. 5). On the other hand, the V1 antagonist did not reduce the effects of VDAVP. It did not significantly modify the heart rate, mean arterial pressure and total peripheral resistance response, and significantly increased the cardiac output response at all time points. Nitroprusside infusion in untreated dogs reduced mean arterial pressure and total peripheral resistance, and increased cardiac output and heart rate.

![Figure 5](https://example.com/image1.png)

**Figure 5.** Mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR) and heart rate (HR) for 1 minute before (CTL) and 4 minutes after iv injection of VDAVP, 200 ng/kg, in six dogs without treatment (triangles), under V1 blockade with d(CH2)5Tyr(Me)AVP, 10 µg/kg (circles), or under V1 + V2 blockade with d(CH2)5-D-Tyr(Et)AVP, 10 µg/kg (squares). An asterisk indicates a value significantly different from control (Duncan multiple range test). Values are means ± se.

![Figure 6](https://example.com/image2.png)

**Figure 6.** Mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR), and heart rate (HR) for 1 minute before (CTL) and 5 minutes during an iv infusion of nitroprusside, 3 µg/kg per min, in six dogs without treatment (triangles), under V1 blockade with d(CH2)5Tyr(Me)AVP, 10 µg/kg (circles), or under V1 + V2 blockade with d(CH2)5-D-Tyr(Et)AVP, 10 µg/kg (squares). An asterisk indicates a value significantly different from control. Values are means ± se.
rate (Fig. 6). Both antagonists significantly accentuated the fall in blood pressure, but they did not differ from each other in their effect on any variable at any single time point. This result suggests that their effect was related to their ability to block the vasoconstrictor effects of AVP released by the blood pressure fall induced by nitroprusside. It will be noted specifically that the combined V₁ + V₂ antagonist did not prevent in any way the increase in cardiac output and heart rate associated with nitroprusside administration.

Discussion

Many previous studies have shown that blockade of the vasoconstrictor effects of AVP with specific antagonists under conditions of increased vasopressin release is associated with hemodynamic effects (for a review, see Liard, 1984). Thus, Schwartz and Reid (1983) reported a significant increase in heart rate and cardiac output, as well as a fall in total peripheral resistance, following administration of the V₁ antagonist d(CH₅)₅Tyr(Me)AVP to dehydrated, conscious dogs. Our results confirm these findings. They further show that the hemodynamic effects of V₁-receptor blockade in dehydrated dogs appear to be due only in part to suppression of the vasoconstrictor effects of endogenous vasopressin, since a V₁ antagonist and a combined V₁ + V₂ antagonist elicited significantly different cardiovascular actions.

Some of the effects of the two analogs used in our study were very similar. Renin increased in both instances, as did regional blood flows in several vascular beds, notably the skin, the fat, and the skeletal muscle. These results suggest that increased AVP levels in dehydrated dogs inhibit renin release, even though renin levels are significantly increased, and reduce some regional blood flows through V₁-mediated effects. Thus, even though skin and fat blood flows were not decreased below control values in 48 hour-dehydrated dogs, vasopressin apparently still acted as a vasoconstrictor in these vascular beds. Presumably, such factors as local control mechanisms return blood flow toward normal values despite the prolonged presence of excessive amounts of vasoconstrictor agents.

On the contrary, part of the increase in cardiac output, as well as the increase in renal, liver, and bone blood flows that followed V₁ blockade, do not appear to result directly from blockade of AVP-induced vasoconstriction, but instead seem to reflect interaction of endogenous AVP with V₂-like receptors. Indeed, the combined V₁ + V₂ blockade with the antagonist d(CH₅)₅-D-Tyr(Et)AVP significantly blunted the increase in cardiac output and heart rate, as well as the fall in total peripheral resistance, when compared to V₁ blockade with d(CH₅)₅Tyr(Me)AVP, and it totally abolished the increase in renal, liver, and bone blood flow. We showed in the present study that d(CH₅)₅-D-Tyr(Et)AVP is a very effective blocker of the V₁ receptors, since it completely prevented the pressor effect of bolus injections of 50 and 500 ng/kg AVP. Also, the pA₂ values for the anti-vasoressor effects of the two analogs used in the present study are very similar [8.62 and 8.41, respectively (Manning and Sawyer, 1983)], indicating that d(CH₅)₅Tyr(Me)AVP and d(CH₅)₅-D-Tyr(Et)AVP are both powerful and about equivalent V₁ antagonists. Therefore, the difference in cardiac output response to the two antagonists cannot be attributed to a lesser degree of blockade of V₁-receptors by d(CH₅)₅D-Tyr(Et)AVP. The finding that V₁ blockade in dehydrated dogs increased renal, liver, and bone blood flow would be particularly difficult to explain as a result of suppression of vasopressin-induced vasoconstriction, since these three vascular beds are among the least sensitive to this effect (Liard et al., 1982). Previous studies using V₁-receptor blockade have also reported significant changes in liver and/or renal hemodynamics under conditions of increased endogenous release of AVP (Aisenbrey et al., 1981; Vari et al., 1985).

We used the analog d(CH₅)₅-D-Tyr(Et)AVP because of its antagonistic properties against the antidiuretic effects of AVP, as shown in rats (Manning et al., 1982) and in dogs (Kinter et al., 1984, and present study). We suggest, therefore, that the difference between the hemodynamic effects resulting from the administration of these two analogs in dehydrated dogs is due to V₁ blockade by d(CH₅)₅-D-Tyr(Et)AVP. Similarly, the lack of tachycardia following injection of 40–50 ng/kg AVP in conscious dogs pretreated with the combined V₁ + V₂ antagonist (present study) and its presence in dogs pretreated with the V₁ antagonist (Liard and Spadone, 1984) would also be explained by the extent of V₁ blockade. The analog d(CH₅)₅Tyr(Me)AVP does not have any V₂-blocking activity, being a very weak antidiuretic agonist (Kruszynski et al., 1980). It could be argued that our observations in dehydrated dogs are the result of a nonspecific action of the V₁ or of the combined V₁ + V₂ antagonist not present in the other compound. However, d(CH₅)₅Tyr(Me)AVP, when administered to normally hydrated dogs, has no or little hemodynamic effects during the period in which we conducted our measurements (30 minutes after administration) (Schwartz and Reid, 1983; Liard and Spadone, 1984; Rose et al., 1984; Hassel et al., 1984, 1985). Similar results have been obtained in other species, including rat (Charcopos et al., 1982), rabbit (Elliott et al., 1985) and man (Busien et al., 1984), suggesting that this analog is devoid of intrinsic cardiovascular effects in the absence of elevated AVP levels. Furthermore, in the study by Schwartz and Reid (1983), another analog was used to block the vasoconstrictor effects of AVP in dehydrated dogs with results identical to those of d(CH₅)₅Tyr(Me)AVP. Therefore, the cardiac
output increase and the fall in total peripheral resistance which accompany the injection of d(CH₂)₅Tyr(Me)AVP in dehydrated dogs seem closely associated with its selective ability to block V₂-mediated effects of AVP, rather than with some nonspecific action. As for the combined V₁ + V₂ antagonist d(CH₂)₅-D-Tyr(Et)VAVP, we found in the present study that this analog did not exert any hemodynamic effect in conscious normally hydrated dogs. Furthermore, it did not decrease in an unspecific manner the fall in total peripheral resistance and increase in cardiac output induced by administration of a vasodilator (nitroprusside), but affected it in the same way as the V₁ antagonist. On the other hand, the combined V₁ + V₂ blocker completely prevented the increase in cardiac output and fall in total peripheral resistance induced by a specific V₂ agonist, whereas the V₁ blocker did not. Our results suggest that a substantial portion of the hemodynamic effects of V₁ blockade in dehydrated dogs is due to unmasking agonistic effects of AVP on V₂-like receptors, in a manner similar to what we observed previously with a specific antidiuretic agonist (Schwartz et al., 1985). As in our previous study, we found no evidence that the renal handling of water was involved in any way in the observed effects, despite the fact that V₂-receptors have been primarily described in renal tubular cells. From our measurements of the volume of urine in the bladder in protocol A, we conclude that no major volume loss occurred within 30 minutes of administration of the combined V₁ + V₂ antagonist. Also, hematocrit did not change significantly, indicating that no major plasma volume reduction took place after V₁ + V₂ blockade. Finally, the increase in cardiac output which followed V₁ blockade was almost immediate, so that its prevention by V₂ blockade could not have resulted from a volume effect. Thus, if V₂ blockade accounts for the effect of d(CH₂)₅-D-Tyr(Et)VAVP in blunting the increase in cardiac output due to V₁-receptor blockade, it is not through a volume-mediated renal action.

The V₂-like receptors that would be responsible for mediating hemodynamic responses after V₁ blockade have not been identified. Even though they do not appear to involve water excretion, these receptors could be located in the kidney and trigger cardiovascular reflexes or the release of humoral agents such as prostaglandins or kinins. V₂-like receptors could also be in the central nervous system, vascular smooth muscle cells, and/or myocardial cells, among other possibilities. Stimulation of adenylyl cyclase, which is a well-established step in the renal effects of AVP, would, if present in the heart and vascular smooth muscle, increase cardiac contractility and decrease vascular resistance, since dibutylryl cyclic adenosine 3',5'-monophosphate (cAMP) produces positive inotropic and chronotropic effects and causes dilation of peripheral vessels in the dog (Okaaki, 1977). However, binding studies in smooth muscle cells have shown only one class of high-affinity binding sites that correspond to V₂-receptors (Penit et al., 1983).

Relatively large rates of infusion of V₂ agonist or of AVP following V₁ blockade must be used to increase cardiac output and reduce vascular resistance in conscious dogs (Schwartz et al., 1985). In the present study, only moderate levels of AVP appeared to exert such effects after V₁-blockade in 48 hours of dehydration. This finding may be related to the prolonged exposure to increased levels of vasopressin under the present experimental conditions. Alternatively, the mechanisms of the increased cardiac output and reduced peripheral resistance may be different in both instances despite the striking similarity of the hemodynamic patterns. Whether or not interaction with V₂-like receptors contributes to counteract the vasoconstrictor effects of AVP in dehydrated dogs cannot be ascertained from our studies, since it is possible that V₂-mediated hemodynamic effects are not expressed in the presence of V₁ agonism. However, administration of a selective V₂ antagonist could conceivably lead to further vasoconstriction in the presence of increased levels of AVP.

Even though the analogs used in this study were carefully selected for their specific actions on V₂-and/or V₁-receptor-mediated effects of AVP, we cannot exclude the possibility that other characteristic of one or the other antagonist accounts for their different hemodynamic effects in dehydrated dogs. Both analogs are antioxytocic (Kruszynski et al., 1980; Manning and Sawyer, 1983). The analog d(CH₂)₅Tyr(Me)AVP has been shown to block the corticotrophic releasing factor-like activity of arginine-vasopressin (Knepel et al., 1984), but the activity of d(CH₂)₅-D-Tyr(Et)VAVP was not tested in the same system. Other functional properties of these two compounds may prove to differ and to be importantly involved in the responses described in the present study. However, it appears that the results obtained while using antagonists of the vasoconstrictor effects of AVP to determine the role of vasopressin in cardiovascular control may involve more than one mechanism. The possibility that part of the influence of these antagonists on hemodynamic variables may be due to unmasking the action of AVP on V₂-like receptors should be considered. This point may be of interest when vasopressor antagonists of vasopressin are administered to patients with hypertension (Gavras et al., 1984) and congestive heart failure (Nicod et al., 1985), even though important species differences are known to exist with respect to the actions of vasopressin analogs (Stassen et al., 1983).
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