The purpose of these studies was to determine how plasma levels of arginine vasopressin (pAVP) are related to workload, plasma osmolality (pOsm), blood volume (BV) and plasma angiotensin II (pAII) in exercising dogs. Measurements were made in dogs running on a treadmill at 7.5 km. hr^{-1} at slopes of 0, 10% and 20% when they were hydrated ad lib and when they had been deprived of drinking water and also in dogs running on a 20% slope after an IV infusion of hypertonic NaCl. Dehydration increased pOsm by 6.6% and reduced BV by 10% in resting dogs. In dehydrated animals, pAVP, pAII and pOsm were elevated above hydrated levels at rest and during exercise at all three workloads. In hydrated dogs, pOsm rose during exercise at 10% and 20% slopes but pAVP rose above resting levels only at the highest workload and pAII was not affected by exercise. In dehydrated dogs, pOsm and pAVP rose during exercise at 10% and 20% slopes and pAII was elevated at the 20% slope. BV decreased during exercise at the highest workload in both hydrated and dehydrated animals. After hypertonic NaCl, pAVP rose during exercise but pOsm and pAII did not. The results suggest that both osmotic and nonosmotic factors contribute to the release of AVP in exercising dogs and that exercise leads to a leftward shift in the relationship of pAVP to pOsm which could be a result of reduced blood volume.

Key Words: dehydration — exercise — arginine vasopressin — blood volume — dogs

INTRODUCTION

In humans, circulating levels of arginine vasopressin (AVP) are elevated during exercise and the plasma concentration of AVP (pAVP) is related to the intensity of the exercise (1—4). The stimuli which lead to the release of AVP in exercise are not completely understood and may include elevated plasma osmolality, reduced plasma volume and increased plasma levels of Angiotensin II (pAII) (1, 3). Hydration by ingestion of fluids before or during exercise reduces the rise in pAVP during exercise (4, 5).

The state of bodily hydration also has a marked effect on cardiovascular
and thermoregulatory adjustments to exercise in both panting and sweating mammals. In dehydrated exercising dogs, body temperature is elevated and upper respiratory water loss and blood flow both are reduced below hydrated levels (6, 7). In hypovolemic humans exercising in a warm environment, cutaneous blood flow, cardiac output and stroke volume all are lower than in the normovolemic state and body temperature is higher (8, 9). It has been suggested that AVP might play a role in the alterations in thermoregulation in dehydrated exercising humans (9), but the effects of dehydration on pAVP in exercise have not been studied systematically.

It is not known how exercise of different intensities affects pAVP in mammals other than humans. Studies in other species might help to clarify the mechanisms of release of AVP in exercise and eventually the role which it might play in body fluid balance and cardiovascular regulation during exercise. The present studies were undertaken to determine how exercise affects pAVP and pAII in this species and how dehydration and elevated body fluid osmolality influence plasma levels of these hormones in exercise.

MATERIALS AND METHODS

Eight adult male mongrel dogs weighing between 24 and 31 kg (28±1 kg, M±SEM), were used. All of the animals had been in the laboratory for at least 1 year and during this time each animal ran on the treadmill 3 or 4 times per week at the workloads used in this study.

Effect of workload and hydration state on pAVP and pAII in exercise.

At an air temperature of 22°C, measurements of plasma concentrations of arginine vasopressin and angiotensin II were made before, during and after exercise at three different workloads first when each animal was hydrated ad lib and again when it had been dehydrated by removal of drinking water for 48 hr combined with a one-hour treadmill run (7.5 km. hr⁻¹, 10% slope) at the beginning and at 24 hr of the dehydration period. Exercise tests at the different workloads were made in random order and at least 10 days apart for each animal. The three workloads used in the experiments were as follows:

1. 7.5 km. hr⁻¹, level treadmill, 60 min run (five dogs)
2. 7.5 km. hr⁻¹, 10% slope, 60 min run (six dogs)
3. 7.5 km. hr⁻¹, 20% slope, 30 min run (eight dogs)

Effect of hypertonic NaCl infusions on pAVP in exercise.

When the first series of experiments showed that pAVP was elevated in hydrated animals only at the highest exercise intensity, another series of experiments was done to determine how this exercise affected pAVP in hydrated dogs with elevated pOsm. Measurements of pOsm and pAVP were made in eight animals after they had received
an IV infusion (1.9 ml. min⁻¹ into the external jugular vein) of hypertonic NaCl (30% NaCl, 1.2 ml. kg⁻¹). Plasma AII was measured in three of these animals. The animals rested for 30 min after the end of the infusion and then ran at 7.5 km. hr⁻¹ on a 20% slope for 30 min at 22°C air temperature. We found previously that IV infusion of a small volume of concentrated NaCl (2.5 ml. kg⁻¹ of 30% NaCl) does not change the blood volume in resting dogs (7) and confirmed this in three animals in the present study using the Evans Blue technique.

Before each experiment, a 20 ga, 2 inch A-Cath (Delmed, Inc.) was inserted percutaneously into the left external jugular vein of the dog and attached to a length of polyethylene tubing which was closed with a stopcock and taped to the back of the neck to allow blood sampling while the animal was running. A copper-constantan thermocouple was inserted into the rectum for measurement of body core temperature. Then the animal rested quietly on the treadmill for 30 min. In experiments in which hypertonic saline was infused, the rest period began when the infusion was completed. At the end of the rest period, a pre-exercise blood sample was drawn and the exercise period was started. Blood samples were taken at 30 and 60 min of exercise on the level treadmill and at a 10% slope and at 30 min of exercise during runs at a 20% slope. In each experiment, a post-exercise blood sample was taken 15 min after the end of the exercise period. Body temperature was measured at the time of blood sampling using a Bailey model BAT-12 thermocouple thermometer.

Each blood sample was 8 ml in the experiments in which AVP and AII were both measured and 4 ml in the hypertonic infusion experiments where only AVP was measured. Hematocrit (Hct) was measured in triplicate by the micro method and corrected for trapped plasma by a factor of 0.96. After filling the hematocrit tubes, the remaining sample was divided into two parts, one to be used for measurement of AII and one for measurement of AVP and pOsM. For measurement of AII, four ml of whole blood was immediately mixed with angiotensinase inhibitor (10) and centrifuged separately from the rest of the sample. After centrifugation, plasma was removed from both portions of the sample and aliquots which had not been exposed to AIIase inhibitor were saved for measurements of plasma osmolality using a Wescor vapor pressure osmometer. Separate one ml plasma samples which had and had not been exposed to AIIase inhibitor were extracted immediately using C₁₈ reversed-phase 1 ml mini-columns (Analytichem, Harbor City, Ca.) for later measurement of AII and AVP, respectively. Red cells which had not been exposed to AIIase inhibitor were resuspended in normal saline and injected back into the animal as soon as the plasma had been removed.

Measurements of pAVP and pAII.

Extraction of angiotensin II from plasma using reversed-phase mini-columns has been described and fully validated elsewhere, as has the angiotensin II radioimmunoassay protocol and antiserum employed in the present studies (11). Extraction of AVP from plasma used an identical protocol. Recovery of AVP in this extraction protocol was determined by the addition of biological concentrations of unlabelled AVP to plasma with subsequent radioimmunoassay quantification to be 88.8±7.4% (M±SEM).

The antiserum used in the AVP assay was raised by immunizing rabbits against thyroglobulin-coupled AVP homogenized with Freund's complete adjuvant. The antiserum is used in a non-equilibrium assay at a final dilution of 1:300,000. The sensitivity of the assay is 0.2 pg/tube. Cross-reactivity properties of the antiserum have been evaluated for several related peptides. At 50% displacement cross-reactivity with arginine-vasotocin was < 0.01%, with pressinoic acid was < 0.005% and with oxytocin and
angiotensin II < 0.001%. Interassay coefficient of variation in this assay is 9.0%, intraassay coefficient of variation is 8.2%. The assay employed a commercial 111-I-AVP preparation (New England Nuclear, Billerica, Mass.) and was separated by second antibody (goat anti-rabbit gamma globulin, Research Products International, Mount Prospect, III.) and polyethylene glycol.

**Blood Volume Measurements.**

To determine how the period of water deprivation affected blood volume, we measured plasma volume, hematocrit and plasma protein concentration in five resting animals when they were hydrated and again when they were dehydrated. In separate experiments, hematocrit and plasma protein concentrations were measured before and at the end of a 30 min period of running at 7.5 km.hr⁻¹ on a 20% slope in order to estimate the changes in blood volume occurring running at the heaviest workload in the hydrated and dehydrated states. Plasma volume was measured using Evans Blue dye as described previously (7) and plasma protein concentration was measured by refractometry.

At the end of the series of experiments, oxygen consumption (Vo₂) during running at each of the three workloads was measured in four of the dogs, in the hydrated state, using a plastic respiratory mask (6) and an open-circuit method as described by Seeholzer et al. (12).

Data were analyzed using two-way ANOVA for repeated measures, two-way ANOVA with replication (13), and paired t tests.

**RESULTS**

During periods of water deprivation, the animals lost 9.6 ± 0.15% of the initial body weight and plasma osmolality rose 21 ± 1 mosm. (kg⁻¹H₂O⁻¹) above the hydrated level (mean values using the average of all periods of water deprivation for each dog). In dehydrated exercising animals, pAVP, pAII, pOsm and rectal temperature were elevated above the hydrated level at all three workloads. Hematocrit was elevated above the hydrated level only at the two higher workloads (Fig. 1A, B and C). The change in hematocrit and in rectal temperature during exercise was significant for all three workloads and for both hydrated and dehydrated dogs.

During one hour of running on the level treadmill there was no change in pAVP, pAII or pOsm in either hydrated or dehydrated animals (Fig. 1A). Neither pAVP nor pAII changed during exercise on a 10% slope in hydrated animals, but pOsm rose significantly (Fig. 1B). In dehydrated animals, pAVP rose during the 10% run (Fig. 1B). Plasma AVP and osmolality rose significantly in hydrated animals running on a 20% slope, but pAII was not elevated (Fig. 1C). In dehydrated animals at this workload, pAVP, pAII and pOsm all rose significantly during the run.

In dogs which had received hypertonic saline, pOsm was elevated above hydrated levels (P < 0.01) but was not different from dehydrated
**Fig. 1.** Effect of exercise on pAVP, pAII, pOsm, hematocrit and body temperature in hydrated (solid lines) and dehydrated (dashed lines) dogs. The exercise periods are marked with rectangles. Treadmill speed was 7.5 km. hr⁻¹ in all experiments. The slope was 0 in A, 10% in B, and 20% in C. Tₘ was 22°C. Points are M±SEM. Asterisks indicate significant differences (2-way ANOVA with replication) between hydrated and dehydrated runs (** P < 0.01; *** P < 0.001). P values show significance of change (repeated measure 2-way ANOVA) during exercise for each parameter.
pOsm and pAVP was significantly higher than pAVP in resting hydrated animals (P < 0.01) and lower than pAVP in resting dehydrated animals (P < 0.001) (Fig. 2). Plasma osmolality did not change during exercise in the animals which had received a hypertonic infusion, but pAVP changed significantly (P < 0.01). Plasma AII did not change during exercise after the hypertonic infusion in the three animals in which it was measured. Figure 2 shows the relationship between mean levels of pOsm and pAVP in resting and running animals in the three different conditions (hydrated ad lib, dehydrated, and after an infusion of hypertonic saline).

Fig. 2. Log-linear plot of pOsm-pAVP relationship in 8 resting and running dogs (30 min at 20% slope) in three different hydration states: Hyd = hydrated ad lib; NaCl = after IV infusion of hypertonic NaCl; Dehyd = water-deprived animals. Points are M±SEM. Hyd and Dehyd values are also plotted on Fig. 1C. Dehyd animals are hypovolemic. Hyd and NaCl animals are normovolemic.

Dehydration led to a 17% reduction in plasma volume and an 11% increase in plasma protein concentration in resting animals (Table 1). Calculation of the blood volume in intact dogs from measurements of plasma volume and hematocrit is uncertain because the ratio of whole body hematocrit to venous hematocrit (F_\text{cells}) is variable and depends on the degree of splenic contraction. Reeve et al (14) reported that this ratio is about 1 in conscious resting dogs and falls to 0.9 or below after injections of adrenaline which cause splenic contraction. Assuming that F_\text{cells} was 1 in resting dogs, then the average blood volume was reduced by 10%
Table 1. Effect of dehydration on intravascular volume at rest and plasma protein concentration in exercise.

<table>
<thead>
<tr>
<th></th>
<th>Hydrated Rest</th>
<th>30 min run</th>
<th>Dehydrated Rest</th>
<th>30 min run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt.</td>
<td>30.0±1.0</td>
<td></td>
<td>27.3±0.9</td>
<td></td>
</tr>
<tr>
<td>Plasma volume (ml)</td>
<td>1422±83</td>
<td></td>
<td>1181±76**</td>
<td></td>
</tr>
<tr>
<td>Blood volume (ml)</td>
<td>2422±98</td>
<td></td>
<td>2172±84*</td>
<td></td>
</tr>
<tr>
<td>Plasma Protein (g. 100 ml⁻¹)</td>
<td>7.5±0.1</td>
<td>+ + +</td>
<td>8.4±0.1***</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

1 7.5 km. hr⁻¹, 20% slope. N = 5. Values are M±SEM.

* P < 0.05; ** = P < 0.01; *** = P < 0.001, difference between hydrated and dehydrated values (paired t test).

+++ = P < 0.001, difference between rest and exercise values (paired t test).

When they were dehydrated. During 30 min of exercise at a 20% slope, plasma protein concentration increased above resting levels by 6.3% in hydrated animals and by 5.6% in dehydrated animals. Hematocrits measured in these animals before, during and after exercise were not different from those measured in the larger group of animals and presented in Figure 1.

\( \dot{V}_O_2 \) (ml. kg⁻¹. min⁻¹) in hydrated animals during exercise at the three different workloads was 30±2 on the level treadmill, 41±2 on a 10% slope and 54±3 on a 20% slope.

DISCUSSION

Body fluid osmolality is the major factor controlling AVP release in resting mammals, and pAVP is highly correlated with plasma osmolality (15, 16). In resting humans and dogs, the regression of pAVP on pOsm is affected by blood volume. Hypovolemia shifts the curve to the left with a decrease of the osmotic threshold for AVP release and/or an increase in the slope of the line; and hypervolemia shifts the curve to the right (15, 17, 18, 19). Our measurements in resting dogs showed this pattern, dehydrated animals with a reduced blood volume having higher pAVP at the same pOsm than animals which had received hypertonic NaCl. These volume effects are apparently mediated by cardiac stretch receptors (19).
In exercising humans, reductions in plasma volume and elevations in osmolality occur simultaneously and both are related to exercise intensity (1, 2). Convertino et al. (1) found that the change in pAVP in men doing graded exercise was correlated with changes in pOsm but not with changes in plasma volume and concluded that reduced plasma volume in exercise might be a secondary stimulus to AVP release. Wade and Claybaugh (4) found that changes in pAVP were not always correlated with changes in either plasma osmolality or volume in exercising men. Plasma AVP levels in our hydrated and hypertonic dogs exercising at the highest workload indicate that both osmotic and nonosmotic factors are involved in AVP release in exercise in this species. In hydrated dogs, pAVP in exercise was higher and pOsm was lower than in resting dogs which had received a hypertonic infusion. In hypertonic dogs, pAVP rose in exercise and pOsm did not. The plot of pAVP against pOsm in resting and exercising animals (Fig. 2) suggests that the pOsm-pAVP relationship is shifted to the left in normovolemic dogs doing heavy exercise. A decrease in circulating blood volume during exercise or a reduction in central blood volume consequent to peripheral pooling could lead to such a shift.

Only a few studies have addressed the question of whether the circulating blood volume in dogs is affected by exercise. Sarelius (20) concluded from Evans Blue disappearance curves that plasma volume did not change and the volume of circulating red cells increased in intact greyhounds doing light exercise for 30 min. Morimoto et al. (21) found no change in blood volume (Het/Hb method) in splenectomized dogs running for 15 min at 35% and 50% of \( \dot{V}_{O_2,\text{max}} \). If the increase in plasma protein concentration is an accurate reflection of decreased plasma volume in our intact dogs running on a 20% slope, and if \( F_{\text{cells}} \) is 0.9 in exercising dogs, the blood volume at 30 min of exercise fell below resting levels by 4.5% in hydrated animals and 3.5% in dehydrated animals. However, the change in plasma protein concentration may underestimate the change in the plasma volume. In resting dogs, the increase in plasma protein concentration after dehydration (+11%) was less than the decrease in plasma volume measured using Evans Blue dye- (—17%). Such disproportionate changes in plasma protein concentration and plasma volume occur in humans after exercise dehydration and may reflect protein movement out of the vascular compartment (22). Thus, although a precise calculation of blood volume changes during exercise in our animals is not possible, the data do indicate that circulating blood volume fell during exercise in both hydrated and dehydrated dogs and was 9—10% lower when they were dehydrated than when they were hydrated.

Pooling of blood in cutaneous veins as body temperature rises has been
postulated to be a significant factor leading to the progressive fall in cardiac stroke volume in humans doing sustained exercise in the heat (23). Stroke volume is lower in hypovolemic subjects than in hydrated ones and this has been attributed to reduced central blood volume and cardiac filling pressure (9, 23). A reduction in cardiac filling pressure consequent to peripheral pooling could accentuate the effects of exercise hypovolemia on the release of AVP. In exercising dogs, elevations in body temperature lead to increased blood flow to the skin of the extremities and to the mucosal surfaces of the nose, mouth and tongue (24). Although blood flow through the carotid arteries is about 10% of the cardiac output in running dogs (24, 25), pooling of blood in the nose, mouth and tongue seems unlikely since the head is elevated above the heart when the dog is running. Pooling might occur in cutaneous veins of the legs of exercising dogs as a result of thermoregulatory dilation, and Pagney et al. (26) have suggested that the tilt of the body in dogs running up a slope could promote pooling of blood in the posterior part of the body.

Elevations in pAII in dehydrated, exercising dogs at a workload which does not lead to increased pAII in hydrated animals might be a result of renal vasoconstriction. Renal blood flow does not fall during exercise in intact, fully hydrated dogs but in splenectomized dogs, renal blood flow is reduced in exercise (27) and this may be related to the inability of the splenectomized dog to maintain blood volume during exercise by splenic contraction. Dehydrated exercising dogs may have a redistribution of cardiac output which is similar to that occurring in splenectomized dogs. On the other hand, Zambraski et al. (28) have shown that the elevation in plasma renin in exercising, intact dogs, is mediated by increased sympathetic nerve activity, and this could be occurring in hypovolemic exercising animals at lower workloads than in normovolemic animals.

Both pAVP and pAII increased in dehydrated dogs doing heavy exercise, but in hydrated animals and animals which had received an infusion of hypertonic saline, pAVP rose after 30 min of running on a 20% slope but pAII did not. This indicates that an elevation of pAVP in exercising dogs can occur with no elevation in pAII. Convertino et al. (1) found a poor correlation between the change in pAVP and the change in plasma renin activity in exercising humans and concluded that AII is not an important stimulus for AVP release in exercise.

The finding that pAVP in hydrated dogs did not rise above resting levels during running at the two lower workloads but was elevated during heavier exercise is consistent with observations that pAVP in exercising humans is related to workload (1, 2, 4). In humans exercising for 6 min periods at progressively heavier workloads, pAVP is elevated above resting levels when the energy cost of the exercise reaches 50% $V_{O_{max}}$ and pAVP
continues to rise as exercise intensity increases (1, 2). We did not measure $V_{O_2\text{max}}$ in our animals, but their oxygen consumption during running on the level treadmill and on a 10% and 20% slope represents about 18%, 25% and 34% of the maximal oxygen consumption measured by Seeherman et al. (12) in dogs which had undergone an exercise training program. From these data we cannot conclude that pAVP is released in exercising dogs at a lower relative workload than in exercising humans, since our animals ran for 30 min and exercise duration is known to affect pAVP. Wade and Claybaugh (4) found that men running at 70% of maximum heart rate showed a significant increase in pAVP after 60 min but not after 20 min. The increase in pAVP as exercise continues could be related to pooling of blood in thermoregulatory vascular beds as discussed above.

Plasma levels of renin and AII in exercising humans are also related to workload and to exercise duration (1, 2, 4, 29). Plasma renin during exercise at a fixed workload decreases with exercise training as $V_{O_2\text{max}}$ increases (2). In our hydrated dogs, pAII was not elevated during exercise even at the heaviest workload. In contrast, Zambraski et al. (28) found that plasma renin levels in dogs rose to 1.5X resting levels during 15 min of exercise at 5 mph, 25% slope. Since pAII would be expected to parallel plasma renin during exercise (29), the difference in the responses of the two groups of dogs may be due to the heavier workload used by Zambraski et al., or it could be related to differences in the state of training of the animals, since our dogs had been running regularly on the treadmill for a year.

Body temperature rose during exercise at all three workloads and was always higher when the dogs were dehydrated than when they were hydrated. The elevation in body temperature of dehydrated exercising dogs has been observed previously and appears to be a consequence of reduced evaporation by panting and reduced blood flow to the nose and mouth (6). Dehydrated dogs also show a marked reduction in the water lost by drooling from the lips and tongue during heavy exercise (7). Doris (30) examined the possibility that the hyperthermia observed in dehydrated animals exposed to heat was caused by AVP. He found no effect of intravenous or intracerebroventricular infusions of AVP on evaporation or body temperature in resting cats in a warm environment. However, Liard et al. (31) showed that AVP infusions lead to reduced capillary blood flow in the skeletal muscle and skin of resting dogs. If AVP produces vasoconstriction in the mucosal surfaces of the nose and mouth of the dog or in cutaneous vascular beds of humans, then this hormone might play a significant role in the hyperthermia which occurs when they exercise in both of these species when they are dehydrated.
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