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Characterization of the effects of the vasopressin V2 receptor on sweating, fluid balance, and performance during exercise

Tamara Hew-Butler,1 Jed Hummel,1 Brian C. Rider,1 and Joseph G. Verbalis2

1Exercise Science Program, Oakland University, Rochester, Michigan; and 2Endocrinology and Metabolism, Georgetown University Medical Center, Washington, DC

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Hew-Butler T, Hummel J, Rider BC, Verbalis JG. Characterization of the effects of the vasopressin V2 receptor on sweating, fluid balance, and performance during exercise. Am J Physiol Regul Integr Comp Physiol 307: R366–R375, 2014. First published June 18, 2014; doi:10.1152/ajpregu.00120.2014.—A regulatory effect of arginine vasopressin (AVP) on sweat water conservation has been hypothesized but not definitively evaluated. AVP-mediated insertion of sweat and salivary gland aquaporin-5 (AQP5) water channels through activation of the vasopressin type 2 receptor (V2R) remains an attractive, yet unexplored, mechanism that could result in a more concentrated sweat with resultant decreased water loss. Ten runners participated in a double-blind randomized control treadmill trial under three separate pharmacological conditions: a placebo, V2R agonist (0.2 mg desmopressin), or V2R antagonist (30 mg tolvaptan). After a familiarization trial, runners ran for 60 min at 60% of peak speed followed by a performance trial to volitional exhaustion. Outcome variables were collected at three exercise time points: baseline, after the steady-state run, and after the performance run. Body weight losses were <2% across all three trials. Significant pharmacological condition effects were noted for urine osmolality ([F = 84.98; P < 0.0001] and urine sodium concentration ([Na+]_p) [F = 38.9; P < 0.0001], which verified both pharmacological activation and inhibition of the V2R at the kidney collecting duct. Plasma osmolality and [Na+]_p demonstrated significant exercise ([F = 26.0 and F = 11.1; P < 0.0001] and condition ([F = 5.1 and F = 3.8; P < 0.05]) effects (osmolality and [Na+]_p, respectively). No significant exercise or condition effects were noted for either sweat or salivary [Na+]_p. Significant exercise effects were noted for plasma [AVP] ([F = 22.3; P < 0.0001], peak core temperature ([F = 103.3; P < 0.0001]), percent body weight change ([F = 6.3; P = 0.02], plasma volume change ([F = 21.8; P < 0.0001]), and thirst rating ([F = 78.2; P < 0.0001]). Performance time was not altered between conditions ([P = 0.80). In summary, AVP acting at V2R does not appear to regulate water losses from body fluids other than renal excretion during exercise.

arginine vasopressin; sweat sodium; V2 antagonist; running

ARGININE VASOPRESSIN (AVP) is the primary neuroendocrine regulator of water metabolism, acting to maintain plasma osmolality within the normal physiological range of 275–295 mosmol/kg H2O (38). Osmotic regulation of plasma tonicity generally occurs through activation of the vasopressin type 2 receptor (V2R) in the kidney. Activation of V2R initiates the translocation and subsequent insertion of aquaporin-2 (AQP2) water channels into the apical membrane of the collecting duct principal cells. This allows for water reabsorption along osmotic gradients and concomitant antidiuresis when plasma tonicities are high (46).

Unlike this well-characterized interaction of V2R-stimulated translocation of AQP2 channels in the kidney, a regulatory effect of AVP on sweat water conservation has yet to be determined. The capability of water reabsorption within an estimated 1.6 to 4.0 million sweat glands (41) is a teleological appealing possibility, which could maximize water conservation during prolonged periods of thermoregulatory stress. Exercise performed at intensities above 50% of V˙O2 maximum can shift up to 92% of all water (and 87% of all sodium losses) away from urine production toward requisite thermoregulatory sweat production (6, 29, 32). This primary shift in water and sodium excretion routes would be of increasing homeostatic importance during endurance exercise, particularly when performed in hot environments. Thus the capacity for osmotically mediated water conservation to occur in sweat glands would be an attractive effector mechanism aimed at maintaining fluid homeostasis during sustained periods of thermoregulatory stress.

Sweat glands appear to have acute fluid and sodium regulatory elements such as aquaporin-5 (AQP5) water channels (4), Na+/H+ exchangers (10), adrenergic nerve terminals, and β-adrenergic receptors (35), cystic fibrosis transmembrane conductance regulator chloride channels (CFTR-Cl), and epithelial sodium channels (ENaC) (9, 35). Much of the available evidence support changes in sweat sodium concentration ([Na+]_s) to reflect changes in sweat rate (11). However, two studies recently reported statistically significant positive associations between sweat [Na+]_s and plasma AVP concentrations ([AVP]_p) (5, 13). These independent investigations hypothesize that increases in sweat [Na+]_s might possibly be mediated by an AVP-dependent mechanism, perhaps by increasing water reabsorption either through vasopressin receptor type 1a (V1aR) or V2R stimulation. The presence of AVP receptors within human sweat glands is only weakly supported by indirect evidence (37). Prior investigations looking specifically at the effects of vasoconstriction on sweat rate and composition do not support activation of the V1aR as a plausible mechanism for any association between sweat [Na+]_s and [AVP]_p (7, 33, 34, 45). Therefore, AVP-mediated insertion of AQP5 water channels through activation of the V2R within the apical membrane of the sweat gland remains an enticing, yet unexplored, mechanism.

AQP5 water channels have been identified within the apical membrane of secretory cells located in salivary, sweat, lacrimal, and airway submucosal glands as well as in corneal,
Thereafter, the speed on the treadmill was increased by 0.5 miles per hour (mph) every 6 s until the participant could no longer keep pace with the treadmill (volitional exhaustion).

Trials 2, 3, and 4 were then conducted utilizing the randomized exercise test lab protocol (Fig. 1A). These three trials utilized the same exact protocol, differing only in the pharmacological intervention used to assess the role of the AVP V2R on sweat sodium concentration and performance. In randomized, double-blind order (both participant and investigator blinded to the intervention), either a placebo pill, the V2R antagonist tolvaptan (Samsca, 30 mg tablet), or the V2R agonist desmopressin (DDAVP, 0.2 mg tablet) was ingested along with a CorTemp Core Temperature Sensor 2 h before commencement of the exercise trial (time 0) with 240 ml of bottled water. All tablets were placed within identical colored capsules, with the investigator from Georgetown University (J. G. Verbalis) creating the randomization key for each participant. Ten coded packets (one per study participant, each containing a placebo, V2R agonist, and V2R antagonist tablet labeled in separate envelopes as trial 2, 3, or 4) were then sent to Oakland University where the lead investigator (T. D. Hew-Butler) distributed one packet to each participant after completion of the familiarization trial (trial 1). The randomization key was unlocked only after all data collection was completed.

No food was allowed following ingestion of the capsule and sensor, but ad libitum water intake was allowed according to thirst. Thirty minutes before the trial began (time 0), each participant arrived to the exercise lab where he or she was prepped with side-by-side sweat patches on the upper back and a temperature sensor reading confirmed. The CorTemp data logger was then attached to each participant’s waist, enclosed within a zippered running belt for continuous monitoring. Immediately preceding the exercise test, all participants

MATERIALS AND METHODS

Participants

Ten healthy (no acute or chronic medical conditions requiring regular prescription medication use), habitual runners (>50 km weekly running distance for >6 wk before study participation) between the ages of 18–60 yr were recruited to participate in this trial. Exclusion criteria were 1) history of kidney problems; 2) inability to sense thirst; 3) difficulty swallowing; 4) history of gastrointestinal disorders; and 5) history of fainting associated with venipuncture. Oral and written informed consent was obtained from each participant before the trial began. This study was approved by both the Oakland University and Georgetown University Medical Center Institutional Review Boards. The study protocol was registered in ClinicalTrials.gov as identifier NCT02084797.

Experimental Protocol

All consenting participants presented to the exercise lab on four separate occasions, at the same time of day, every other week. Each participant was instructed to refrain from exercise 24 h immediately preceding and competitive events of >21 km within 3 days of each lab trial. The first lab visit (trial 1) was a treadmill familiarization trial during which an incremental test to volitional exhaustion was performed to determine each participant’s VO2peak. This VO2peak test was then utilized in trials 2, 3, and 4 as the performance test. Specifically, after a 5-min warmup, each participant ran for 1 min at a self-selected comfortable speed, equivalent to the average speed of an easy run. Thereafter, the speed on the treadmill was increased by 0.5 miles per
were asked to completely void their bladder. The urine was collected into a prelabeled plastic container that was later measured for volume (ml). After the urine void, 1 ml of saliva was collected via the passive drool technique. Then 5 ml of venous blood were withdrawn from an antecubital vein with the participant in a seated position. Each participant was weighed in running clothes without shoes on a WW-42D impedance scale (Weight Watchers, New York City, NY) immediately before mounting the treadmill. All of the measures collected before treadmill running began have been designated as “baseline” measures.

Each randomized exercise test (Fig. 1B) consisted of 60 min of “steady-state” running at a speed corresponding to 60% of each participant’s V\(_{\text{O2peak}}\) running speed. After completion of the 60-min steady-state run segment, the left upper back sweat patch was quickly removed, 1 ml of saliva was collected, and 5 ml of blood withdrawn via venipuncture from an antecubital vein with the participant seated. The participant was then asked to completely void his or her bladder and all urine was collected in a prelabeled plastic container. After voiding was completed, each participant was again weighed before remounting the treadmill for the performance segment of the trial. All of the measures collected immediately following steady-state running were designated as “steady-state” measures. Data collection following the steady-state run was performed within 5 min of commencing the performance segment.

Each participant then immediately underwent a performance trial: running for 1 min at a speed according to 60% V\(_{\text{O2peak}}\) then increasing the treadmill speed 0.5 mph every 60 s until volitional exhaustion. Each participant was blinded to their performance time and speed between the trials. Blood, sweat (right upper back patch), saliva, and urine were then collected immediately upon completion of the performance segment of the trial. A treadmill-modified whole body washdown was performed at the end of the randomized exercise test to determine whole body sweat sodium loss (1, 42). All of the measures collected after completion of the performance segment of the trial were designated as “performance” measures.

Thirst rating was assessed before the trial started (baseline) and immediately postexercise and performance testing using a 10-point anchored scale. On the scale, “0” was the lowest possible rating (not thirsty at all) while “10” was the highest possible rating (extremely thirsty).

If any research participant needed to urinate during the 60-min steady-state run (which was possible using the V2R antagonist), those individuals were allowed to dismount the treadmill and empty their bladder (into a container for further analysis and volume measurement). The time lost was then subsequently added to the overall running time so that all participants ran for a total of 60 min on the treadmill during the steady-state portion of the run. Since the average time to volitional exhaustion during the performance test (V\(_{\text{O2peak}}\) test) was ~10 min (10.4 ± 0.9 min in the pilot trial) it was deemed unlikely the participants needed to urinate during the performance stage of the trial. Bottled water was freely available for each participant throughout the trial. All core temperature data were downloaded from the data logger after the trial was completed, with the peak temperature for each stage of the trial recorded.

Outcomes

The primary outcome variables included biochemical measurement of sodium concentration in sweat, blood, and urine during each randomized trial (placebo, V2R agonist and V2R antagonist). Secondary outcome variables included measurement of exercise performance (time during the incremental test to exhaustion) and peak core temperature. Tertiary variables included measurement of body weight, plasma volume, [AVP]\(_{\text{p}}\), plasma oxytocin concentration ([OT]\(_{\text{p}}\)), and thirst rating.

Biochemical and Fluid Balance Analyses

Plasma samples. All venous blood samples were collected in chilled lithium heparin tubes. All blood samples were immediately centrifuged at 3,000 rpm for 10 min. The separated plasma was then placed into two 1.5-ml Eppendorf tubes and immediately stored at −80°C until batch analysis could be performed at Georgetown University Medical Center. Plasma [Na\(^+\)] and potassium concentration ([K\(^+\)]) was measured using ion-selective electrodes (Beckman Synchron EI-ESE, Fullerton, CA). Osmolality was measured using a vapor pressure osmometer (VAPRO 5520, Wescor, Logan, UT). Changes in plasma volume (PV) were estimated by comparing pre- and post-intervention measurements of plasma protein (PP) using a clinical refractometer (Schuco Clinical Refractometer 5711-020, Japan)(44). Plasma [AVP]\(_{\text{p}}\) and oxytocin ([OT]\(_{\text{p}}\)) were measured by specific RIAs following acetone-ether extraction as described previously (47). The standard curve for AVP is linear between 0.5 and 10.0 pg/tube with the use of a synthetic AVP standard (PerkinElmer Life Sciences, Boston, MA). The minimum detectable concentration of AVP in extracted plasma was 0.5 pg/ml. The AVP antiserum (R-4) displayed 1% cross-reactivity with OT. The standard curve of the OT assay was linear between 0.25 and 5.0 pg/tube with the use of a synthetic OT standard (PerkinElmer Life Sciences). The minimum detectable concentration of OT in extracted plasma was 0.25 pg/ml. The OT antiserum (Pitt-Ab2) displayed 1% cross-reactivity with AVP.

Sweat samples. Sweat was collected via two different techniques: 1) sweat patch collection, and 2) whole body washdown technique (WBW). For the sweat patch collection, the upper back was cleansed with alcohol, rinsed with distilled water, and allowed to air dry. After the area was clean and dry, two sweat patches were applied side-by-side to the upper back (to the left and right of the thoracic spine), just below the shoulder blades. Each patch consisted of one sheet of 8- by 5 cm × 5 cm sterile gauze covered with one sterile sheet of 10 cm × 12 cm Tegaderm. For sample collection, after the steady-state exercise, the left sweat patch was carefully removed and the contents of the gauze poured directly into an Eppendorf tube that was immediately closed and frozen at −80°C until further analyses could be performed. After the performance stage of the test, the right patch was similarly removed with the sweat immediately collected, frozen, and stored. All assessments of [Na\(^+\)] and [K\(^+\)] were measured as in the plasma samples. For the WBW technique (1, 42), after completion of the performance stage of the trial, each participant was placed in a plastic kiddie pool within a fully enclosed walk-in tent. With the participant seated in the middle of the pool, one researcher poured ~1.5 liters of distilled water over the participant’s head and body. The participant was then asked to remove all clothing in the privacy of the tent and then pour the remaining amount of distilled water (3.78 liters total) over his or her body, leaving the clothes and the water jug within the pool. The participant was provided a clean towel to dry him or herself off and asked to leave the towel within the kiddie pool. After the participant redressed, the contents of the kiddie pool were swirled around vigorously for 60 s and two 1.5-ml Eppendorf tubes were filled with samples of water obtained from the contents of the kiddie pool. These whole body washdown samples were then stored at −80°C until electrolyte analyses could be performed. Sweat [Na\(^+\)] and [K\(^+\)] were measured as in the plasma samples.

Saliva samples. All salivary samples were collected using the passive drool technique, in which all participants allowed saliva to accumulate within their mouths. Each participant then allowed the accumulated saliva to flow into a Dixie cup until ~1 ml of saliva was collected. The saliva was then immediately expelled into an Eppendorf tube, closed, and stored at −80°C until electrolyte analyses could be performed. Saliva [Na\(^+\)] and [K\(^+\)] were measured as in the plasma samples.

Urine samples. All urine samples were collected in plastic containers. Two 1.5-ml samples were removed and placed into Eppendorf tubes, which were immediately closed and stored at −80°C until electrolyte analyses could be performed.
electrolyte analyses could be performed. Urine \([\text{Na}^{+}], [\text{K}^{+}],\) and osmolality were measured as in the plasma samples. After these sample aliquots were removed and stored, all remaining urine was measured for volume (in ml) before being discarded.

Fluid balance parameters. Upon entering the laboratory, all participants were given free access to bottled water throughout the trial and asked to drink according to thirst. The volume of water was quantified by measuring the remaining contents and subtracting this volume from the known volume of water ingested by counting bottles emptied during the trial. An estimate of total fluid losses during the trial was calculated immediately before and after treadmill running by the following balance equation: fluid balance = water ingested (ml) – urine produced (ml) – body weight losses (kg losses were used to approximate sweat losses in ml).

Statistical Analyses

A two-way repeated measures ANOVA was used to examine potential differences in exercise (baseline, steady-state, and performance segments) versus condition (placebo, V2R agonist, and V2R antagonist) for plasma, urine, sweat, and saliva \([\text{Na}^{+}], [\text{K}^{+}],\) and osmolality values. A one-way repeated measures ANOVA was used to examine potential differences in fluid balance and performance across conditions (placebo, V2R agonist, and V2R antagonist). When necessary, a Bonferroni correction with unweighted means was performed post hoc to further identify exercise (row) and/or condition (column) differences between mean values. Linear regression analyses (Pearson’s) were performed to assess relationships between variables. Statistical significance \(\alpha\) level was set a priori at \(P < 0.05\). All data were presented as means ± SD unless otherwise noted. It was previously estimated that 5–8 participants would provide adequate statistical power (\(\beta = 0.2\)) to detect changes in sweat electrolyte concentrations that would exceed the within-participant coefficient of variation based on previous studies (25).

RESULTS

Ten participants successfully completed the study trial (8 males and 2 females). One female reported regular menses and was tested only during the follicular phase of her cycle while the other female ceased menstruation and was tested every other week, like the male subjects. Since previous pilot testing demonstrated no sex differences in any of the variables of interest (13), male and female data were combined for analyses. The mean age of the participants was 35.5 ± 13.1 yr with 8.8 ± 8.7 yr of running experience and a body mass index (BMI) of 22.5 ± 3.3 kg/cm². The average \(\dot{V}O_2\text{peak}\) of all participants was 58.7 ± 6.2 ml·kg\(^{-1}\)·min\(^{-1}\), with a peak treadmill running speed of 11.0 ± 1.4 mph and average time to \(\dot{V}O_2\text{peak}\) of 11.2 ± 2.4 s. The average speed that represented 60% of \(\dot{V}O_2\text{peak}\) (the designated steady-state running speed) was 6.6 ± 0.8 mph.

Changes in \([\text{Na}^{+}]\) and \([\text{K}^{+}]\) from plasma, urine, saliva, and sweat samples over both exercise (baseline, steady-state, and performance) and condition (placebo, V2R agonist, and V2R antagonist) are shown in Figs. 2 and 3. For plasma \([\text{Na}^{+}]\) (Fig. 2A), there was a significant exercise effect \([F(2,79) = 11.14]\) and condition effect \([F(2,79) = 3.81]\). For plasma \([\text{K}^{+}]\) (Fig. 2B), there was a significant exercise effect \([F(2,79) = 11.14]\) and condition effect \([F(2,79) = 3.81]\). For plasma \([\text{Na}^{+}]\) (Fig. 2A), there was a significant exercise effect \([F(2,79) = 11.14]\) and condition effect \([F(2,79) = 3.81]\). For plasma \([\text{K}^{+}]\) (Fig. 2B),
there was a significant exercise effect \( F(2,79) = 6.60 \). For urine \([\text{Na}^+]\) (Fig. 2C), there was a significant interaction between exercise and condition \( F(4,79) = 3.73 \) with a significant exercise effect \( F(2,79) = 7.76 \) and condition effect \( F(2,79) = 38.29 \). For urine \([\text{K}^+]\) (Fig. 2D), there was a significant exercise effect \( F(2,79) = 11.74 \) and condition effect \( F(2,79) = 52.29 \). For saliva \([\text{Na}^+]\) (Fig. 3A), there was a significant exercise effect \( F(2,79) = 11.61 \). There were no significant interaction, condition, or exercise effects for saliva \([\text{K}^+]\) (Fig. 3B), sweat \([\text{Na}^+]\) (Fig. 3C), or sweat \([\text{K}^+]\) (Fig. 3D) as measured by the sweat patch.

For plasma osmolality (Fig. 4A), there was a significant exercise effect \( F(2,77) = 26.00 \) and condition effect \( F(2,77) = 5.13 \). For urine osmolality (Fig. 4B), there was a significant condition effect \( F(2,77) = 84.98 \). For \([\text{AVP}]_p\) (Fig. 4C), there was a significant exercise effect \( F(2,27) = 22.33 \). There were no interaction, exercise, or condition effects for \([\text{OT}]_p\) (Fig. 4D).

To further assess whether or not there was an effect of V2R manipulation on sweat (patch) and saliva \([\text{Na}^+]\) content, mean differences from the placebo condition were calculated individually for both the V2R agonist and V2R antagonist conditions after the steady-state and performance exercise time points (Fig. 5). For sweat \([\text{Na}^+]\), a nonsignificant increase was seen with V2R antagonist treatment, whereas a nonsignificant decrease was seen with V2R agonist treatment (Fig. 5A). For saliva \([\text{Na}^+]\), greater increases were seen during V2R antagonist treatment compared with

Fig. 3. Two-way repeated-measures ANOVA results for saliva and sweat sodium (\([\text{Na}^+]\)) and potassium (\([\text{K}^+]\)) concentrations. Exercise is represented on the \(x\)-axis (Baseline, Steady-State, and Performance) and represents the temporal order in which the samples were taken. Condition (Placebo, V2R Agonist, and V2R Antagonist) is represented on the \(y\)-axis and represents the values obtained during each of the three separate pharmacological treatments. All data are represented by means (symbols) and standard deviation (bars). The standard deviation for the placebo trial (open circle) is represented by the wide “T” and solid line; the V2R Agonist trial (open square) is represented by the more narrow “T” and dashed line; while the V2R Antagonist (open triangle) is represented by the bold “T” and dotted line to distinguish between the overlapping error bars. A statistically significant Exercise Effect was seen in saliva \([\text{Na}^+]\) (A) without a significant difference between conditions in post hoc analyses.

For fluid balance and exercise performance parameters, one-way ANOVA analyses revealed significant differences in total urine produced and total water ingested, but not in overall water balance, performance time, or plasma volume change (Table 2). When a two-way repeated measures ANOVA was performed for plasma volume change across both exercise and condition, a significant exercise effect was noted \( F(1,53) = 21.83; P < 0.0001 \). For body weight change, there was also a significant exercise effect \( F(1,52) = 6.26; P = 0.02 \).

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V2R agonist treatment, but these differences were not statistically significant (Fig. 5B).

For thirst rating, there was a significant exercise effect \([F(2, 81) = 78.21; P = 0.0001]\) and condition effect \([F(2, 81) = 4.49; P = 0.01]\) between the V2R antagonist versus the placebo condition during the steady-state point. For the whole body washdown sweat collection, the mean sweat \([\text{Na}^+]\) was 14.1 ± 6.6, 12.7 ± 4.8, and 12.4 ± 3.8 meq/l for the placebo, V2R agonist, and V2R antagonist conditions, respectively \((P = 0.75)\). A weak correlation \((r = 0.39; P = 0.05)\) was demonstrated in sweat \([\text{Na}^+]\) between the sweat patch versus WBW technique.

**DISCUSSION**

The primary hypothesis that sweat water content, as reflected by sweat \([\text{Na}^+]\), is regulated by the vasopressin V2R at the level of the sweat gland was not supported by the results of this study. Confirmation of V2R stimulation by the selective V2R agonist desmopressin was evidenced by urine osmolality values that were higher than the values obtained in either the placebo and V2R antagonist condition at all exercise points tested (Fig. 4B). Similarly, confirmation of V2R antagonism by the selective V2R antagonist tolvaptan was evidenced by urine osmolality values that were significantly lower than those obtained in either the placebo and V2 agonist condition at all exercise points tested (Fig. 4B). Blocking the V2R augmented urinary free-water excretion, as verified by a urine volume production that was threefold greater than in the placebo and V2R agonist trials (Table 2). The lack of a significant decrease in urine volume with desmopressin treatment likely reflects near-maximal exercise-induced antidiuresis in the placebo

Table 1. *Linear correlations \((r)\) with endogenous [AVP]_P under the three randomized trial conditions*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>V2R Agonist</th>
<th>V2R Antagonist</th>
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</thead>
<tbody>
<tr>
<td>Plasma osmolality, mosmol/kg H2O</td>
<td>0.20</td>
<td>0.69*</td>
<td>0.69*</td>
</tr>
<tr>
<td>Plasma [Na(^+)], meq/l</td>
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<td>0.61*</td>
<td>0.55*</td>
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<td>−0.23</td>
<td>−0.06</td>
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<tr>
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<td>0.42*</td>
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<tr>
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<td>−0.45*</td>
<td>0.60*</td>
</tr>
<tr>
<td>Plasma Volume Δ, %</td>
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<td>−0.62*</td>
<td>−0.69*</td>
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<tr>
<td>Body Weight Δ, %</td>
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<td>−0.42</td>
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<tr>
<td>Plasma [Oxytocin], pg/ml</td>
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<td>0.81*</td>
<td>0.29</td>
</tr>
<tr>
<td>Thirst Rating, 1–10</td>
<td>0.27</td>
<td>0.50*</td>
<td>0.45*</td>
</tr>
</tbody>
</table>

\([\text{AVP}]_P\), plasma arginine vasopressin concentrations. Three randomized trial conditions: Placebo, pretreatment with the V2R Agonist desmopressin; and pretreatment with the V2R Antagonist tolvaptan. *\(P < 0.05\).
state. Thus the intended activation and blockade of the V2R by the treatment protocols were successfully confirmed by urinary output measures, and particularly highlighted by the changes in urine osmolality (Fig. 4B). These expected changes in urinary free water excretion, however, were not accompanied by parallel changes in either sweat or salivary secretions. It therefore appears unlikely that V2R activation stimulates AQP5 water channel activity in sweat or salivary glands, in contrast to AVP-mediated V2R activation with subsequent insertion of AQP2 into the lumen of the kidney collecting duct principal cells.

V2R effects on AVP secretion were physiologically compensated by osmotically appropriate changes in endogenous AVP secretion (Table 1). Furthermore, changes in [AVP]p coincided with osmotically appropriate changes in behavioral thirst ratings (Table 1). Hyperosmolality associated with maximal exercise intensity was likely attributed to the 9–10% decline in plasma volume, as documented previously (50). Of note, hyponatraemia (plasma [Na+] > 145 meq/l) was documented in seven athletes after the steady state and eight athletes after the performance run during the V2R antagonist trial (Fig. 2A). Drinking according to thirst, particularly in the V2R antagonist trial, may have been attenuated by stomach fullness (39) or distraction from completing the assigned treadmill task. The augmented hyponatraemia demonstrated in the V2R antagonist condition, however, did not adversely affect overall water balance, body weight change, or performance compared with both the placebo and the V2R agonist conditions (Table 2).

V2R Effects on Plasma Variables

Plasma [Na+] increased with increasing exercise intensity, which accounted for ~20% of the overall variance seen in plasma [Na+]. This corresponding hyponatraemia appeared to further stimulate endogenous AVP secretion, with plasma AVP levels statistically higher at the performance time point in the V2R antagonist condition (3.6 ± 2.9 pg/ml) compared with both the placebo (2.0 ± 1.6 pg/ml) and V2R agonist (2.5 ± 1.6 pg/ml) conditions (Fig. 4C). The significant increase in both plasma [Na+] and [AVP]p would be expected secondary to the significantly increased urinary free water losses seen under the V2R antagonist condition. Parallel changes were not seen between plasma [Na+] and [K+] (Fig. 2, A and B), which confirm independent regulation of the body’s main extracellular and intracellular cations during exercise (40).

Plasma oxytocin concentrations did not mirror any discernable osmotic pattern related to plasma, urine, sweat, or saliva [Na+] or [K+] (Fig. 4D). Previous investigations have demonstrated an electrophysiological response in human immortalized sweat glands (NCL-SG3 cells) when exposed to oxytocin
V2R Effects on Sweat Composition and Volume

Sweat [Na\(^+\)] did not mirror the same pattern as urine [Na\(^+\)] in response to the V2R agonist and antagonist (Fig. 3C). Thus it is clear that V2R does not influence sweat sodium concentration, as originally hypothesized. The average sweat [Na\(^+\)] of ~80 meq/l as measured in our participants is similar to those reported by other investigators using the same region and technique (13, 15). Sweat [K\(^+\)] values (Fig. 3D) were around the physiological range of ~4 meq/l, which suggested the “leaching” effect of epidermal electrolytes was minimal (49).

The WBW technique is considered the gold standard measurement of sweat electrolyte content during exercise (1, 2, 42). However, our sweat [Na\(^+\)] values were sevenfold lower than the patch measurements (between 12 and 14 meq/l) and ~50% lower than previously reported (2, 30). The large discrepancy between our patch and WBW results may have resulted from sweat droplets escaping from the measurement area; electrolytes trapped in clothing, towel, and/or the collection pool; contamination from cleaning solutions or prior use; or other unknown factors.

V2R Effects on Saliva

Salivary [Na\(^+\)] mirrored the pattern of plasma [Na\(^+\)] under the three pharmacological conditions (i.e., highest with the V2R antagonist and lowest with the V2R agonist; Fig. 3A). This suggests water absorption occurred in salivary glands after steady-state and performance running from a mechanism other than V2R activation. Weakly significant correlations were found between salivary [Na\(^+\)] versus plasma [Na\(^+\)] (r = 0.45), which offer limited support for the use of salivary measurements as a potential surrogate for the determination of changes in plasma [Na\(^+\)] in real time (48) during exercise (26), possibly due to removal of parasympathetic impulses that modify salivary secretion rates (26). However, the absolute measurements of plasma and salivary [Na\(^+\)] differed by a magnitude of approximately sevenfold, which would impose any clinical utility of using salivary measures to diagnose dysnatremia. Changes in salivary [K\(^+\)] did not mirror changes in plasma [K\(^+\)] (Fig. 3B) and should not be used as a surrogate marker of plasma [K\(^+\)] change.

V2R Effects on Urine Composition and Volume

Urine [Na\(^+\)] and osmolality values were highest during the V2R agonist condition and lowest during the V2R antagonist condition, as pharmacologically expected. Urine [Na\(^-\)] and osmolality increased after the performance trial under the V2R antagonist condition but not following the placebo or V2R agonist conditions (Figs. 2C and 4B). This paradoxical decline in urine concentrating ability has been previously noted in conjunction with relative decreases in urine excretion (16). Our data demonstrate a reversal of this paradoxical renal finding only during the V2R antagonist condition, suggesting that either the sensitivity of the V2R receptor was altered during high-intensity exercise (8, 23, 24), or the V2R was downregulated in response to dehydration (22). Urine output was also sustained in the V2R antagonist trial, suggesting V2R antagonism can override the competing effects of limited renal blood flow and reduced glomerular filtration rate in the production of urine during exercise (16, 27, 36).

V2R Effects on Overall Fluid Balance

Urineywater retention (V2R agonist condition) and water losses (V2R antagonist condition) were adequately compensated by drinking according to thirst during each study trial. There were no statistically significant differences between fluid balance and percent body weight change between the three study conditions (Table 2). As expected, fluid intake was greatest during the V2R antagonist condition with total water ingestion ~50% higher than either the total amount of water ingested during the placebo and V2R agonist condition to compensate for the threefold increase in urinary losses (Table 2).

V2R Effects on Core Temperature and Performance

The only statistically significant difference in peak body temperature was seen between the placebo versus V2R agonist conditions after the performance time point (38.4°C vs. 38.9°C, respectively). This finding appears to contradict data obtained from rats suggesting that central AVP administration acts as an antipyretic agent, at least in response to fever (31). There was no difference in exercise performance between the three pharmacological conditions (Table 2). However, treadmill running time to exhaustion was extended by 32 s (5%) during the V2R antagonist over the placebo trial despite the increased tonicity (hypernatremia) and associated cellular dehydration, which persisted after the steady-state run into the performance test.

Limitations

We utilized both J) the customary measurement (sweat patch) and 2) gold standard measurement (WBW) to measure changes in sweat [Na\(^+\)] as a primary outcome measure. Nonetheless, our inability to detect subtle, real-time, regulatory changes in sweat gland output remains a study limitation. The difference in urine osmolality between the V2R antagonist versus the placebo and V2R agonist conditions were sufficiently robust, however, to negate even modest measurement errors in the interpretation of our sweat data. Other potential study limitations include lack of control of room temperature, daily exercise, or food intake, all of which could potentially affect the results.

Perspectives and Significance

These data indicate that the V2R does not modulate water losses from sweat or saliva during moderate to high-intensity exercise stress. Despite the teleological advantage of reducing sweat water and sodium losses during exercise, this appears not to be a major homeostatic mechanism during acute exercise. The present study used pharmacological manipulation with a receptor-specific agonist and antagonist to activate and inhibit the AVP V2R, thereby overriding the effects of endogenous AVP secretion. As such, these results provide indirect evidence that the V2R does not affect translocation of AQP5 channels.
that are present in sweat and salivary glands. In contrast, this study verified that V2R activation or blockade produced physiologically appropriate, osmotically induced, secondary changes in endogenous [AVP] and thirst stimulation through activation or blockade of AQP2 channel insertion in the kidney collecting duct. Although the V2R antagonist produced mild hyponatremia unaccompanied by greater plasma volume losses at the end of the exercise trial, overall water balance, body weight change, and exercise performance were not significantly different between conditions.

Future basic science and translational studies should assess the contribution of muscarinic activation on the relationship between AQP5 translocation and sweat water output (18, 28, 41). Alternatively, the translocation of AQP5 water channels in sweat gland epithelium may be triggered by mechanical stimuli related to cell volume changes that are secondary to plasma tonicity changes (43). For example, stimulation of the muscarinic type 3 receptor by the acetylcholine receptor agonist carbachol has been shown to cause an unexpected decrease in cell volume with increased AQP5 trafficking (18). Similarly, rats exposed to hypertonic stress demonstrate an increase in AQP5 protein expression in lung, submandibular, and lacrimal glands through an ERK-dependent pathway (14). Hypotonicity has also been shown to reduce AQP5 expression through mechanical activation of transient receptor potential vanilloid 4 channels (21, 43). Thus perhaps our original hypothesis should now be extended in the direction of investigating a more direct relationship between plasma tonicity and sweat water output (Fig. 5). While central activation of muscarinic receptors may initiate eccrine sweat secretion, it appears that hypotonicity increases, and hypotonicity decreases, AQP-mediated plasma water absorption independently of V2R stimulation. Future sweat water output studies should target exercising humans and horses as unique thermoregulatory models to best translate molecular AQP5 water channel insights into a more coordinated understanding of whole body fluid homeostasis, physical performance, and secretory gland disease (3, 18).

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REFERENCES

14. Liu X, Bandypadhyay B, Nakamoto T, Singh B, Liedke W, Melvin JE, Ambudkar I. A Role for AQP5 in activation of TRPV4 by hypotho-

DISCLOSURES

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AUTHOR CONTRIBUTIONS


