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Acutely elevated vasopressin increases circulating concentrations of cortisol and aldosterone in fasting northern elephant seal (*Mirounga angustirostris*) pups

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Summary

The physiological actions of vasopressin (VP) in marine mammals are not well defined. To help elucidate its hormonal and renal effects in this group of mammals, northern elephant seal (*Mirounga angustirostris*) pups (*N*=7; 99±4 kg) were first infused with 0.9% saline (control; 220 ml), followed 24 h later with VP (as a 20 ng kg^{-1} bolus, then 2 ng kg^{-1} min^{-1} for approximately 35 min in 225±16 ml saline). During both control and VP periods, blood samples were collected prior to infusion, and 15, 30, 60, 120 min and 24 h after infusion to examine the hormonal responses of the pups to VP. Renal responses were quantified from 24 h urine samples obtained prior to infusion (control) and 24 h post-infusion. Compared to the control period, infusion of VP increased plasma concentrations of cortisol over a 120 min period and aldosterone over 30 min, while plasma renin activity (PRA) was decreased for a 120 min period. The plasma urea:creatinine ratio was elevated following infusion of VP. Urine output and osmotic clearance were increased by 69±18% (mean ± S.E.M.) and 36±10%, respectively, but free water clearance and glomerular filtration rate were not significantly altered 24 h post-infusion of VP. Solute (osmolality, Na^+, K^+ and Cl^-) excretion and fractional excretion of electrolytes were also increased when compared to control values. The increase in cortisol concentration suggests that VP may possess corticotropin releasing hormone-like activity in elephant seals. If osmotic diuresis and natriuresis are typical consequences of elevated [VP] in fasting pups, then not increasing VP normally during the fast may serve as a protective mechanism to avoid the potential loss of Na^+ induced by elevated [VP]. Therefore, under natural fasting conditions, pups may be highly sensitive to small changes in [VP], resulting in the maintenance of water and electrolyte balance.

Key words: aldosterone, cortisol, corticotropin-releasing factor, glomerular filtration rate, natriuresis, osmotic clearance, northern elephant seal, *Mirounga angustirostris*.

Introduction

After weaning, pups of the northern elephant seal *Mirounga angustirostris* undergo a 2–3 month fast, during which time they abstain from drinking seawater and are able to maintain their water balance by conserving the water liberated from oxidation of their large fat stores. During the fast, plasma osmolality and volume remain unaltered, indicating osmotic homeostasis and water balance (Castellini et al., 1990; Costa and Ortiz, 1980; Ortiz et al., 1996, 2000). Whether or not vasopressin (VP) is responsible for mediating this conservation of body water is not yet well established, especially since circulating VP concentrations remain relatively low and unchanged (Ortiz et al., 1996, 2000, 2002b). The lack of an increase in plasma [VP] during the prolonged fasting period in northern elephant seal pups may be anomalous among mammals because abstinence from water generally induces an elevation in circulating [VP]. For example, in dogs, water deprivation induces an increase in circulating [VP] (Claybaugh, 1976; Wade et al., 1983), thereby helping to abate the potential imbalances in water and electrolyte homeostasis. The lack of a fasting-induced increase in plasma [VP] in seal pups therefore suggests that fasting pups may be highly sensitive to low concentrations of VP or that pups maintain water balance *via* other unexamined mechanisms.

The primary action of VP is to facilitate the reabsorption of solute-free water from the collecting duct of the kidney, and this action may be associated with the reabsorption of urea (Klein et al., 1997). A number of studies provide compelling evidence that tubular water resorption is mediated *via* VP in seals. Fasting northern elephant seal pups infused with hypertonic saline exhibited chronically elevated plasma VP concentrations. Furthermore, excreted [VP] was elevated and negatively correlated with free water clearance (C_{H_2O}), and positively correlated with excreted cAMP levels, suggesting that VP mediated tubular water reabsorption (Ortiz et al.,

2002b). The intravenous infusion of pitressin in a water-loaded harbor seal *Phoca vitulina* resulted in an immediate decrease in urine flow, which was associated with a concomitant increase in urinary electrolyte concentrations (Bradley et al., 1954). Pitressin infusions also increased urine osmolality and osmotic clearance (C_{osm}), suggesting that free water reabsorption was increased (or C_{H_2O} was reduced) (Page et al., 1954). Under force-fasted conditions, Baikal (*P. sibirica*) and ringed (*P. hispida*) seals exhibited an increase in excreted [VP] associated with a concomitant decrease in urine output and increase in urine osmolality (Hong et al., 1982). A positive and significant correlation between urine osmolality and excreted [VP] was also observed, further suggesting that the increase in urine osmolality was attributed to an increase in tubular water reabsorption *via* VP stimulation (Hong et al., 1982). In force-fasted grey seals *Halichoerus grypus*, plasma osmolality and [VP] increased in conjunction with an increase in urine osmolality (Skog and Folkow, 1994).

In addition, VP interacts dynamically with other physiological systems in mammals. Aside from its role in tubular resorption of water, VP has also been implicated in elevating glomerular filtration rate (GFR) in rats (Bouby et al., 1996; Roald et al., 2000) and Na^+ excretion in dogs (Bie et al., 1984; Buckalew and Dimond, 1976; Chan and Sawyer, 1961; Johnson et al., 1979; Kompanowska-Jezierska et al., 1998; Smith et al., 1979; Sondeen and Claybaugh, 1989). Although infusion of hypertonic saline in fasting northern elephant seal pups induced a chronic (24 h) elevation in plasma [VP], GFR and Na^+ excretion, the contribution of elevated [VP] to an increase in GFR and Na^+ excretion could not be ascertained (Ortiz et al., 2002b).

Previous studies of pitressin infusions in seals suggest that VP possesses an antidiuretic function; however, those studies did not describe other hormonal or renal effects of the infusion. Therefore, to reconcile some of the previously observed effects of the hypertonic saline-induced elevation in [VP] as well as to describe the effects of VP on peripheral physiological systems, we quantified hormonal and renal responses of fasting northern elephant seal pups to infused VP.

Materials and methods

Details of the animal handling and catheterization procedures have been described previously (Ortiz et al., 2002a,b) and will only therefore be mentioned briefly here.

Animals

Seven northern elephant seal pups *Mirounga angustirostris* Gill 1866 (99±4 kg; 4 males, 3 females), between 5 and 8 weeks postweaning, were transported from Año Nuevo State Park (approximately 30 km north of Santa Cruz, CA, USA) to Long Marine Laboratory, University of California, Santa Cruz. Upon arrival at the marine laboratory, pups were weighed using a hanging-load cell and placed in a sandpit overnight. The following morning, a catheter was inserted into the extradural spinal vein after the pup had been sedated with 0.01 ml kg^{-1} body mass tiletamine HCl and zolazepam HCl (Telazol; Fort Dodge Animal Health, Fort Dodge, IA, USA). Following the catheterization procedure, pups were allowed to recover for approximately 22 h prior to the collection of control data. The catheter served as the sole route by which materials were infused or blood was collected. Immediately following the catheterization and every day (3–4 days) until the catheter was removed, each pup received 1 g cefazolin sodium (Fort Dodge Animal Health, Fort Dodge, IA, USA) to minimize the risk of a bacterial infection from the catheter. The catheterized pup was then placed in a metabolic cage with a urine collection pan underneath attached to a collection flask.

Control infusion

During the control infusion, pups received 220 ml of sterile isotonic saline calculated to be the approximate volume of vehicle to be given on the day of the hormone infusion. Control blood samples were collected prior to (pre-infusion) and 15, 30, 60 and 120 min, and 24 h post-infusion, with reference to the end of the infusion period. The 24 h sample also served as the pre-infusion sample for the VP infusion trial.

Vasopressin infusion

On the day following the collection of control blood samples, pups were infused with a bolus (20 ng kg^{-1} in 5 ml isotonic saline) of arginine VP (AVP; Phoenix Pharmaceuticals, Belmont, CA, USA) followed by a constant infusion of 0.6 ng kg^{-1} ml^{-1} for a 34 min period in sterile, isotonic saline (225±16 ml). Based on a percentage total body water (TBW) pool size of approximately 38%, determined empirically for similarly sized pups (Ortiz et al., 2002a, 2003), the infused volumes amounted to 0.60±0.02% of the pups' TBW pool. Post-infusion blood samples were taken at 15, 30, 60 and 120 min, and at 24 h after the end of the infusion period (as with the control sampling schedule).

Blood samples and plasma analyses

All blood samples were obtained from the indwelling catheter into 20 ml syringes. Prior to the collection of each blood sample, a 3 ml sample was drawn into a 20 ml syringe with 10 ml of sterile isotonic saline to clear the catheter line of any residual blood that could potentially contaminate the samples drawn. Blood was transferred into pre-chilled collection tubes containing either lithium heparin or EDTA. After 30 s of gentle rocking, duplicate samples of whole blood were removed in capillary tubes and spun in a microcentrifuge to determine hematocrit (Hct) (%). The remaining blood was centrifuged for 15 min (1500 ***g*** at 4°C), and plasma collected and frozen at –20°C for later analyses.

All assays were conducted using commercially available radioimmunoassay kits validated previously for use with northern elephant seal plasma (Ortiz et al., 2002a,b, 2000). Aldosterone (DPC, Los Angeles, CA, USA), cortisol (DPC), VP (AVP; Phoenix Pharmaceuticals), and angiotensin II (AII; Phoenix Pharmaceuticals) were analyzed from heparinized plasma, and plasma renin activity (PRA; Dupont-NEN, MA,

USA) was determined from EDTA-treated plasma. Prior to being assayed, VP and AII were extracted from the plasma using a C-18 column extraction procedure as previously described (Zenteno-Savin and Castellini, 1998). Final concentrations were not corrected for incomplete extractions. All samples were run in duplicate in each assay. Hormone assays displayed an intra-assay percentage coefficient of variation (%CV) of 7–9% and interassay %CV of 6–11% (including urinary hormones). Electrolytes (Na^+, K^+, Cl^-), creatinine, blood urea-nitrogen and total proteins were analyzed from heparinized plasma and were measured on a clinical auto-analyzer (Roche Diagnostics, Somerville, NJ, USA). Osmolality was determined using a freezing point osmometer (Fiske, Norwood, MA, USA).

Urine analyses

In each study, urine volume in the collection flask was measured after a 24 h collection period and a 3 ml portion was filtered and frozen for later analyses. Urine samples were collected without the use of preservatives. Because urine samples could only be collected as the pups naturally voided, obtaining paired plasma–urine samples was not possible in our hands. In order to maintain consistency in collection of urine, a cumulative 24 h sample was therefore collected. Urine samples were analyzed for electrolytes (Na^+, K^+, Cl^-), creatinine, osmolality and urea-nitrogen using the same techniques as with the plasma samples. The same commercial assays used to measure the plasma hormones were used to measure the extracted urinary hormones. Urinary VP, AII and urodilatin (Phoenix Pharmaceuticals) were extracted as for the plasma, but in a volume of 0.5 ml. Aldosterone and cortisol were extracted as previously described (Ortiz et al., 1999). As with the plasma extractions, final urinary concentrations were not corrected for by incomplete extractions. Prostaglandin E_2 (PGE_2; Assay Designs, Ann Arbor, MI, USA) was analyzed by enzymatic immunoassay following a 1:10 dilution of urine with assay buffer. The PGE_2 kit displayed significant cross-reactivity with the urinary elephant seal PGE_2, as indicated by the significant parallelism exhibited between the standards and the diluted urine pool.

Calculations

For all variables, excretion values were calculated as urinary concentration × daily urine volume. GFR was estimated by standard creatinine clearance. Osmotic clearance (C_{osm}; ml h^{-1}) was calculated as:

$$C_{osm} = U_{osm}\dot{V}/P_{osm}\,, \quad (1)$$

where U_{osm} and P_{osm} represent urinary and plasma osmolality, respectively, $\dot{V}$ is urine flow rate (ml h^{-1}). Free water clearance (C_{H2O}) was calculated as the difference between $\dot{V}$ and C_{osm}.

Fractional excretion (FE; %) was calculated as:

$$FE = ([x]_U\dot{V}) / ([x]_P GFR) \times 100\,, \quad (2)$$

where $[x]_U$ and $[x]_P$ represent urinary and plasma concentrations, respectively, of a compound x.

Statistics

Means for plasma values during the post-infusion period were compared to those during the control period by two-way analysis of variance (ANOVA) adjusted for repeated measures over time. If significant group × time interactions were not observed, means during the post-infusion period were compared to pre-infusion values by one-way ANOVA adjusted for repeated measures. Excretion values between control and post-infusion periods were compared by paired *t*-test. Fisher's PLSD test was administered *post-hoc* if significance was determined. Values (means ± S.E.M.) were considered significantly different at $P<0.05$. Statistical analyses of means were made using Statview (SAS, 1998).

Results

Because the volumes infused were such a small percentage (<1%) of the pups' TBW pool size, the infused saline was probably not sufficient to alter plasma volume, and thus urine output. Also, the lack of a change in hematocrit (57±1 and 56±1%), osmolality (311±1 and 314±3 mosm l^{-1}) and total proteins (5.6±0.2 and 5.3±0.1 mg dl^{-1}) between control and post-infusion periods, respectively, suggests that plasma volume was not altered following the infusion of saline during control and experimental periods. Therefore, the observed responses could be attributed to the effects of VP and not blood volume expansion.

Vasopressin data

Plasma VP concentrations were not significantly altered during the control sampling period; however, mean concentrations increased by approximately 13-fold after 15 min and threefold by 30 min post-infusion, remaining significantly ($P=0.0032$) higher than control values for at least 120 min post-infusion (Fig. 1). Total excreted [VP] increased 10.5-fold above control values following the infusion (Fig. 1).

Plasma data

Plasma aldosterone and cortisol concentrations were elevated and PRA reduced following the infusion (Fig. 2); however AII (12.9±2.6 and 15.2±2.8 pg ml^{-1}) was not significantly altered between control and post-infusion periods (not shown). Plasma aldosterone returned to control concentrations within 60 min while plasma cortisol and PRA returned to control concentrations within 24 h (Fig. 2). Electrolytes (Na^+: 155±1 and 153±1 mmol l^{-1}; K^+: 4.4±0.1 and 4.6±0.1 mmol l^{-1}; Cl^-: 106±1 and 106±1 mmol l^{-1}), blood urea-nitrogen (1.9±0.1 and 1.9±0.1 mmol l^{-1}) and creatinine (74.5±3.3 and 71.4±1.6 μmol l^{-1}) were not altered between control and post-infusion periods, respectively. During the control period the plasma urea:creatinine ratio (U:C) was unchanged (26.1±0.8); however, following the infusion of VP, the U:C ratio increased by 15% ($P=0.0076$) above pre-infusion levels (24.5±1.6) after 120 min (28.1±1.8) and remained elevated after 24 h (27.7±1.8) (not shown).

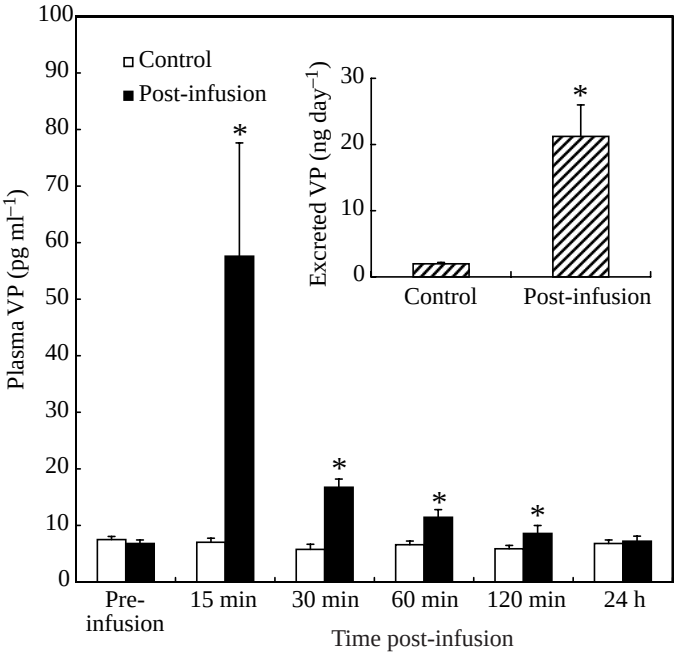

Fig. 1. Plasma vasopressin (VP) levels during control and post-infusion sampling periods. Values are means ± S.E.M. (N=7). Insert: total VP excreted over a 24 h period in controls and post-infusion. *Significantly different (P<0.05) from control value.

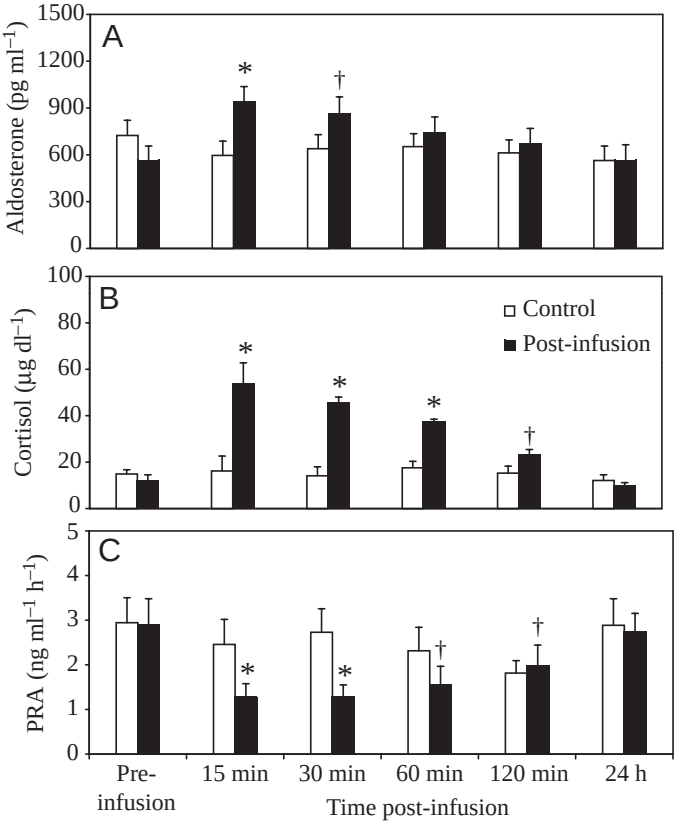

Fig. 2. (A) Plasma aldosterone (Al) and (B) cortisol concentrations, and (C) plasma renin activity (PRA), in response to the infusion of VP. See Materials and methods for details. Values are means ± S.E.M. (N=7). *Significantly different (P<0.05) from control; †significantly different (P<0.05) from Pre.

Urine output and excretion data

Compared to the control values, following infusion of VP, urine output over a 24 h period (162±17 and 278±36 ml day^{-1}) and osmotic clearance (698±64 and 884±95 ml day^{-1}) increased by 69±18% and 36±10%, respectively; however, free water clearance (–533±53 and –602±72 ml day^{-1}) and GFR (80±5 and 78±7 l day^{-1}; not shown) were not significantly altered between control and post-infusion periods, respectively (Fig. 3). Electrolyte (Fig. 4) and osmolal excretion (212±21 and 277±29 mosmol day^{-1}) as well as fractional excretion of electrolytes were significantly increased 24 h post-infusion (Table 1). Urea excretion (46±5 and 41±7 mmol day^{-1}) and fractional excretion of urea (Table 1) were not significantly altered following infusion of VP. Excreted cortisol and urodilatin levels were elevated following the infusion of VP; however aldosterone, AII and PGE_2 levels were not significantly altered (Table 2).

Discussion

Because seals primarily inhabit a hyperosmotic environment and naturally endure prolonged periods of food and water deprivation, this group of mammals is an intriguing model with which to address comparative, environmental and evolutionary questions about renal salt and water handling. Moreover, the physiological role played by VP in water homeostasis in marine mammals has been a topic of study for decades and remains largely unresolved (for a review, see Ortiz, 2001). The peripheral effects of VP on other hormonal systems also have yet to be examined in marine mammals. Although vasopressin is classically recognized as the primary antidiuretic hormone in mammals, it appears to induce a number of other physiological changes. Despite the fact that the present study cannot confirm an antidiuretic function of VP in fasting seals, the results identify at least two of the many potential functions seen in other mammals that involve VP, suggesting that VP may be as dynamic in marine mammals as in terrestrial mammals. The two functions in fasting northern elephant seal pups that appear to be associated with VP are (1) a natriuresis and (2) stimulation of a neuroendocrine response *via* the pituitary–adrenal axis. It is also possible that VP may acutely increase GFR in fasting seals.

The extradural vein of the pups was catheterized, so that frequent serial blood samples could be obtained in order to examine the acute peripheral effects of VP. We recognize, however, that the collection of 24 h urine samples only provides an opportunity to quantify the net effects of acutely elevated [VP] on kidney function and does not provide an examination of more acute and dynamic renal changes. Therefore, the renal responses to acutely elevated [VP] are discussed in this context, especially since the 22 h period between the 120 min and 24 h post-infusion blood samples

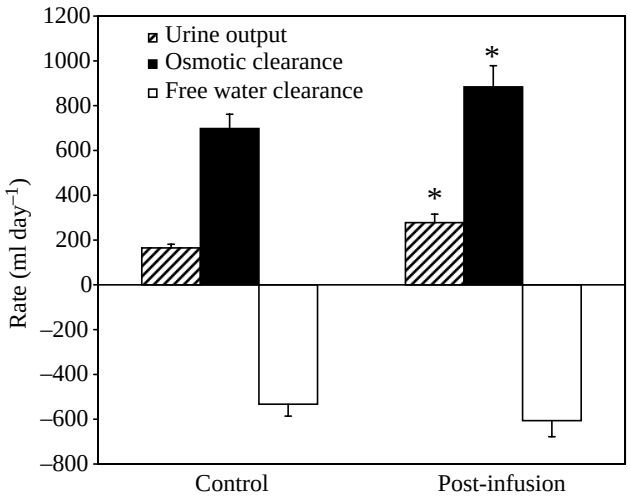

Fig. 3. Urine output, osmotic clearance (C_{osm}) and free water clearance (C_{H2O}) over a 24 h period in control and post-infused pups. See Materials and methods for details. Values are means ± S.E.M. (N=7). *Significantly different (P<0.05) from control values.

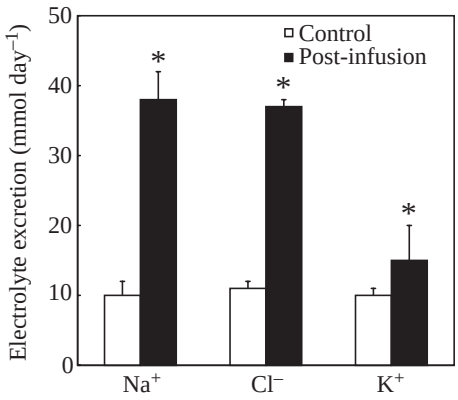

Fig. 4. Excreted electrolytes over a 24 h period in control and post-infused pups. Values are means ± S.E.M. (N=7). *Significantly different (P<0.05) from control value.

probably represents a refractory period in which kidney function was compensating for the peripheral changes induced by acutely elevated [VP]. Although the 24 h post-infusion period was characterized by a diuresis and natriuresis, the kidney may have been in an antidiuretic state during the 2 h post-infusion period when [VP] was significantly elevated. Nonetheless, the observed net diuresis is consistent with previous studies, which have shown that acute infusion of VP increases urine output as well as Na^+ and K^+ excretion in mammals (MacFarlane et al., 1967; Sondeen and Claybaugh, 1989). The diuresis observed in the present study can be attributed to an increase in solute excretion, or osmotic diuresis, and not to an increase in free water clearance. We have previously shown that under a chronic (24 h) state of hyperosmolality, circulating [VP] remained elevated until the condition of hyperosmolality was rectified, during which time free water clearance was reduced, indicating that VP

Table 1. *Fractional excretion of electrolytes and urea over a 24 h period before (control) and post infusion of vasopressin*

	Fractional excretion (%)	
	Control	Post-infusion
Na^+	0.091±0.018	0.328±0.034*
K^+	2.7±0.3	4.3±0.3*
Cl^-	0.167±0.038	0.452±0.058*
Urea	30.0±2.6	27.4±3.1

Values are means ± S.E.M.
*Significantly different (P<0.05) from control values.
See Materials and methods for details.

Table 2. *Hormones excreted during a 24 h period before (control) and post infusion of vasopressin*

	Amount excreted per 24 h	
	Control	Post-infusion
Aldosterone (ng)	1044±165	923±142
Angiotensin II (ng)	10.4±1.5	11.5±1.2
Cortisol (μg)	168±36	224±35*
Prostaglandin E_2 (nmol)	531±169	392±86
Urodilatin (ng)	4.8±0.5	9.4±1.4*

Values are means ± S.E.M.
*Significantly different (P<0.05) from control values.
See Materials and methods for details.

mediated the tubular resorption of water (Ortiz et al., 2002b). In the present study, the effect of acutely elevated [VP] was no net change in free water clearance. This distinction between acutely and chronically elevated circulating VP concentrations may be the underlying factor responsible for the contrasting VP-related responses observed between the two studies. Therefore, the antidiuretic function of VP in fasting seals may depend on the duration of exposure of target tissues to VP. Thus, the physiological functions of VP in fasting seals appear to be dynamic, depending on whether the elevation in [VP] is chronic or acute.

Infused VP has been shown to induce natriuresis in a variety of terrestrial mammals (Fejes-Tóth and Szenasi, 1981; Kompanowska-Jezierska et al., 1998; MacFarlane et al., 1967), which is consistent with the present study. However, excreted K^+ and Cl^-, FE_{K+}, and FE_{Cl^-} were also elevated, suggesting that the actions of VP on electrolyte handling are non-selective. Infusion of pitressin in the harbor seal was also associated with an increase in excreted Na^+ and K^+ (Bradley et al., 1954). Despite the natriuretic effect of VP, the loss of Na^+ amounted to approximately 700 mg, or the amount of Na^+ in approximately 65 ml of seawater. Although the present study reveals that VP may function as a natriuretic, this relatively small loss of Na^+ appears to be physiologically insignificant. However, over the course of 2–3 months, acute elevations in [VP] may result in significant loss of Na^+. Therefore, under

naturally fasting conditions, [VP] may not increase over the course of the fast, as expected, in order to abate the natriuretic actions of VP. Thus, the kidneys may be keenly sensitive to low concentrations of VP, thereby maintaining both water and electrolyte homeostasis.

Previously in fasting seal pups, following plasma volume expansion by infusion of isotonic saline and hypernatremia induced by infusion of hypertonic saline, circulating and excreted ANP concentrations were not elevated (Ortiz et al., 2002b), suggesting that another natriuretic mechanism is employed in these seals. The kidney-derived natriuretic factor, urodilatin, has been reported to possess greater natriuretic activity than ANP (Drummer et al., 1996; Goetz et al., 1990). Therefore, in the present study, we examined the response of urodilatin instead of ANP to VP stimulation. Excreted urodilatin was elevated post-infusion, suggesting that the natriuretic function of VP may be mediated *via* a physiological cascade incorporating urodilatin.

VP has been implicated in the elevation of GFR in rats (Bouby et al., 1996; Roald et al., 2000), so we hypothesized that creatinine excretion would increase in response to acutely elevated [VP]. Although the net effect was no significant change in GFR following infusion of VP, the significant increase in plasma U:C ratio, a recognized indicator of altered filtration rate (Duarte and Preuss, 1993), suggests that filtration rate may have been acutely elevated as a consequence of the increase in [VP].

Vasopressin has been measured as an index of stress and can possess secretory properties similar to that of corticotropin releasing hormone (CRH) (Aguilera and Rabadan-Diehl, 2000; Brooks and Challis, 1989; Kjaer, 1993; Zehnder et al., 1995). As an adrenocorticotropin (ACTH) secretogogue, VP may stimulate the release of cortisol (Brooks and Challis, 1989). In the present study, plasma cortisol concentration was increased approximately 4.5-fold by 15 min post-infusion and remained nearly double after 2 h, while plasma aldosterone concentration increased by approximately 66% after 15 min, suggesting that VP possesses corticotropin releasing factor-like action in northern elephant seal pups. Alternatively, the increase in plasma aldosterone may have been in response to the increased natriuresis resulting in the maintenance of plasma Na^+ concentrations. During natural fasting conditions, plasma cortisol increases linearly over the course of the fast (Ortiz et al., 2001a,b) despite the lack of an increase in [VP] (Ortiz et al., 1996, 2000), suggesting that the fasting-induced increase in cortisol is not VP mediated.

The reduction in plasma renin activity (PRA) following the infusion of VP is consistent with that observed in terrestrial mammals (Johnson et al., 1979; Merrill and Cowley, 1986; Reid et al., 1983); however, the lack of a decrease in AII was unexpected since a decrease in renin may also be associated with a decrease in AII (Morton et al., 1982). The dissociation between renin and AII in the present study suggests that (1) the production of AII is unaffected by the observed reductions in renin or (2) the reduction in AII is delayed beyond the 24 h period examined in fasting northern elephant seal pups. Also, under natural fasting conditions, PRA and aldosterone are linearly increased over the first 5 weeks of the fast while VP concentrations remain relatively low and unchanged (Ortiz et al., 2000). The renin–angiotensin–aldosterone system (RAAS) probably contributes significantly to the conservation of water and electrolytes during the fast, so not increasing VP levels during the fast may be beneficial to the pups by not attenuating the response of RAAS. This relationship between VP and PRA provides another plausible explanation for fasting northern elephant seal pups to have developed an increased sensitivity to low VP concentrations.

In mammals, VP stimulates urea transporters to enhance urea resorption into the renal medulla and, thus decreases FE_{urea}, while glucocorticoids have been shown to downregulate VP-regulated urea transporters, resulting in an increase in FE_{urea} (Klein et al., 1997). Despite the increase in circulating and excreted cortisol, the elevated concentrations of VP may have been sufficient to alleviate the glucocorticoid-mediated downregulation of VP-regulated urea transporters, resulting in no net alteration in FE_{urea}.

In summary, the increase in plasma and excreted cortisol levels suggests that VP may function as a potent CRF in these mammals, as in terrestrial mammals. Plasma aldosterone may have increased acutely in response to the natriuresis. The suppression of PRA by VP suggests that during the fast [VP] is not elevated to alleviate the inhibition on renin because, under natural conditions, PRA and aldosterone are concomitantly increased over the first 5 weeks of the fast (Ortiz et al., 2000). Acutely elevated [VP] induced a net osmotic diuresis accompanied by natriuresis, without affecting net free water clearance. Although GFR measured 24 h post-infusion was not elevated, the increase in plasma U:C ratio suggests that filtration rate may have been elevated during the first 120 min post-infusion, when circulating [VP] was also increased. Excretion of Na^+, K^+ and Cl^- were all elevated, suggesting that electrolyte excretion in response to VP is non-selective in fasting seals. The increase in excreted urodilatin suggests that the VP-induced natriuresis may have been mediated *via* this natriuretic peptide. The functions of natriuretic peptides in marine mammals warrant further investigation. In terrestrial mammals, VP is typically elevated in response to an increase in plasma osmolality and a decrease in blood volume resulting from water deprivation-induced dehydration. However, fasting northern elephant seal pups maintain constant plasma osmolality and blood volume, thereby precluding an increase in [VP]. Nonetheless, the peripheral actions of acutely elevated [VP] observed in the present study are consistent with those observed in terrestrial mammals, suggesting that VP is as dynamic in fasting seals as in terrestrial mammals.

Abbreviations

AII	angiotensin II
ANOVA	analysis of variance
AVP	arginine VP
C_{H_2O}	free water clearance

C_{osm}	osmotic clearance
CV	coefficient of variation
FE	fractional excretion
Hct	hematocrit
GFR	glomerular filtration rate
PGE_2	prostaglandin E_2
PRA	plasma renin activity
RAAS	renin–angiotensin–aldosterone system
S.E.M.	standard error of the mean
TBW	total body water
U:C	urea:creatinine ratio
U_{osm}, P_{osm}	urinary and plasma osmolality
$\dot{V}$	urine flow rate
VP	vasopressin

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